

NTP Brief on Bisphenol A (BPA)

John R. Bucher, PhD
Associate Director, NTP

FDA Science Board Subcommittee Meeting
September 16, 2008

Center for the Evaluation of Risks to Human Reproduction (CERHR)

- Michael D. Shelby, PhD – Director
- Paul Foster, PhD – Deputy Director
- Kris Thayer, PhD – Staff Scientist
- Diane Spencer, MS – CERHR Staff

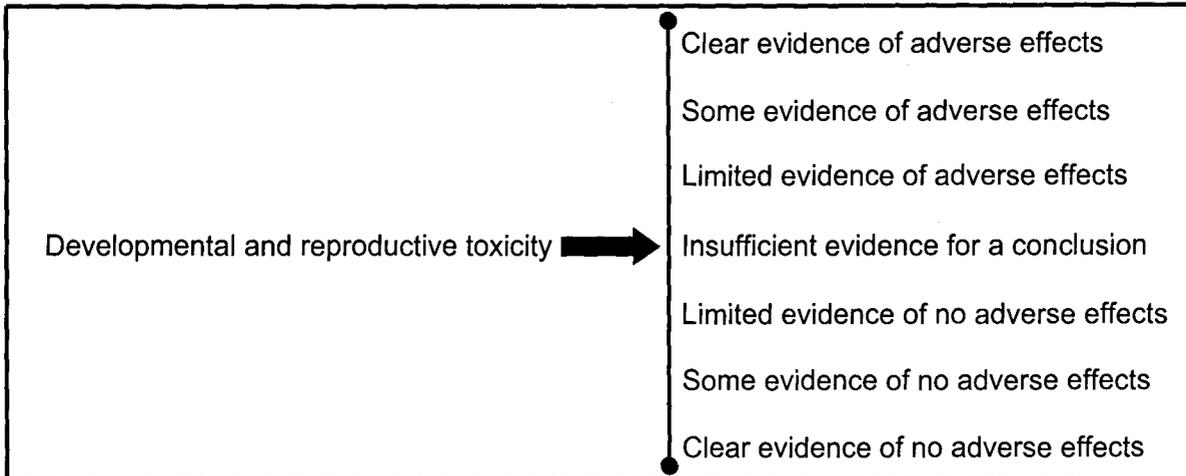
Outline of Presentation

- CERHR process
- NTP Brief on BPA conclusions
- Specific discussion points
 - Human exposure assessment
 - Mechanistic assumptions
 - Metabolism and route of exposure considerations
 - Developmental effects on brain and behavior
 - Developmental effects on the prostate gland
 - Developmental effects on the mammary gland
 - Puberty and sexual maturation

CERHR Process

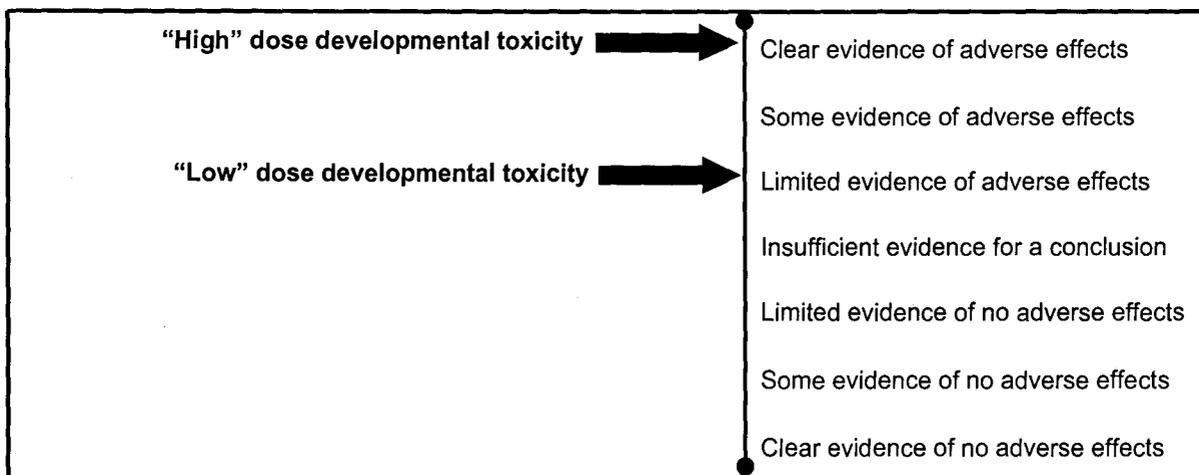
- Nomination and selection
 - December 2005 – CERHR announces intent to evaluate BPA
- CERHR expert panel evaluation
 - March & August 2007 – Public expert panel meetings
 - November 2007 – Final expert panel report released
- NTP Brief
 - April 14, 2008 – Draft NTP Brief released
 - June 11, 2008 – Peer-review of draft NTP Brief
 - September 3, 2008 – Final NTP Brief released as part of NTP-CERHR Monograph
 - Literature review cut-off date: June 1, 2008
- 8 opportunities for public comment during evaluation (5 for written comments, 3 for oral)

