





"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/BI6 mice
- 2 and 200 µg/kg/day BPA (oral to dam GD3 PND21; 4-5 litters/group)
- Acceleration in age at first estrous at 200 μg/kg/day BPA



Modified Figure 1 from Ryan BC & Vandenbergh JG (2006) *Horm Behav.* Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. 50(1): 85-93.





Puberty - "Low" Dose Positive Mouse Studies (Howdeshell *et al.* 1999)

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



Modified Figure 1 from Howdeshell KL et al. (1999) Nature. Exposure to bisphenol A advances puberty. 401(6755): 763-764.



Puberty - "Low" Dose Positive Mouse Studies (Honma et al. 2002)

- **ICR/Jcl** mice
- 2 and 20 µg/kg/day BPA (sc injection to dam GD11-17; 10 litters/group)

4.5

6.1*

7.4*

7.7*

5.8*

5.5*

- Acceleration in age at first estrous at 20 μ g/kg/day
- Increased length of estrous cycle at 2 and 20 µg/kg/day



Modified Figure 1 and Table 4 from: Honma S et al. (2002) Reprod Toxicol. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. 16(2): 117-122.



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 μg/kg/day BPA (oral to dam GD11-17; 7 8 litters/group)
- No effect of BPA on age at vaginal opening
- "Failed" positive control

	Age at vaginal opening (days)	
Dose (μg/kg)	Onset	Completion
Control	28.6 ± 2.6	31.1 ± 2.5
BPA (2)	30.5 ± 1.7	32.8 ± 2.1
BPA (20)	30.8 ± 2.4	33.4 ± 2.2
DES (0.2)	$32.2\pm1.3^{\star}$	$34.7 \pm 1.7^{\star}$

Ashby J *et al.* (1999) *Regul Toxicol Pharmacol.* Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero.* 30(2 Pt 1): 156-166.



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



Modified Figures 1 and 2 from Markey CM *et al.* (2003) *Evol Dev.* Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. 5(1): 67-75.



Puberty - "Low" Dose "Negative" Mouse Studies (Tyl et al. 2008)

- CD-1 multigenerational study
- 3 µg/kg/day 600 mg/kg/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 μg/kg/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 μg/kg/day

Modified Figure 7 from Tyl RW et al. (2008) *Toxicol Sci.* Two-Generation Reproductive Toxicity Study of Dietary Bisphenol A (BPA) in CD-1(R) (Swiss) Mice. May 6. Epub ahead of print.





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
- 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