The Effects of EDCs on Pubertal Development: Current Research Efforts by EPA.

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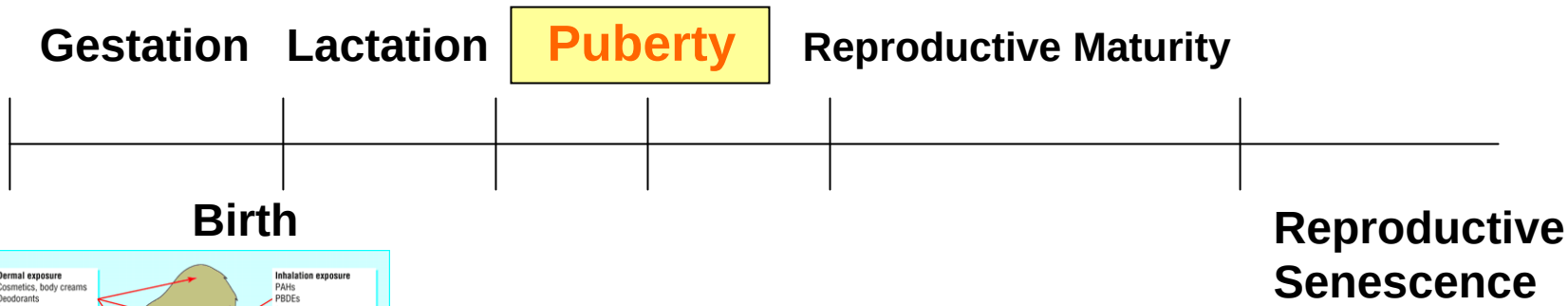
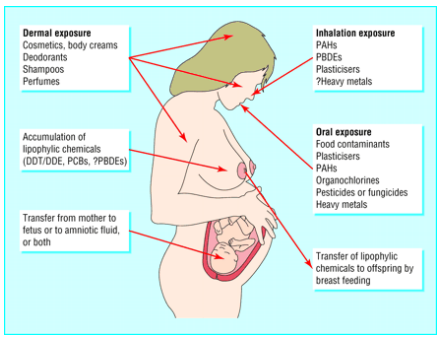
Puberty

- **Puberty is a period of dramatic neuroendocrine development that culminates in reproductive maturation.**
- **Requires extensive interplay between a variety of hormones, organs and tissues.**
- **Period of increased sensitivity to environmental agents.**

Sexual maturation is one point in the life cycle.





Human secular trends for timing of puberty

- There have been many examination surveys conducted in the past.
  - National Health Examination Surveys (NHES) from 1963 to 1970
  - National Health and Nutrition Examination Surveys (I, II and III) from 1940 to 1994
  - Pediatric Research in Office Settings (PROS) from 1992 to 1993

Evaluation of the surveys at a Serono Workshop

- **Serono Symposia International Expert Panel Workshop (Chicago, 2003)**
  - **Questions of the workshop:**
    - **1) Is there a secular trend in puberty timing from 1940 to present in the U.S.?**
      - Girls- Sufficient data to suggest an earlier breast development onset and/or menarche, but not other female pubertal markers.
      - Boys- Data are insufficient to make a conclusion

continued

- **2) Is there enough data to suggest that environmental factors are affecting puberty timing?**
  - Animal data and limited human data indicate that certain EDCs can alter male and female puberty timing.
  - Human studies indicate that increased body fat is associated with earlier puberty in boys and girls.

Suggested Nutritional and Environmental Influences in Humans

- **Body weight gain**
  - **Percentage of overweight children doubled from 70's to 90's (from 6.5% to 11.4%, National Center for Health Statistics)**
  - **Body Mass Index (BMI) is associated with advanced breast development**
  - **However, earlier puberty may be causative of the early weight gain?**
  - **In some cases in boys, there appears to be a delay association with obesity**
  - **Increased fat cells, aromatase increased production of estrogens?**

Suggested Nutritional and Environmental Influences in Humans (continued)