Weight of evidence that BPA causes adverse developmental or reproductive effects in humans*

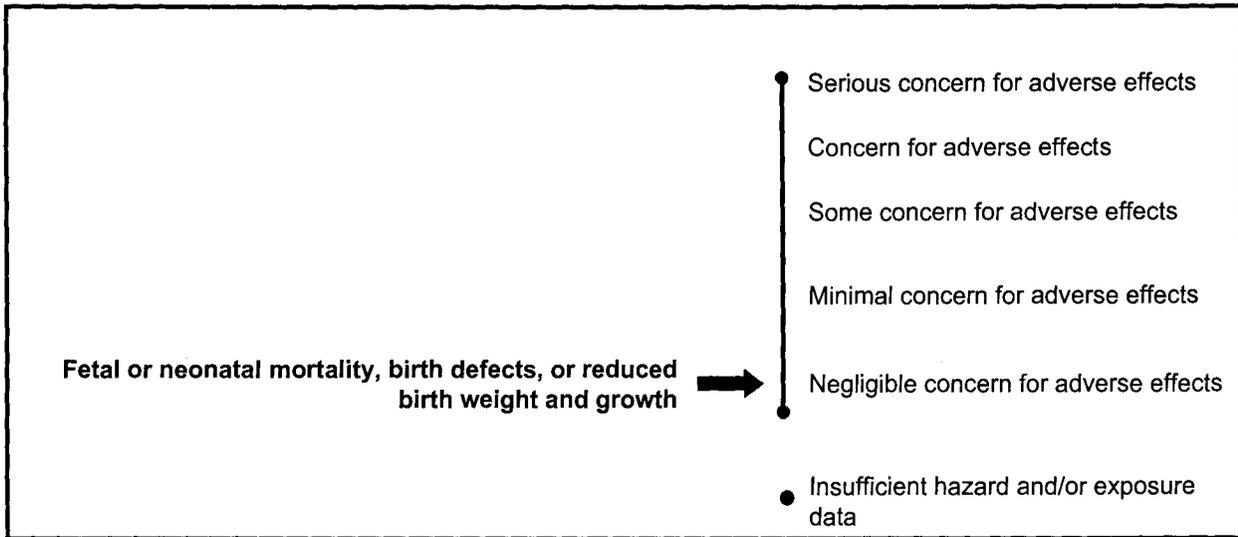


* Based on epidemiological studies in humans

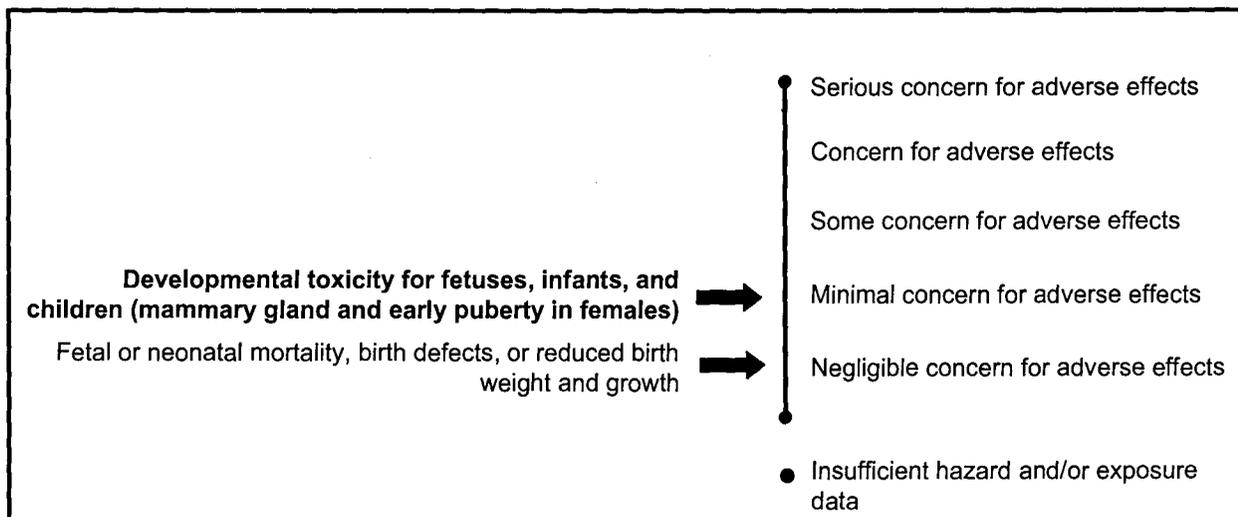
