



# Puberty



## **“Low” Dose Literature on Puberty in Female Rodents**

- Mice - Apparently inconsistent literature
  - 6 “low” dose studies
    - 3 “positive” and 3 “negative” studies
- Rats - Little indication of an effect
  - 8 “low” dose studies
    - 1 “positive” and 7 “negative” studies

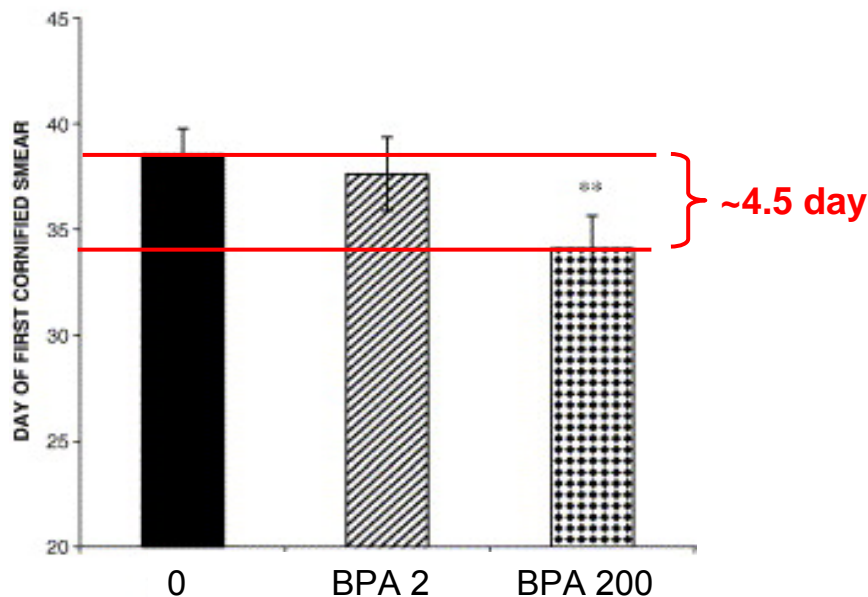


## Puberty – “Low” Dose “Positive” Mouse Studies

- 3 studies reported effects consistent with an acceleration of puberty in female mice
  - Ryan *et al.* 2006, Howdeshell *et al.* 1999, Honma *et al.* 2002
  - Included measurement of puberty (first estrous)
    - First estrous not coincident with other measures of sexual maturation in mice, e.g., vaginal opening
  - 1 to 4.5-day acceleration in puberty-related endpoint

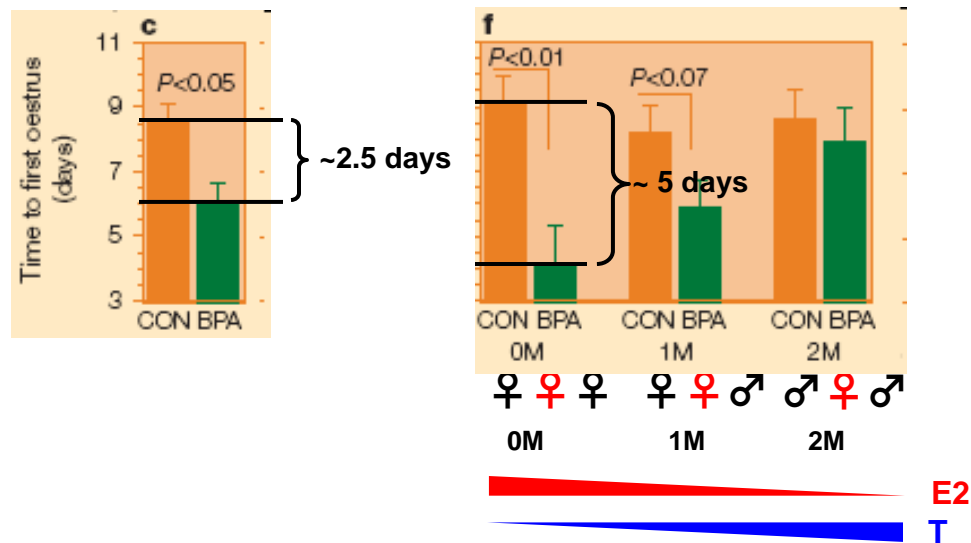
## Puberty - “Low” Dose Positive Mouse Studies (Ryan *et al.* 2006)

- C57/Bl6 mice
- 2 and 200  $\mu\text{g}/\text{kg}/\text{day}$  BPA (oral to dam GD3 – PND21; 4-5 litters/group)
- Acceleration in age at first estrous at 200  $\mu\text{g}/\text{kg}/\text{day}$  BPA



