Study of Phthalates in Pregnant Women and Children

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STAR PROGRESS REVIEW WORKSHOP
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- A large, coherent body of animal studies demonstrates reproductive toxicity of several phthalates

- Phthalate exposure is prevalent in the US population (CDC 2002, 2003)

- Urine assays are now sensitive, reliable and (relatively) inexpensive

No human studies of prenatal phthalate exposure prior to ours

Phthalates and their metabolites

Parent Compound

Dibutyl phthalate (DBP)

Benzyl butyl phthalate (BzBP)

Di-isobutyl phthalate (DiBP)

Diethyl phthalate (DEP)

Di-n-octyl phthalate (DnOP)

Dimethyl phthalate (DMP)

Di-2-ethylhexyl phthalate (DEHP)

Primary Metabolites

Mono-n-butyl phthalate (MBP)

Mono-benzyl phthalate (MBzP)

Mono-isobutyl phthalate (MiBP)

Mono-ethyl phthalate (MEP)

Mono-3-carboxypropyl phthalate (MCPP)

Mono-methyl phthalate (MMP)
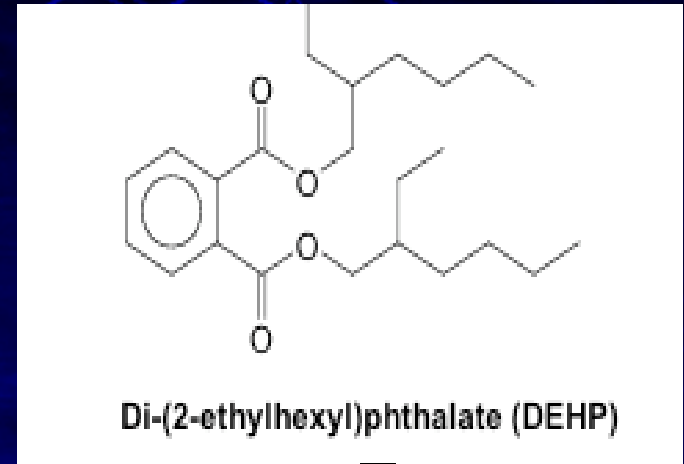
Mono-2-ethylhexyl phthalate (MEHP)

Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)

Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)

Di-2-Ethylhexyl Phthalate (DEHP)

- US production >1 mill lbs/yr
- >95% used as a plasticizer in PVC
- Not chemically bound: migrates from plastic
- Multiple urinary metabolites
  - *MEHHP (52.7%)*
  - *MEOHP (31.8%)*
  - *MEHP (15.5%)*

(Koch 2004)

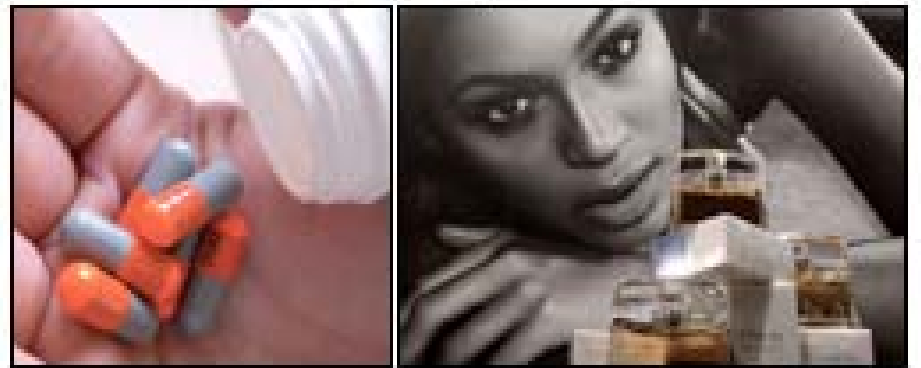Di-(2-ethylhexyl)phthalate (DEHP)

Dibutyl Phthalate (DBP)

- US production:
  >1 million lbs/yr

- Occupational exposure:
  (e.g. nail salons)