Weight of evidence that BPA causes adverse developmental effects in laboratory animals



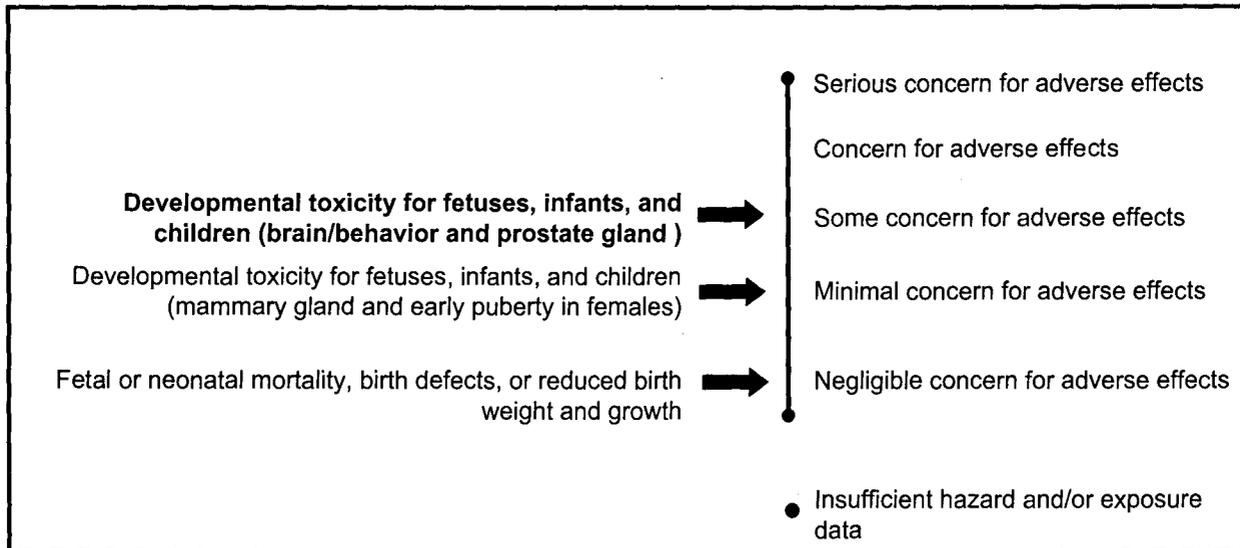
NTP conclusions regarding the possibilities that human development might be adversely affected by exposure to bisphenol A