## Puberty - “Low” Dose Positive Mouse Studies (Howdeshell *et al.* 1999)

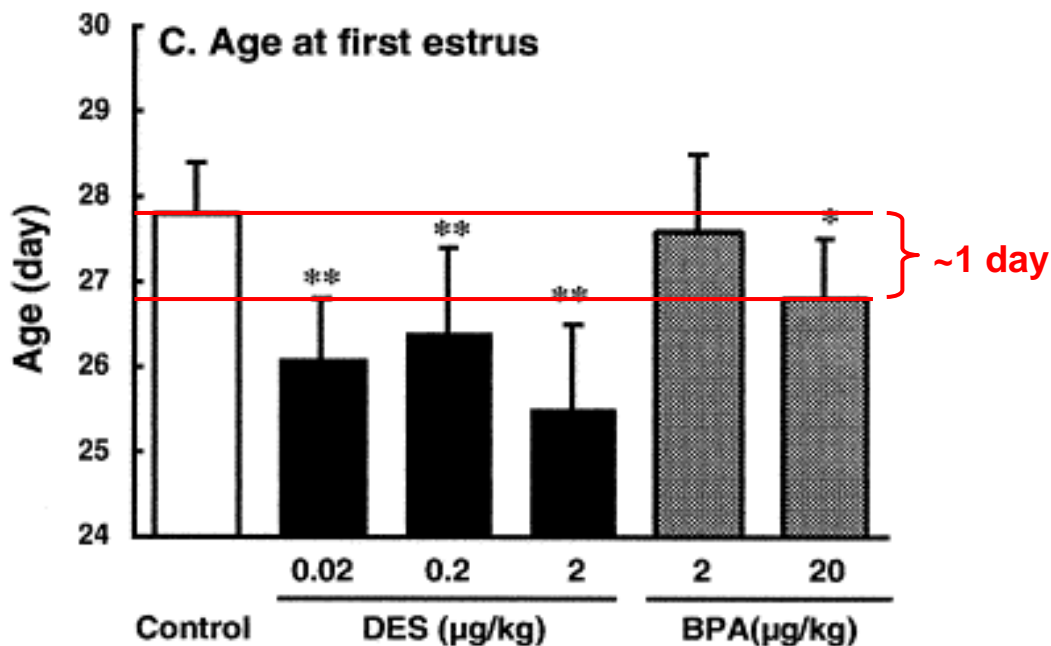
- CF-1 mice
- 2.4  $\mu\text{g}/\text{kg}/\text{day}$  BPA (oral to dam GD11-17; 21 litters/group)
- Shortened interval between vaginal opening and first estrous
  - Interaction with intrauterine position (IUP)





## Puberty - “Low” Dose Positive Mouse Studies (Honma *et al.* 2002)

- ICR/Jcl mice
- 2 and 20  $\mu\text{g}/\text{kg}/\text{day}$  BPA (sc injection to dam GD11-17; 10 litters/group)
- Acceleration in age at first estrous at 20  $\mu\text{g}/\text{kg}/\text{day}$
- Increased length of estrous cycle at 2 and 20  $\mu\text{g}/\text{kg}/\text{day}$



Treatment (ug/kg)	Estrous Cycle Length (days)
0	4.5
DES 0.02	6.1*
DES 0.2	7.4*
DES 2	7.7*
BPA 2	5.8*
BPA 20	5.5*



## Puberty – “Low” Dose “Negative” Mouse Studies

- 3 studies did not detect an acceleration of puberty in female mice
  - Ashby *et al.* 1999,, Markey *et al.* 2003, Tyl *et al.* 2008
  - Used vaginal opening as puberty marker

## Puberty - “Low” Dose “Negative” Mouse Studies (Ashby *et al.* 1999)

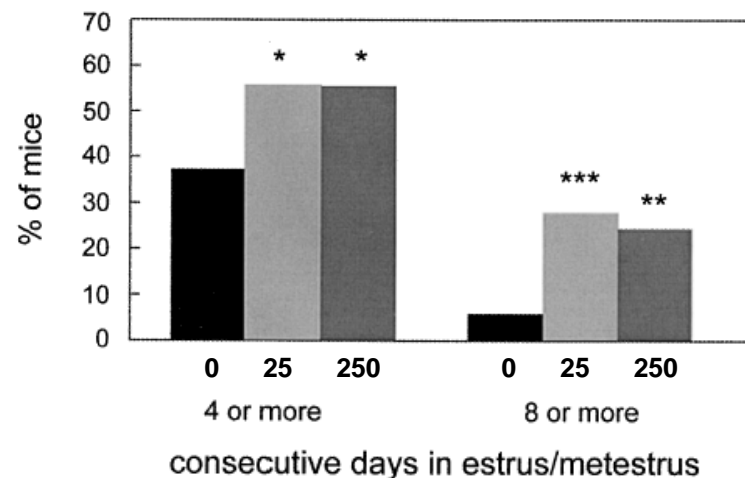
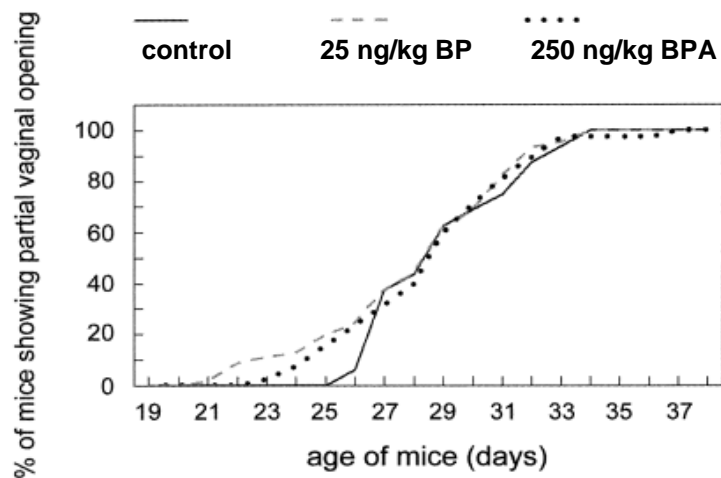
- CF-1 mice
- 2 and 20  $\mu\text{g}/\text{kg}/\text{day}$  BPA (oral to dam GD11-17; 7 - 8 litters/group)
- No effect of BPA on age at vaginal opening
- “Failed” positive control