- In:
  - Cellulose acetate plastics

  - Lacquer, varnish, adhesives

  - Medical coatings and patches
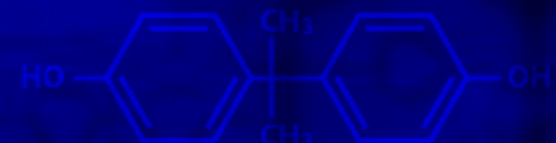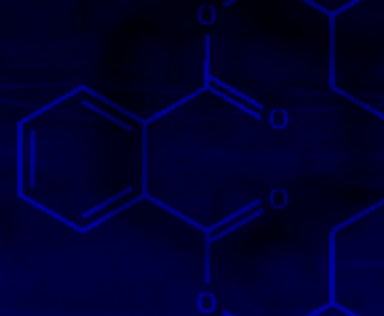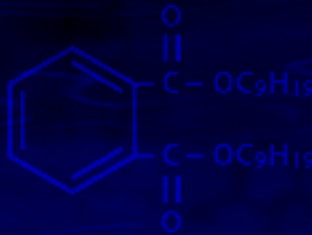
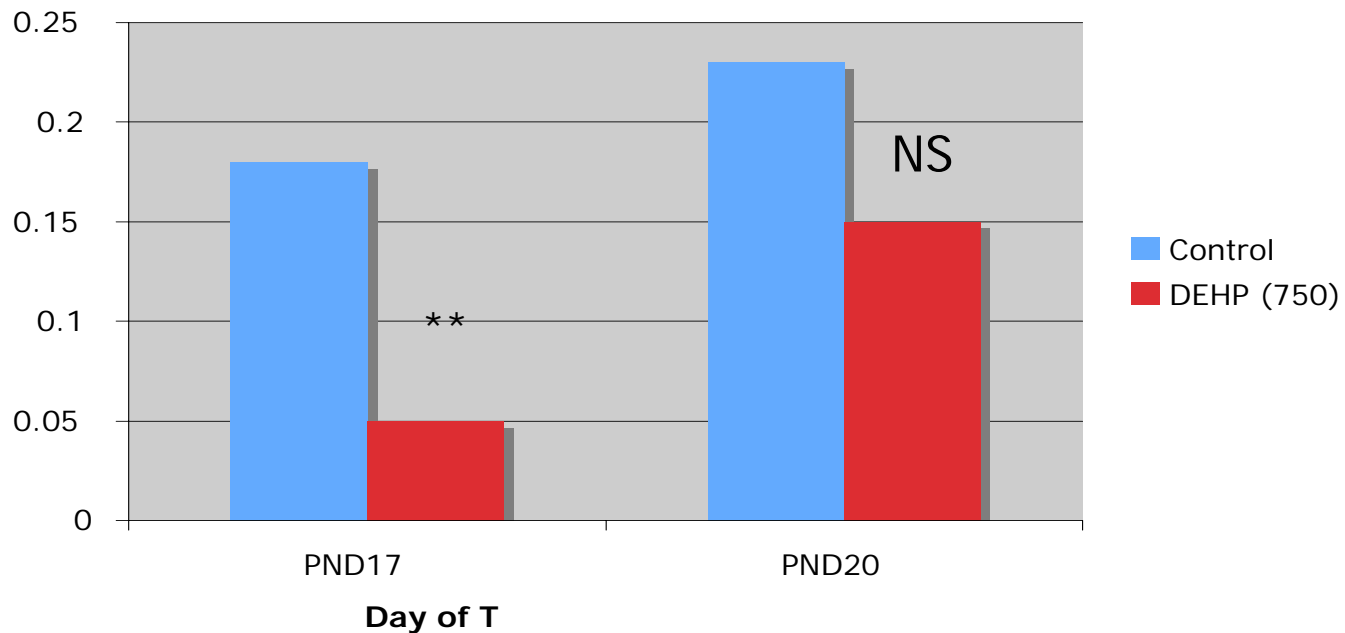 - Cosmetics and nail polish
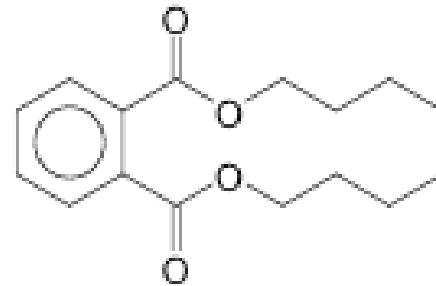
Di-(*n*-butyl)phthalate (DBP)

Overview of Phthalate Toxicology

- Anti-androgenic

- Decrease AGD

- Phthalate Syndrome

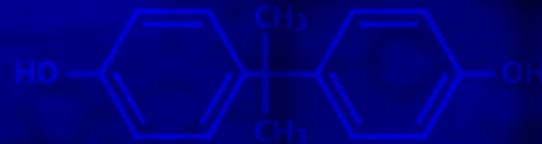# Phthalates decrease fetal testosterone

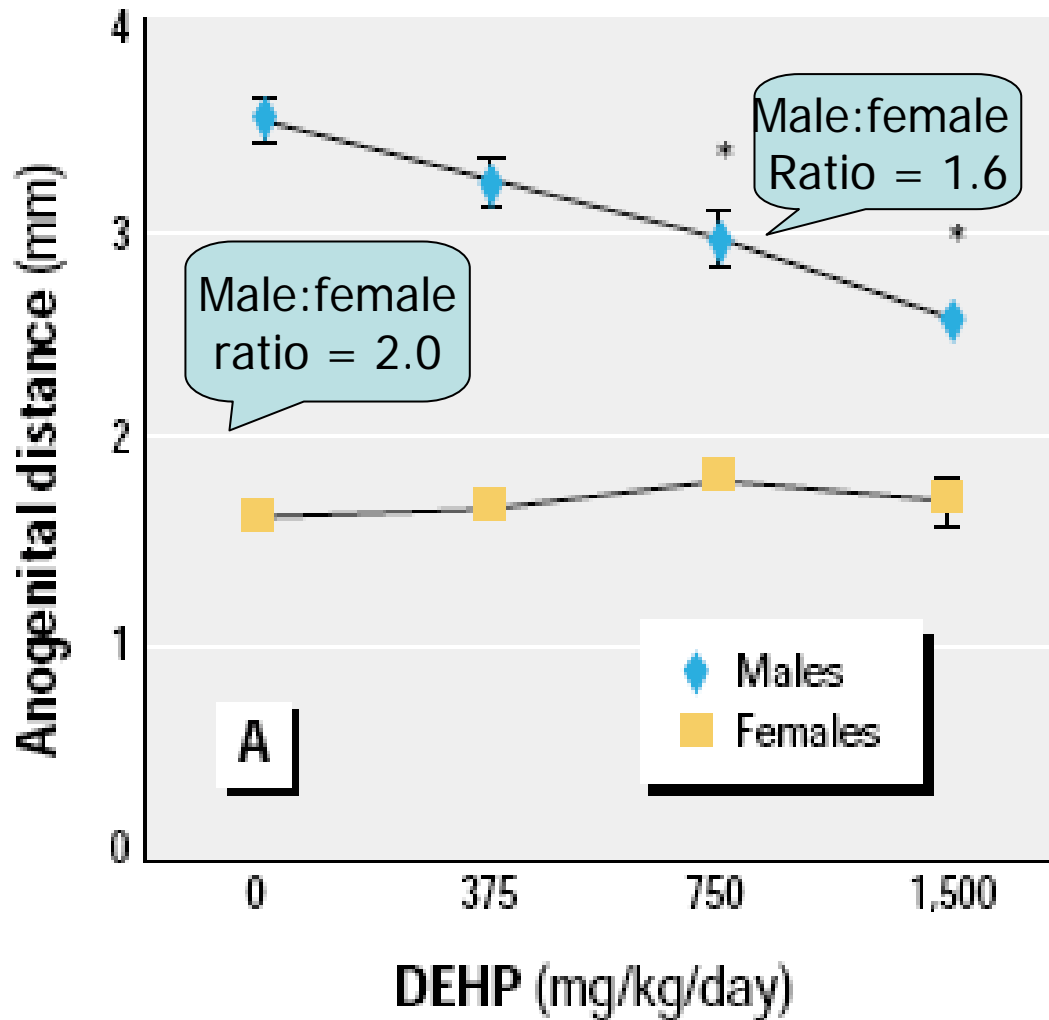**DEHP-induced reduction in fetal testosterone is age dependent**