NTP conclusions regarding the possibilities that human development might be adversely affected by exposure to bisphenol A



NTP conclusions regarding the possibilities that human development might be adversely affected by exposure to bisphenol A



Are People Exposed?

- Yes
 - Estimated daily intakes are highest in infants and children
 - 1 - 13 $\mu\text{g}/\text{kg}/\text{day}$ in formula-fed infants
 - Range encompasses FDA estimated intakes
 - 0.2 - 1 $\mu\text{g}/\text{kg}/\text{day}$ in breastfed infants
 - < 0.300 $\mu\text{g}/\text{kg}/\text{day}$ in adults (95th percentile estimates)

NTP Brief – Mechanistic Assumptions

- NTP did not consider BPA effects solely within the context of relative binding affinity to ER α and ER β
 - 1000 to 10,000-fold lower affinity for ER α and ER β compared to E2
 - Increasing number of studies suggest involvement of other receptor systems
 - Altered expression levels of AR, altered gene expression of dopamine receptors (*in vivo*)
 - Relative high affinity binding to several receptors, i.e., ERR γ , ncmER, GPR30 (*in vitro*)
 - Generally unknown if or how these activities relate to reported *in vivo* effects of BPA

BPA and Estrogen-Related Receptor Gamma (ERR γ)

Table 1
The receptor binding affinity of natural hormones and chemicals for the estrogen-related receptor γ , ERR γ

Natural hormones and chemicals	Binding affinity (IC ₅₀ , nM)	
Bisphenol A (BPA)	13.1 ± 2.34	←
4-Hydroxytamoxifen (4-OHT)	10.3 ± 0.80	
Diethylstilbestrol (DES)	54.3 ± 3.14	←
4-Nonylphenol	194 ± 30.0	
Estrone (E1)	549 ± 0.81	
17 β -Estradiol (E2)	NB ^a	No binding affinity up to 10 μ M
Estrin (E3)	NB	

- Orphan receptor structurally related to ER α and ER β that does not bind E2, expressed in brain and other tissues
- High basal constitutive activity
 - BPA binding preserves this activity
 - DES and 4-OHT repress molecular activity (4-OHT acts as an inverse agonist)

BPA and Membrane ERs

- GPR30
 - 7-transmembrane G-protein-coupled receptor that responds to E2
 - Up regulates adenylyl cyclase and MAPKinase activities, calcium mobilization, cAMP production
 - BPA relative binding affinity of 2-3% compared to E2
 - ER antagonists ICI182,780 and tamoxifen are agonists for GPR30
 - DES does not bind at concentrations up to 10 μ M
- ncmER
 - Non-classical membrane ER described in mouse pancreatic islet cells, regulates Ca²⁺ channels, modulates insulin and glucagon release
 - BPA activates at 1nM (similar to DES)
 - Insensitive to tamoxifen and ICI182,780

Thomas P & Dong J (2006) *J Steroid Biochem Mol Biol.* 102:175-179; Thomas P et al. (2005) *Endocrinology.* 146:624-632; Prossnitz ER et al. (2008) *J Steroid Biochem Mol Biol.* 109:350-353; Alonso-Magdalena P et al. (2005) *Environ Health Perspect.* 113:969-977.

Consideration of Effects

- Focused on effects highlighted by the CERHR Expert Panel and in other recent evaluations
- Are the *in vivo* effects biologically plausible?
- Have the *in vivo* effects been reproduced?
- Do the *in vivo* effects represent adverse health findings in laboratory animals and/or humans?
- What are the potential impacts of any limitations in experimental design?