- **Environmental Chemicals**
  - **Persistent chemicals-Girls exposed to DDE and girls to PCBs prenatally were heavier than unexposed peers at age 14. (Gladen et al., 2000)**
  - **Perinatal lead exposure has been linked to delayed reproductive development (Selevan et al., 2003).**
  - **Polychlorinated aromatic hydrocarbons associated with decreased genital development in boys and decreased breast development in girls (Den Hond et al., 2002).**
  - **Estrogenic compounds, chemicals which alter steroidogenesis (i.e., phthalates) are also suspect.**

continued

- **3) Is altered puberty considered an adverse outcome?**
  - Altered puberty is an adverse outcome, but no decision on magnitude of change.
  - Not enough data on sensitive life stages (*in utero*, perinatal, peripubertal)
  - Early breast development in the absence of other pubertal events is a concern and can result from exposure to estrogenic agents.

EPA Ongoing Research in area of altered pubertal development following environmental exposures.

- **The potential effects of environmental chemicals on pubertal development are of interest to EPA.**
  - **In-house studies (ORD Multiyear Plans- EDC, Human Health, SP2)**
  - **Endocrine Disruptor's Screening and Testing Program (EDSP), OSCP**
  - **Star Grant Research under EDC Multiyear Plan**

Animal models for pubertal development

- **A delay or acceleration in puberty can be easily measured by landmarks of puberty in the rodent. These include preputial separation in the male and vaginal opening in the female.**
- **Mechanisms associated with puberty are conserved across species.**
  - **Rodent models should predict similar outcomes in humans.**

Other measures of pubertal alterations in rodent models.

- **Sex accessory gland development.**
- **Hormone levels which provide mechanistic information (testosterone, estrogen, luteinizing hormone).**
- **Estrous cyclicity in the female.**
- **Maturation of sperm in the male.**

Examples of animal data which shows good correlation with human data

- Den Hond found delayed breast development in humans following exposure to dioxin and Fenton et al. found delayed rat mammary gland differentiation following gestational exposure.
- Human exposure to lead has been shown to be associated with delayed puberty in girls (Selevan et al., 1993) which has also been shown in the rodent (Dearth et al., 2002; Grant et al., 1980)

Mechanisms of pubertal alterations in female rodent models

- **Estrogen agonists- advance puberty (i.e., methoxychlor)**
- **Estrogen antagonists (pharmaceuticals)- delay puberty**
- **Steroidogenesis inhibitors- delay puberty**
- **Aromatase inhibitors- delay puberty**
  - **Block estrogen synthesis (i.e., Ketoconazole and fadrozole delay vaginal opening).**
- **Suppression of hypothalamic-pituitary-gonadal function or suppressed GnRH (i.e., atrazine).**