(Parks et al, 2000)

Anogenital Distance (AGD)

- Sexually dimorphic: In rodents, AGD is about twice as long in males as in females

- In rodents, male AGD is shortened by prenatal exposure to anti-androgens (including: phthalates, flutamide and vinclozilin)
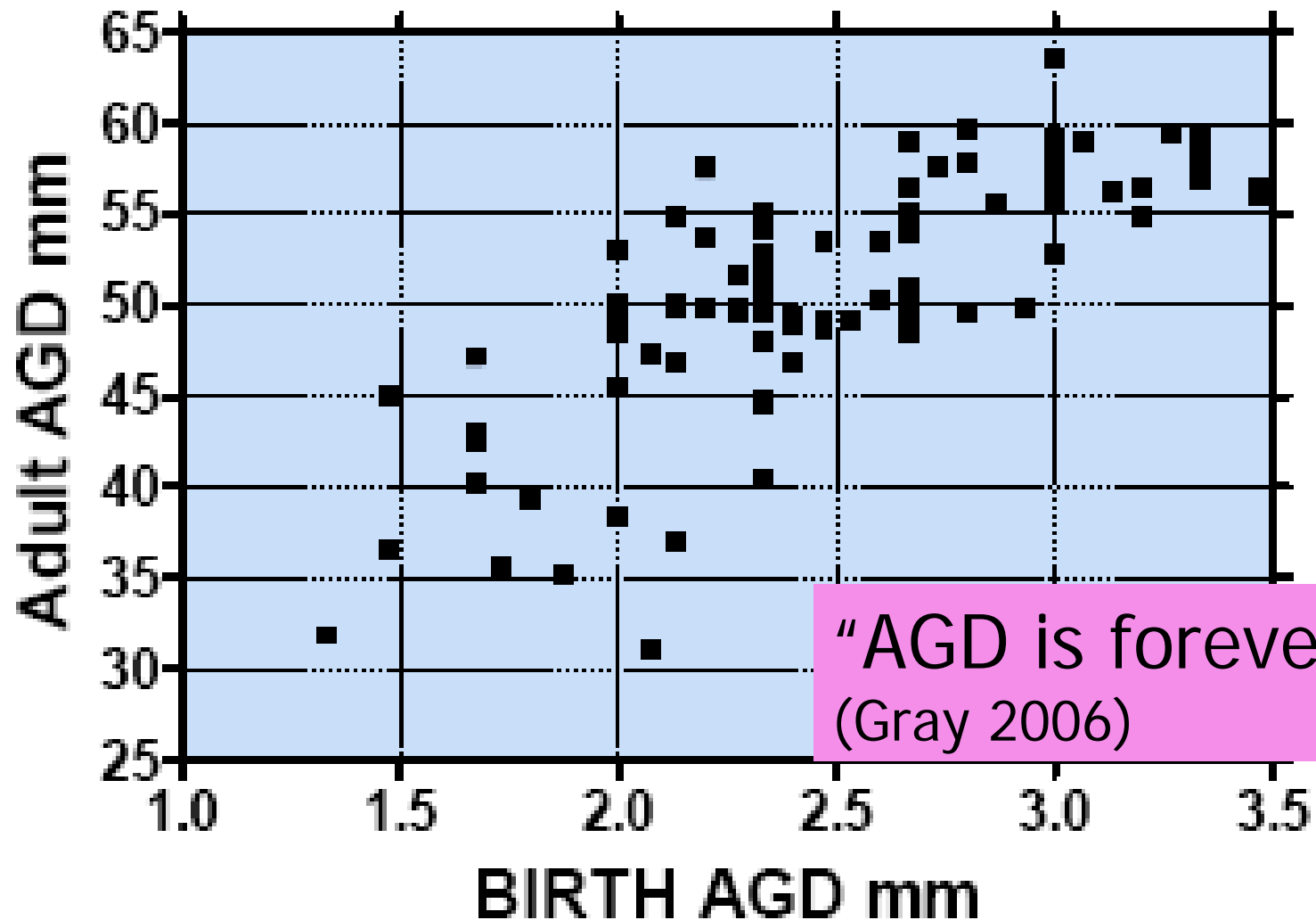
DEHP and AGD (Moore, 2001)

Antiandrogen-treated rats with reduced AGD at birth have reduced AGD as adults.
Hotchkiss et al. 2004

"AGD is forever" (Gray 2006)

Phthalate Syndrome

Malformations of:

- Perineum:
  - **Reduced AGD**
- Epididymis
- Vas deferens
- Seminal vesicles
- Prostate
- External genitalia:
  - Hypospadias
  - Cryptorchidism

Downregulation of

- Fetal testicular testosterone
- Insl-3

Significant ⇓ in testosterone and Leydig cell differentiation @ doses below the NOAEL for DBP

The question we addressed:
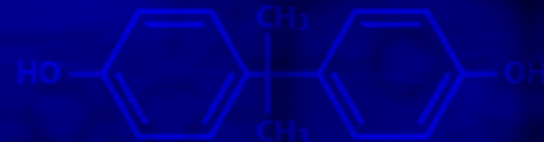
Does prenatal phthalate exposure alter male sexual development in humans?