Consideration of Non-Oral Route of Administration

BPA Metabolism & Route of Administration

- Unconjugated (“free”) BPA biologically active form
- Oral studies considered most relevant for human risks
- BPA glucuronidated in gut and liver
- Adult rodents metabolize BPA more quickly following oral administration compared to subcutaneous (sc) injection
 - Limits utility of sc injection studies in adults
- Neonatal rats metabolize BPA less efficiently than adults at a given administered dose
 - Immaturity of relevant enzyme systems
- Evidence for immaturity of glucuronidation enzymes systems in human fetuses and infants

Consideration of SC Administration Studies

- The NTP considered studies that used sc injection to *neonatal* animals useful in its evaluation
- SC route of administration to adult animals - including pregnant dams - was used only for identifying potential hazards
- BSC peer-review of draft NTP Brief:
 - Ad hoc reviewers and BSC members supported consideration of sc injection studies to neonates

Age-Dependent Metabolism in Rats

- Neonatal rats have higher concentrations of unconjugated BPA (and a longer half-life) than adults at the same oral dose (Domoradzki *et al.* 2004)

	PND 4		PND 7		PND 21		Adult	
	M	F	M	F	M	F	M	F
	2013-times higher at PND 4							
10 mg/kg	162-times higher at PND 4							
Cmax (µg/g plasma)	48.3	10.2	1.1	1.4	0.2	0.2	0.024	0.063
1 mg/kg	162-times higher at PND 4							
Cmax (µg/g plasma)	0.03	0.06	0.04	0.08	0.005	0.006	ND	ND

ND = Not determined

- Assumption that sc administration produces irrelevantly high internal concentrations of BPA compared to oral administration may not apply to neonatal animals because of underdeveloped first pass metabolism

“Low” Dose Effects Used to Support *Some* or *Minimal Concern* for Impacts on Human Development

Brain and behavior

Prostate gland

Mammary gland

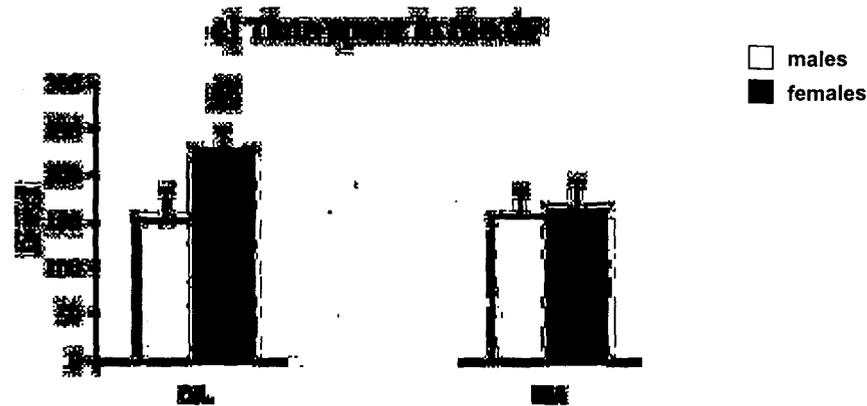
Puberty in females

Brain and Behavior – “Low” Dose Findings

- Based on a number of well-designed studies
 - 3 oral studies report effects at 10 µg/kg/day (Palanza *et al.* 1999, Laviola *et al.* 2005, Gioiosa *et al.* 2007)
 - Other oral studies report effects at < 1 mg/kg/day (Ceccarelli *et al.* 2007, Ryan *et al.* 2006, Della Seta *et al.* 2006, Negishi *et al.* 2004)
 - Maternal behavior, novelty-seeking, exploration, reward response, anxiety, cognition, and emotional behavior
- Collectively, the literature suggests a loss or reduction of sexual dimorphisms in non-reproductive behaviors, brain structures, or biochemical endpoints
 - Certain behavioral responses and effects at the cellular and molecular levels also suggest an effect on the dopaminergic system