Dose ( $\mu\text{g}/\text{kg}$ )	Age at vaginal opening (days)	
	Onset	Completion
Control	$28.6 \pm 2.6$	$31.1 \pm 2.5$
BPA (2)	$30.5 \pm 1.7$	$32.8 \pm 2.1$
BPA (20)	$30.8 \pm 2.4$	$33.4 \pm 2.2$
DES (0.2)	$32.2 \pm 1.3^*$	$34.7 \pm 1.7^*$



## Puberty - “Low” Dose “Negative” Mouse Studies (Markey *et al.* 2003)

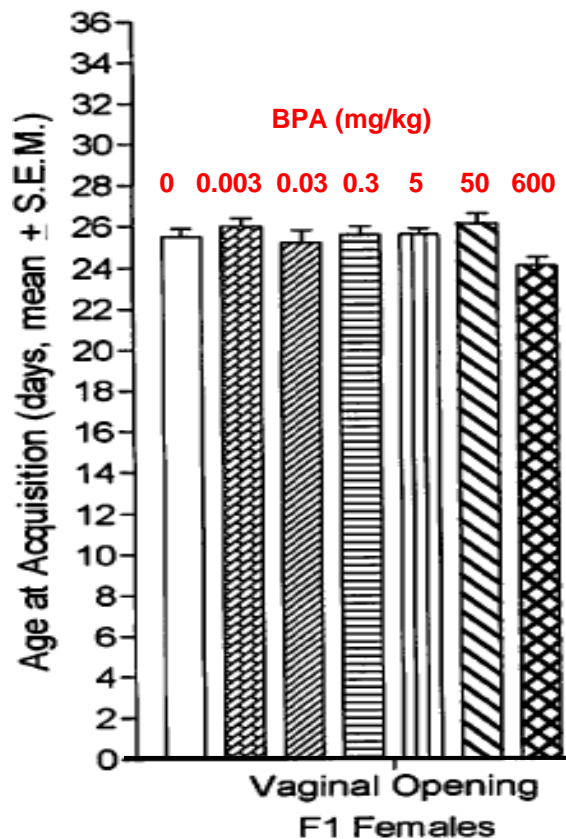
- CD-1 mice
- 25 and 250 ng/kg/day BPA (sc mini-pump in dam GD9-20; 6-10 litters per group)
- No effect on mean age at vaginal opening
  - Some mice showed partial opening ~4 days earlier than controls
  - Longer estrous cycles in BPA-treated animals





## Puberty - “Low” Dose “Negative” Mouse Studies (Tyl *et al.* 2008)

- CD-1 multigenerational study
- 3  $\mu\text{g}/\text{kg}/\text{day}$  – 600  $\text{mg}/\text{kg}/\text{day}$  (oral; 28 litters per group)



- No effect of BPA on vaginal opening at low doses
- Predicted effect in E2 positive control group
- Model may be insensitive to E2 at very low doses
  - 0.2 -100  $\mu\text{g}/\text{kg}/\text{day}$  E2 (Tyl *et al.* 2008. *Tox Sci.* 102(2): 392-412)
  - No effect on duration of estrous cycle at any dose
  - No effect on vaginal opening at doses  $< 30$   $\mu\text{g}/\text{kg}/\text{day}$



## Puberty – Replication

- First estrous versus vaginal opening as measure of puberty
- Little indication of effect at low doses in rats
  - Limits confidence in robustness of effect
  - Could species differences account for the “positive” findings in some mouse studies and “negative” findings in rats?
    - Onset of puberty in mice may be more easily perturbed by external factors, e.g., impact of exposure to a male
    - Species differences in intrauterine position (IUP) effect



## Puberty – Data Limitations

- Each mouse study has its own limitation
  - Sample size, endpoint used, positive control response, route of administration



## Weight of Evidence for Puberty

- **Puberty-related effect in all mouse studies that included first estrous**
- **Possible species and strain differences in sensitivity**

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- **Little indication of effect in rats**
  - **Additional replication**

Clear evidence of adverse effects

Some evidence of adverse effects

**Limited evidence of adverse effects**

Insufficient evidence for a conclusion

Limited evidence of no adverse effects

Some evidence of no adverse effects

Clear evidence of no adverse effects