Study of Phthalates
in Pregnant Women and Children

Designed to assess infant genital development in relation to prenatal phthalate exposure

Study Population

Mothers who:

- Were recruited at a prenatal visit in: Columbia MO, Minneapolis MN, and Los Angeles CA

- Agreed to follow-up study

- Provided a prenatal urine sample

  – *Urine was not requested in first study year*
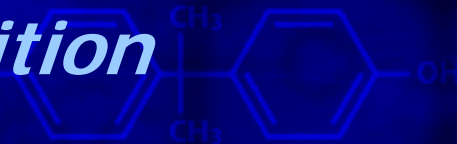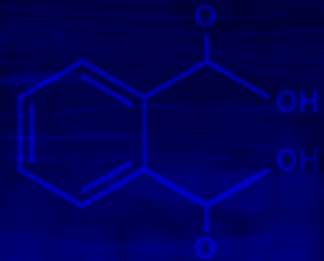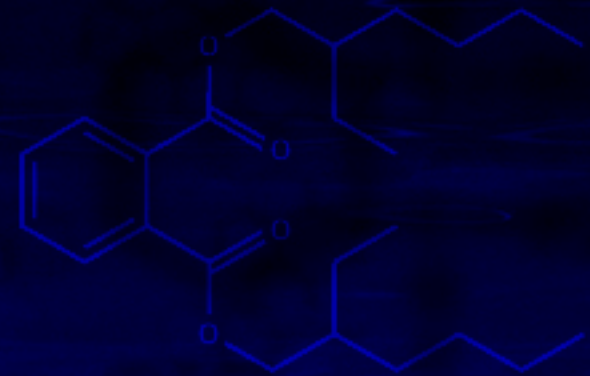
Boys' Physical Exam

- **Anthropometry**

- **Male genital exam**

  - *Anogenital distance*

  - *Testicular descent*

  - *Penile length and width*

  - *Scrotal size and condition*

Measuring Anogenital Distance

This is similar to toxicological measure
AGD is repeatable (CV =7.2%)

- AGD rarely measured in
- Humans

- In girls: AGD
 is distance A to C
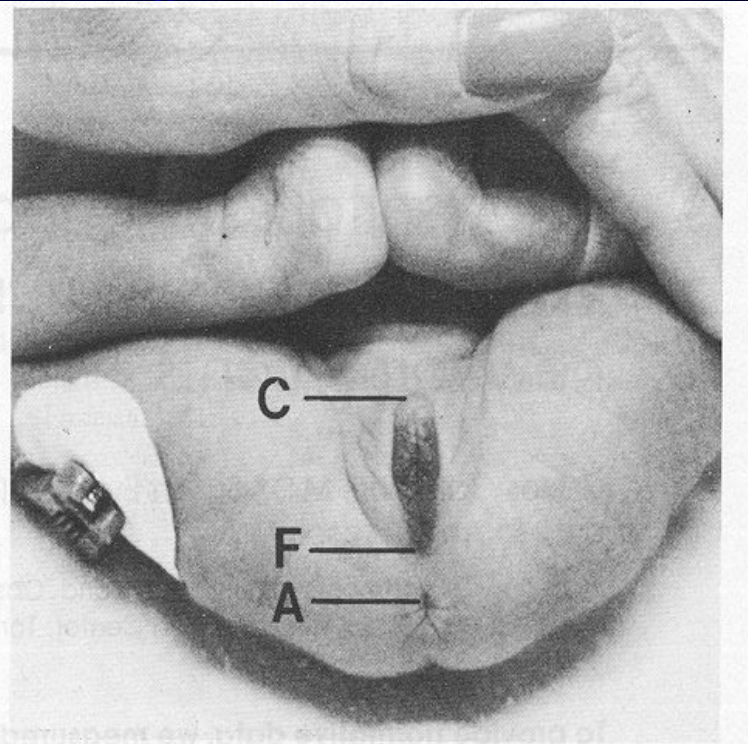
Fig. 1. Perineum of premature infant of 33 weeks gestation. Note position for and sites of measurement. *A*, Anus; *C*, clitoris; *F*, fourchette.

Callegari et al. J of Pediatrics 111:240-243, 1987.

AGD by Sex
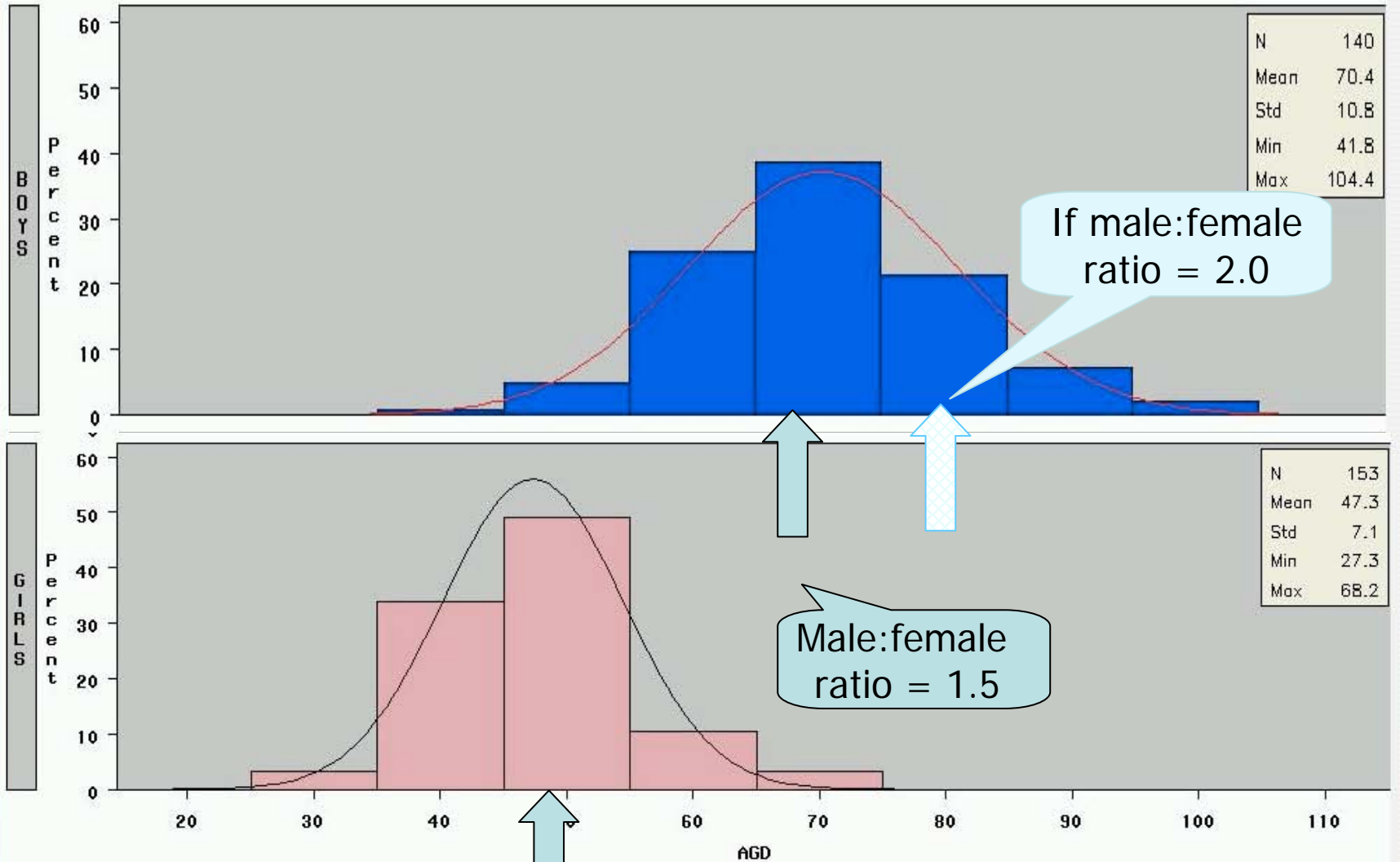
Analysis of Male Anogenital Distance (AGD)