Loss of Sexual Dimorphism – Open Field (OF) Behavior (Gioiosa *et al.* 2007)

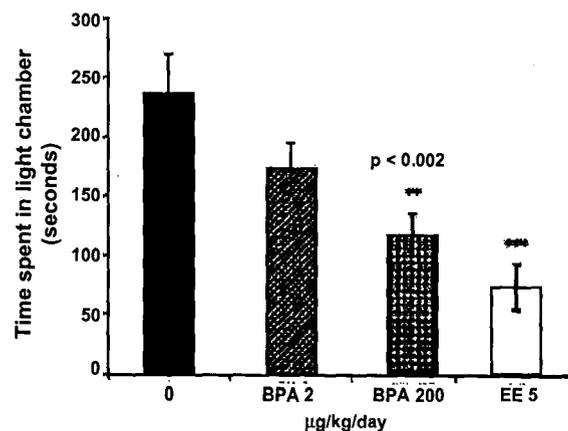
- CD-1 mice
- 10 $\mu\text{g}/\text{kg}/\text{day}$ BPA (oral to dam GD11 - PND8;15-16 litters/group)



Modified Figure 2 from: Gioiosa L. *et al.* 2007. Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. *Horm Behav* 52(3):307-316.

Anxiety Behavior in Female Mice (Ryan *et al.* 2006)

- C57/Bl-6 mice
- 2 and 200 $\mu\text{g}/\text{kg}/\text{day}$ BPA (oral to dam GD3 – PND21; 14-16 litters/group)
- Light/Dark chamber
 - Increased anxiety behavior at 200 $\mu\text{g}/\text{kg}/\text{day}$ BPA



Modified Figure 3 from Ryan BC & Vandenberg JG. 2006. Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Horm Behav* 50(1):85-93.

Brain and Behavior – Reproducibility & Data Limitations

- Difficult to evaluate reproducibility of specific effects
- Effects would not have been detected in guideline-compliant multigenerational studies
 - Behavior not assessed (Tyl *et al.* 2001 & 2008)
 - Rat multigenerational study by Ema *et al.* 2001 included neurobehavioral endpoints, but...
 - Sexual dimorphisms not reported in control animals
 - Did not cover endpoints affected in academic studies
- Adverse consequences of effects unclear
- Relevance for human health also uncertain

Prostate Gland

Prostate Gland – “Low” Dose Findings

- 2 technically well-conducted studies reported effects on the prostate at 10 µg/kg/day
 - “Preneoplastic” prostatic intraepithelial neoplasia (PIN) lesions (Ho *et al.* 2006, sc administration to neonatal SD rats)
 - Morphometric effects related to ductal development (Timms *et al.* 2005, oral administration to pregnant CD-1 mice)
- Findings interpreted as potentially predisposing prostate gland to disease later in life

Prostate Gland – Reproducibility

- NTP 2-year bioassay did not report prostate gland tumors in BPA-treated rats or mice
 - NTP bioassay has never identified a prostate carcinogen
 - Did not include perinatal exposure
- These effects would likely not have been detected in guideline-compliant multigenerational studies
 - No morphometric analysis
 - PIN lesions may not be detected with “conventional” rodent models
 - Some estrogenic effects not detected by H&E staining (Ogura *et al.* 2008)

Prostate Gland – Data Limitations

- Long-term consequences of morphometric changes unclear (Timms *et al.* 2005)
 - Are effects permanent and/or adverse?
- Unclear if the reported PIN lesions progress to cancer (Ho *et al.* 2006)