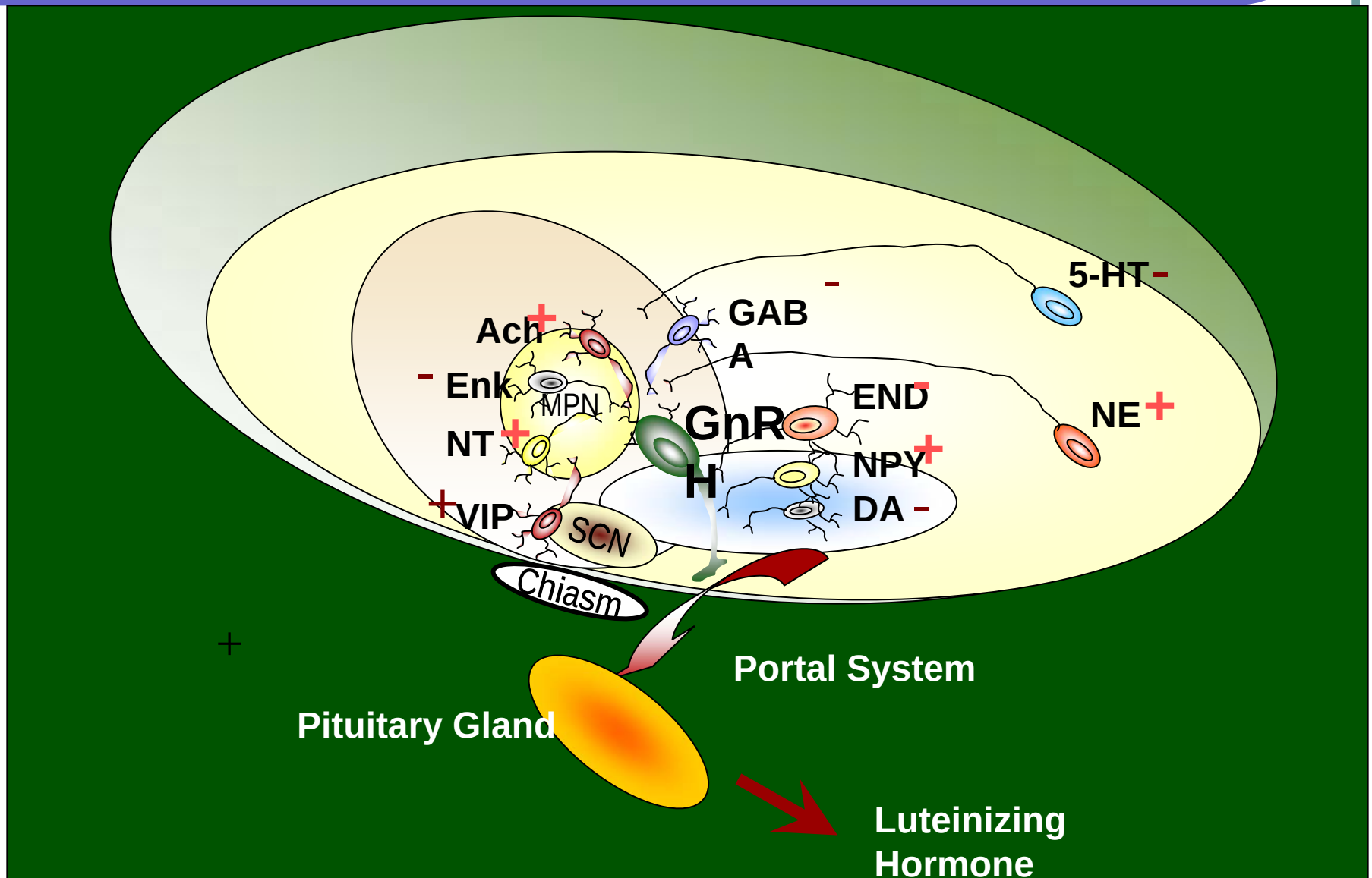
Mechanisms of alterations in puberty in male rodent models.

- **Anti-androgens (vinclozolin, procymidone, linuron, p, p' DDE, prochloraz, polybrominated diphenyl ether mixture of DE-71)**
- **Altered steroidogenesis (phthalate esters, etc.) by inhibition of enzymatic reactions or by altering the mitochondrial transport of cholesterol.**
- **Suppression of hypothalamic-pituitary-gonadal function or suppressed GnRH (i.e., atrazine).**