- AGD increases with both age and weight

- These are strongly correlated

- We used standard growth curves to adjust for body size (CDC, 2000)

- Weight percentile (WT%) calculated for each boy at each visit

Birth to 36 months: Boys
Length-for-age and Weight-for-age percentiles
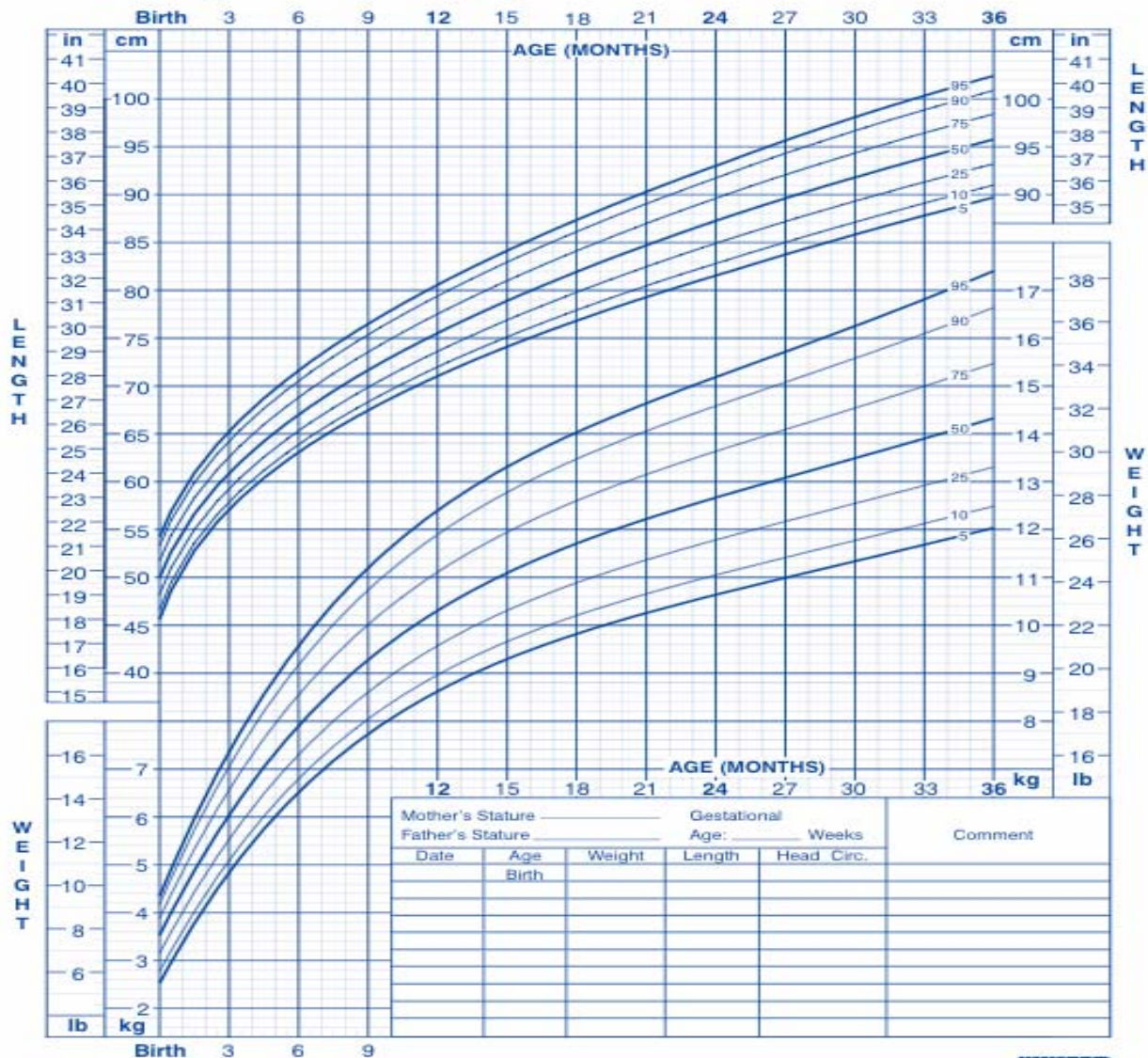
Published May 30, 2000 (modified 4/20/01).
SOURCE: Developed by the National Center for Health Statistics in collaboration with
the National Center for Chronic Disease Prevention and Health Promotion (2000).
http://www.cdc.gov/growthcharts

Analysis of AGD (Continuous)

- *Expected AGD* modeled:

  – Using all visits (mixed model)

  – WT% and age were the only significant predictors

- *AGD and phthalates*

  – Using all visits (mixed model)

  – Log transformed phthalate metabolite concentration used (to normalize data)