Mammary Gland

Mammary Gland – “Low” Dose Findings

- 2 reports of “pre-neoplastic” lesions in mammary gland (Durando *et al.* 2007*, Murray *et al.* 2007*)
 - Ductal hyperplasia and possible carcinoma *in situ*
 - Human correlate lesions have been described as risk factors for invasive breast cancer in women
- Mammary gland identified as BPA target tissue in other studies
 - Increase in undifferentiated structures, altered rate of maturation, epithelial cell proliferation, epithelial-stroma interactions (Colerangle 1997,* Markey 2001,* Markey 2003,* Nikaido 2004,* Muñoz-de-Toro 2005,* Vandenberg 2007,* Wadia 2007,* Moral 2008)
 - Undifferentiated structures considered more susceptible to mammary gland carcinogens

* Studies used sc route of administration to adult animals

Mammary Gland - Reproducibility

- NTP 2-year bioassay did not report effects on mammary gland tumors in BPA-treated female rats or mice
 - Did not include perinatal exposure or whole mounts
- These effects may not have been detected in guideline-compliant multigenerational studies
 - Not assessed, including in rat multigenerational study by Tyl *et al.* 2001
 - No whole mount preparation in mouse (Tyl *et al.* 2008) or rat multigenerational (Ema *et al.* 2001)

Mammary Gland - Data Limitations

- Interpretive
 - Unknown if the reported lesions would progress to cancer
 - SC route of administration to adult animals only useful for hazard identification
- Technical
 - Control for litter effects in Murray *et al.* (2007)
 - Author clarified use of 1 animal/litter in histological examination
 - Use of 100% DMSO in Durando *et al.* (2007) not recommended by sc mini-pump manufacturer
 - NTP not convinced that 100% DMSO can account for reported effects
 - Authors report no indication of DMSO toxicity in public comments
 - Does raise concerns on accuracy of administered dose
- Findings supported “minimal concern”

Puberty

“Low” Dose Literature on Puberty in Female Rodents

- CERHR Expert Panel expressed “minimal concern” for effects on puberty in female mice
 - Two oral mouse studies provided “limited” evidence for an effect (Ryan *et al.* 2006, Howdeshell *et al.* 1999)
- NTP reviewed “low” dose rat and mouse studies
 - Rats studies provided little indication of an effect
 - 8 “low” dose studies in rats
 - 1 “positive” and 7 “negative” studies
 - Mouse studies were inconsistent
 - 6 “low” dose studies in mice
 - 3 “positive” studies (Ryan *et al.* 2006, Howdeshell *et al.* 1999, Honma *et al.* 2002)
 - 3 “negative” studies (Ashby *et al.* 1999, Markey *et al.* 2003, Tyl *et al.* 2008)

Puberty – “Low” Dose Mouse Studies

- Most consistent difference in studies was endpoint used to assess puberty and sexual maturation
 - “Positive” studies assessed first estrous
 - “Negative” studies assessed vaginal opening
- First estrous is considered the appropriate measure of puberty in mice
- First estrous not necessarily coincident with vaginal opening in mice
 - Suggest differential triggers even though both measures are estrogen responsive

Puberty – Data Limitations

- “Negative” studies
 - Use of vaginal opening as indicator of puberty
 - Positive control response
 - “Failed” positive control (Ashby *et al.* 1999)
 - Concern for sensitivity to detect classic estrogenic effects at “low” doses (Tyl *et al.* 2008)
 - Based on positive control response in multigenerational study with 17 β -estradiol (Tyl *et. al.* Toxicol Sci. 102: 392-412)
- “Positive” studies
 - Biological interpretation of a shortened interval between vaginal opening and first estrous (Howdeshell *et al.* 1999)
 - Sample size in Ryan *et al.* 2006 (n = 4 – 5)
 - SC administration to pregnant animals and small magnitude of response (Honma *et al.* 2002)
- Findings supported “minimal concern”

Summary

- The “low” dose studies in laboratory animals provide limited evidence for adverse effects
- These effects occur at BPA exposure levels similar to those experienced by humans; therefore, the possibility that BPA may alter human development cannot be dismissed
- More research is needed to better understand their implications for human health