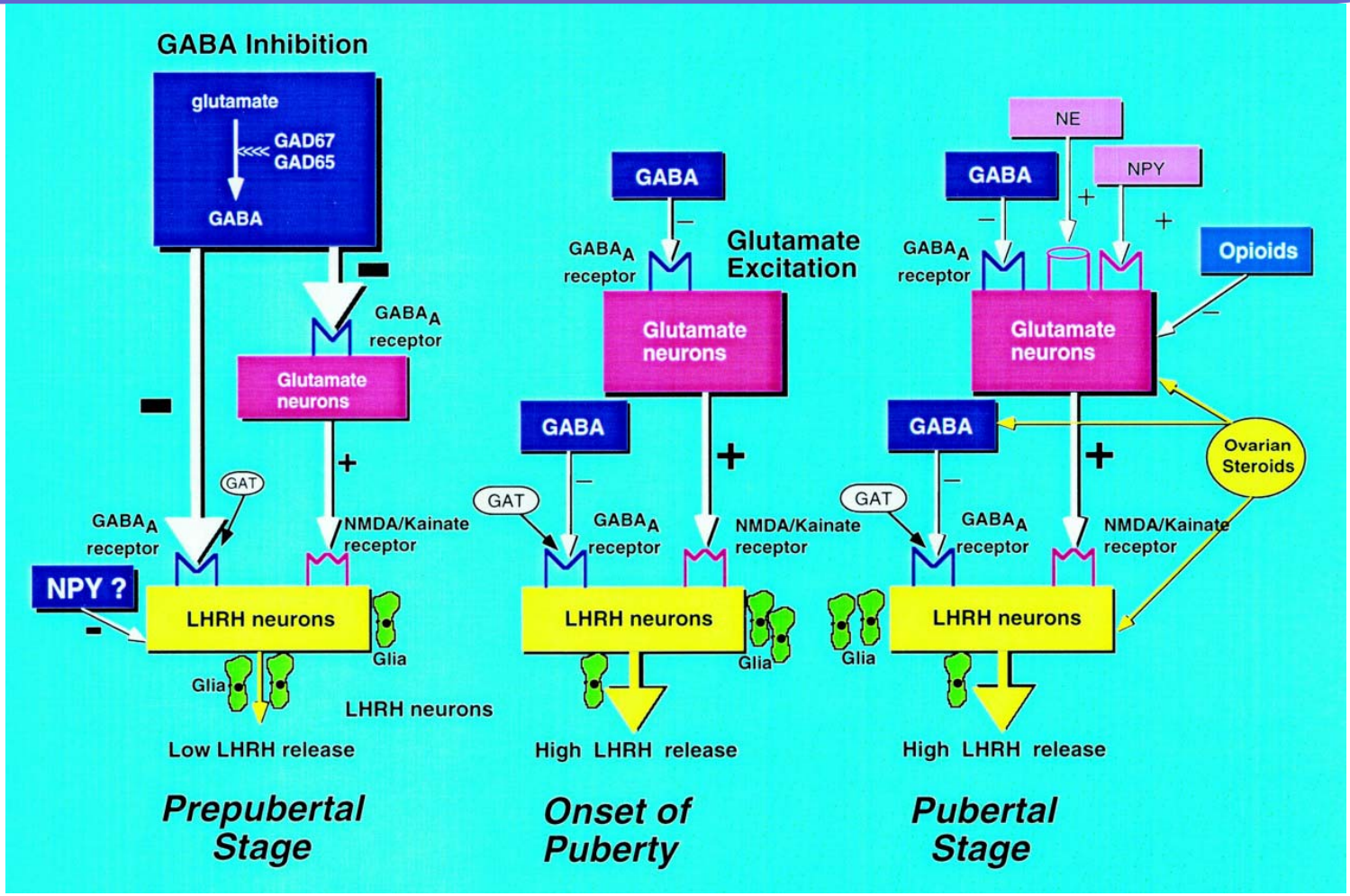
Altered neuroendocrine control of pubertal progression

- **Fewer environmental chemicals targeting brain have been studied.**
  - **Atrazine and metabolites, decreased GnRH, decreased LH, delayed puberty**
  - **Lead, decreased IGF-1, decreased LH, delayed puberty**
  - **List of potential CNS-active compounds is large**

Neural inputs involved in GnRH secretion in the adult, which are All possible target sites for environmental or pharmaceutical insult.



Neuroendocrine regulation of GnRH neuronal activity at the onset of puberty ("central drive" or activation of excitatory inputs).