Phthalate Exposure Assessment

- Samples were collected mid-pregnancy (median 28.6 weeks gestation)

- Concentration of nine phthalate metabolites measured by CDC (blinded to identify of individuals or outcomes

- Analyzed as continuous and categorical variables

  - low (<25th%), medium, high(>=75th%)

- Creatinine (and square-root creatinine) not significant covariates

Concentration of four metabolites in prenatal samples (N=85, ng/mL)

	25th P	Median	75th P	%>LOD
MBP	7.2	13.5	30.9	97
MBzP	3.5	8.3	23.5	94
MiBP	0.7	2.5	5.1	74
MEP	53.3	128.4	436.9	98

Levels somewhat lower than those measured in US female population

AGI by Metabolite Concentration
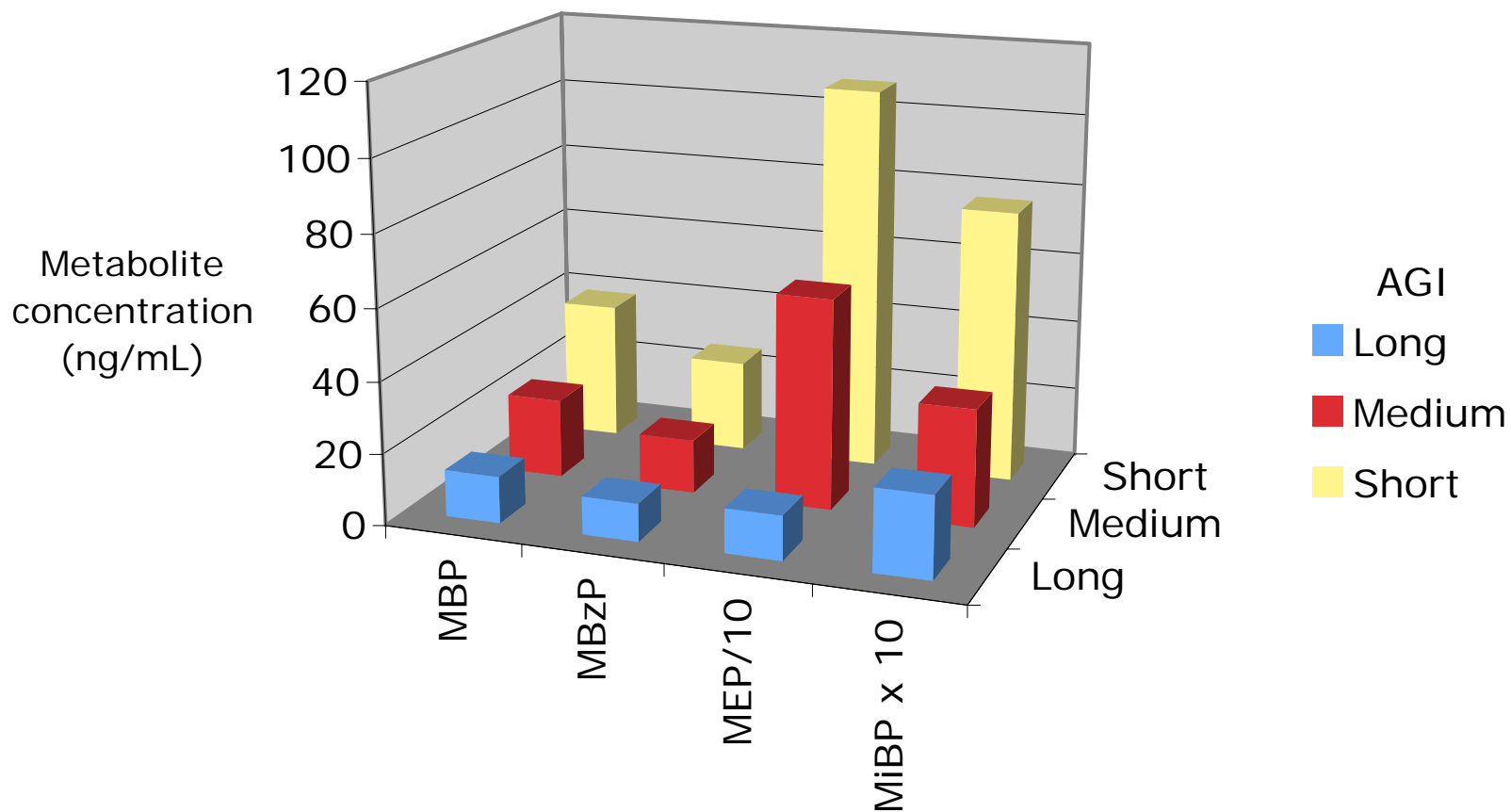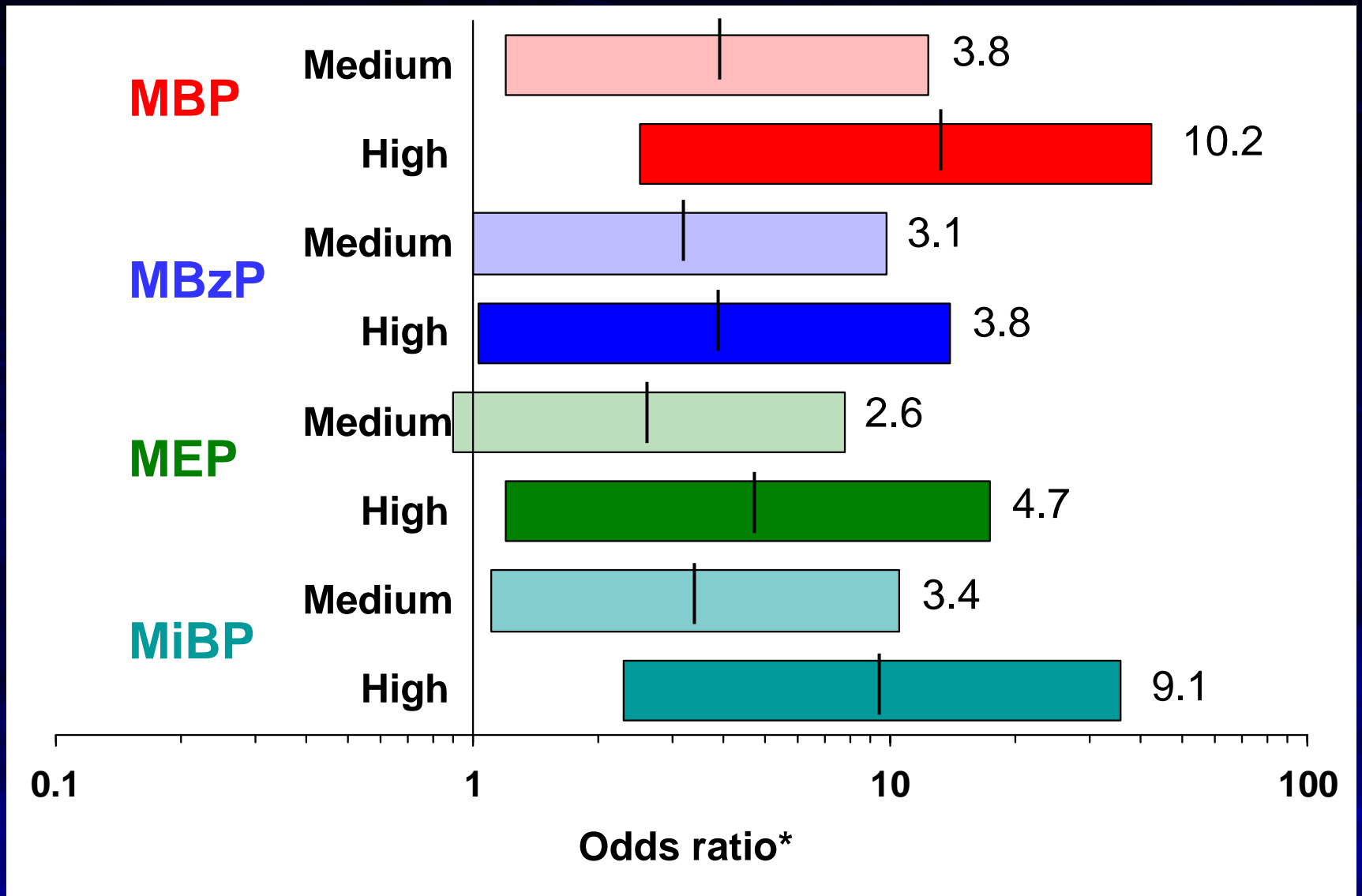
*Odds ratio is relative to low concentration for that analyte (< 25th percentile). Bars represent 95% confidence interval.