Example of Neuroendocrine Effect

ATRAZINE

Hypothalamus
Neurotransmitters/peptides

↓GnRH ↑Dopamine

Pituitary Gland -- ↓Prolactin, ↓LH

Gonads

Effects on Reproductive Function and Development

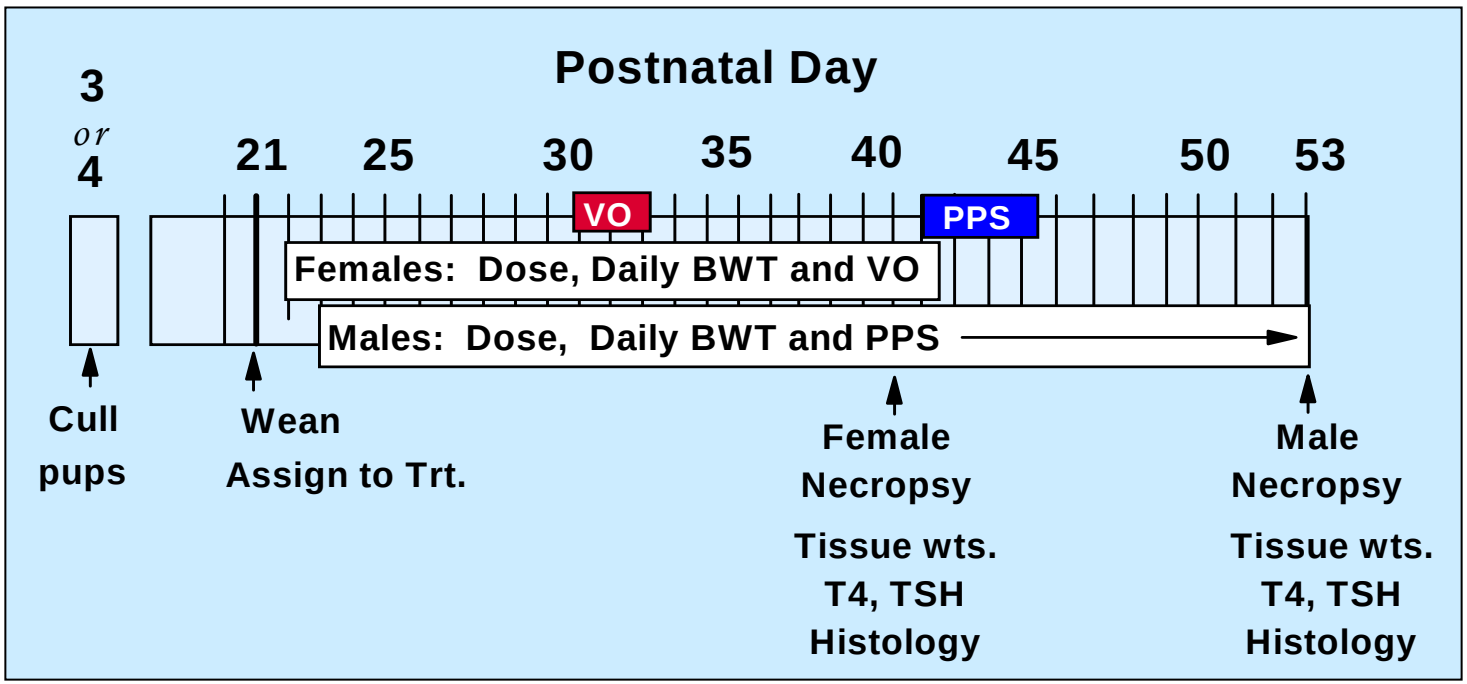
Delayed Puberty in Male and Female Rats

EPA's Endocrine Disruptor's Screening and Testing Program (EDSP)

- In response to 1996 FQPA, the EDSP was formed by EPA to develop *in vivo* and *in vitro* screens for EDCs.
- Two of the *in vivo* screens being considered are the rodent male and female pubertal protocols.

Male and Female Pubertal Protocols

- *Purpose:* To identify chemicals that alter pubertal development and thyroid function through CNS or steroid-mediated mechanisms of actions



Assessment of Pubertal Development and Thyroid Function in Juvenile Male and Female Rats

Purpose

The purpose of these protocols is to quantify the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile male or female rat.

Assessment of Pubertal Development and Thyroid Function in Juvenile Male and Female Rats

Applicability

These assays detect agents that display anti-thyroid, estrogenic/androgenic, anti-estrogenic [estrogen receptor (ER)], anti-androgenic [androgen receptor (AR)] or steroid enzyme mediated activity, or alter luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, growth hormone (GH) secretion or hypothalamic function.

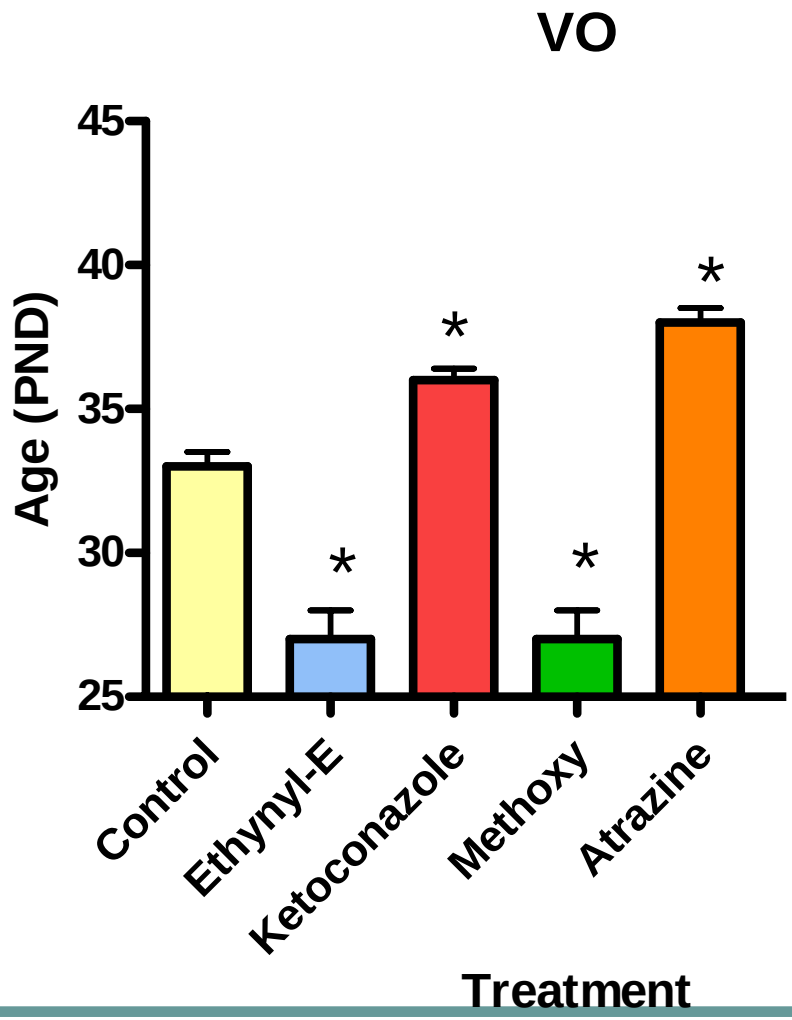
Required endpoints in the female assay

- Body weight gain
- VO
- Organ weights at PND 42
- Ovary wt, uterus wt with and without fluid
- Uterine and ovarian histology, estrous cyclicity from VO to PND 42
- T4, TSH, thyroid weight, thyroid histology