Abbreviations: MBP = Mono-n-butyl phthalate, MBzP = Mono-benzyl phthalate, MEP = Mono-ethyl phthalate, MiBP = Mono-isobutyl phthalate

AGI by boy's age

Boy's age (months)
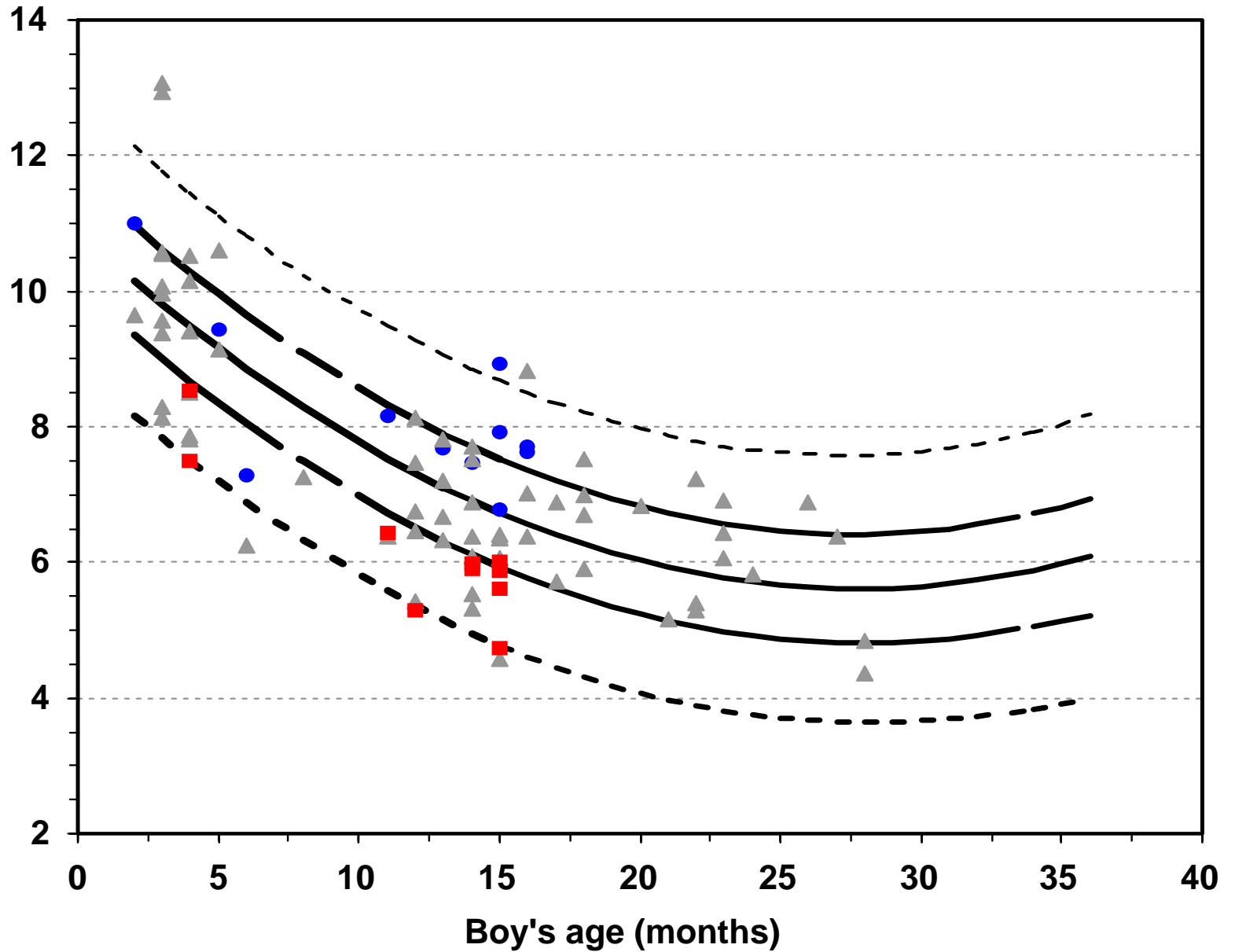
Categorical Analysis of AGD

- **Residual AGD** = Observed AGD - Expected AGD

- Categorized AGD by size of the residual:

  - **"Shorter"** $<25^{th}$ Percentile

  - $25^{th}\% <=$**"Intermediate"** $< 75^{th}\%$

  - 75th percentile$<=$ **"Longer" AGD:**
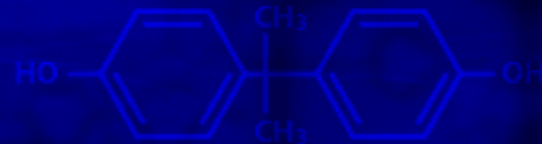
Prenatal MBP and Male AGI

MBP	AGI*		OR (95% CI)
	Smaller	Not	
Low	5	15	1.0 (REF)
Med	24	19	**3.8 (1.2-12.3)**
High	17	5	**10.2 (2.5, 42.2)**

*Smaller = Less than age-adjusted expected value, Not smaller = at least as large as age-adjusted expected value

The problem of mixtures

- Until recently, toxicology examined one phthalate at a time

- But that is not how people are exposed: most people are exposed to multiple phthalates (CDC, 2005)

- New toxicology suggests:
  - *Dose Additivity*
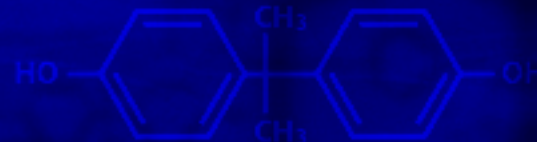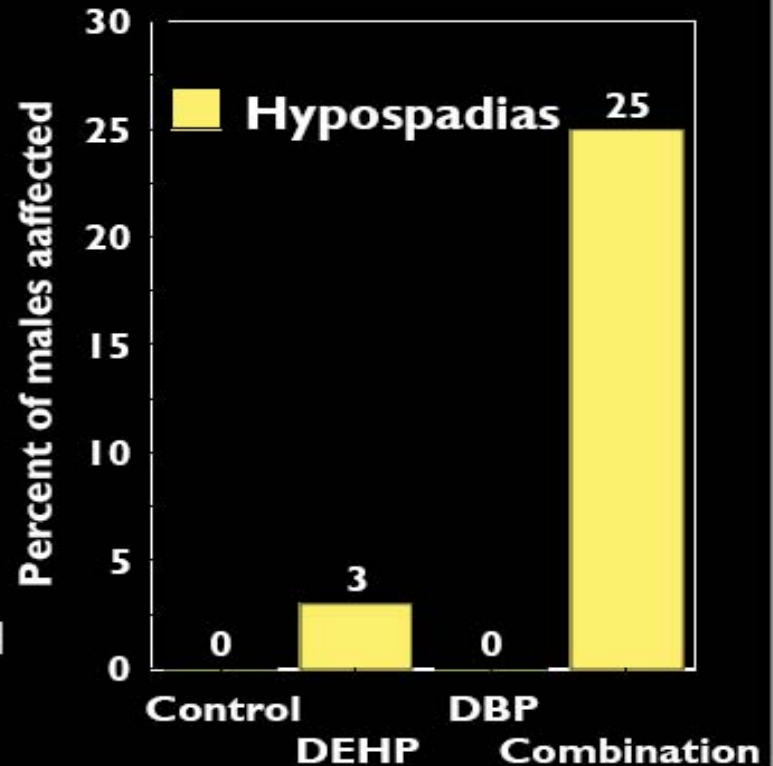
Gray, Personal communication

Joint exposure to four "High Risk" phthalates and AGI

Joint Score	AGI*	
	Smaller	Larger
Highest	9	1
Lowest	1	10
Total	10	11

Odd ratio unstable but very high:

Lower 95% confidence interval > 5.5

*Highest = All (or all but one) of 4 phthalates in top 25%; Lowest = All (or all but one) in lowest 25%

Other findings

- ## Significantly correlated with AGD:
    - *Degree of testicular descent*
    - *Penile volume*
    - *Scrotal size*

This cluster of outcomes consistent with "phthalate syndrome" in rodents

Comparing animal and human studies

Factor	Rodent	Human
Route	Oral	Oral, inhalation, dermal, parenteral
Dose	High, medium	Low
Agent	Single	Mixture

Routes of Exposure

- **Percutaneous**

- **Ingestion**

- **Dermal Absorption**

- **Inhalation**

Measurable in urine, serum,
breast milk, amniotic fluid

Were these changes seen at very high phthalate levels?

- "High" level ($>=75^{th}$ percentile) was compared to levels in NHANES samples

- "High" levels in our study seen in about 25% of adult US females

- How do these compare to EPA reference dose?

Estimated exposure and EPA reference dose (µg/kg/day)

Phthalate	Median	95th%	Reference dose
DEHP	1.32	9.32	20
DEP	6.64	112.3	800
BBzP	0.50	2.47	200
DBP	0.99	2.68	100

(Marsee, et al, 2006)

Clinical implications of the phthalate syndrome??

- **In rodents:**
  - At birth: Shorter AGD, impaired testicular descent, hypospadias
  - Later: Low sperm count, rarely testicular tumors

- **Our study of humans suggests:**
  - At birth: Shorter AGD (some, but most NS, decrease in testicular descent, smaller penile volume)

  Future studies needed to determine clinical correlates in humans

U MO
Robin Kruse,
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Christina Wang, Cathy Mao

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U IA
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