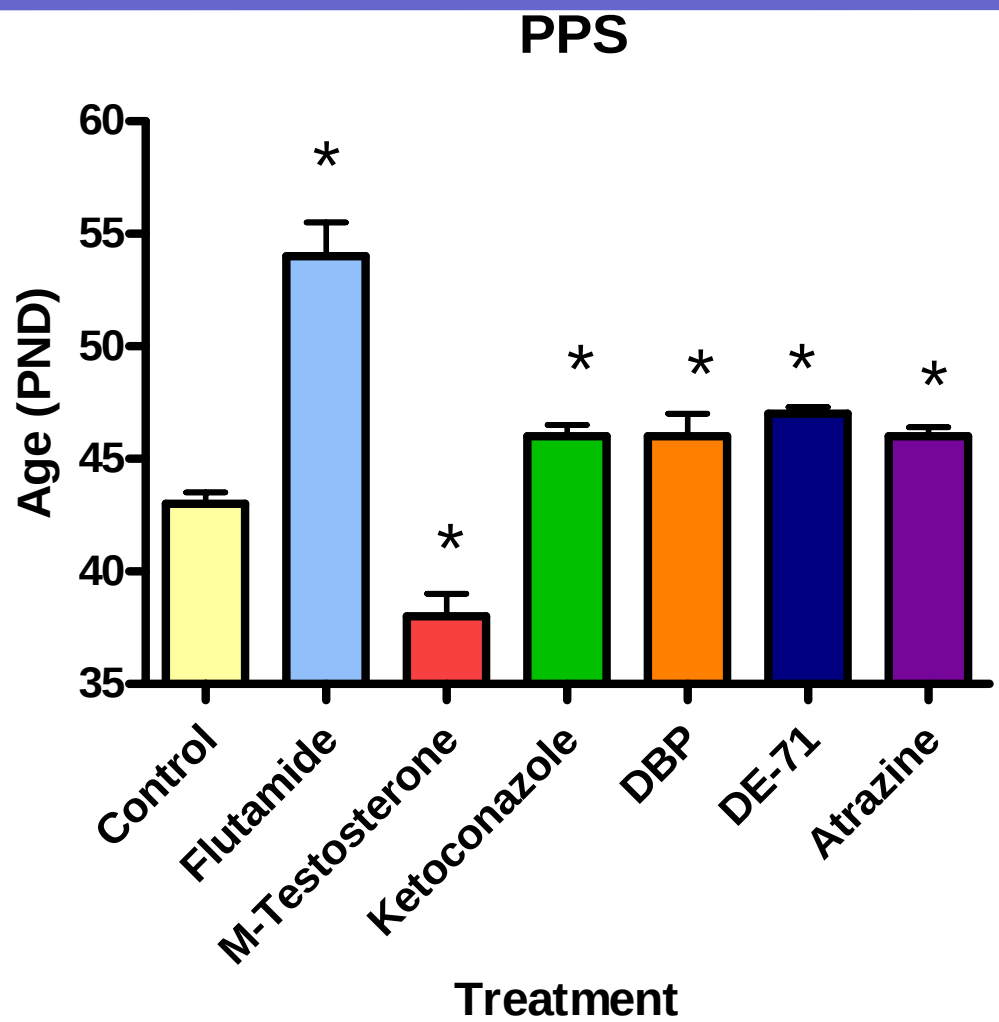
Required endpoints in the male assay

- **Growth**
- **Preputial separation (PPS)**
- **Reproductive tissue weights (Epi, SV, VP, DLP, LABC, Testes)**
- **Liver, adrenal, pituitary and kidney weights**
- **Serum Testosterone**
- **Testicular and Epididymal Histology**
- **T4, TSH, thyroid weight and histology**

Examples of EDCs tested by contract in the female pubertal assay.



Examples of EDCs tested by contract in the female pubertal assay.



Conclusions for correlation

- **There is an apparent correlation between human and animal effects (i.e., lead and PCAHs).**
- **Mechanisms associated with puberty are conserved across species**
  - **GnRH regulation**
  - **Pituitary hormone secretion**
  - **Steroid hormone feedback**

Conclusions of animal models

- **Effects of environmental chemicals on pubertal progression using perinatal and peri-pubertal exposure have been demonstrated.**
- **The male and female protocols developed for EDSP are sensitive to a number of EDCs with known mode/mechanism of action.**

Use of puberty as marker of altered reproduction.

- More studies are needed to address issues of adverse outcomes following changes in puberty.
  - Human epidemiology studies
  - Animal models, link to adverse outcomes
- For human, there are concerns for altered puberty
  - Psycho/Social impact
  - Lifetime exposure to steroids- cancer of breast, ovary, prostate?

Are changes in pubertal timing linked to adverse outcomes in later life?

- This question is important for the risk assessment based on rodent exposures and are relevant to adult reproductive health.
- Other questions or needs that are important in this area:
  - Studies that include estrous cycle data in the female can provide information to elucidate pseudoprecocious puberty or to indicate acyclicity following a normal time of vaginal opening.
  - Reproductive capability of the perinatally exposed offspring can provide information on impacts of altered puberty.
  - Does the endocrine milieu of the rodent return to normal?
  - Is the supressed growth of the reproductive tract a transient effect?
  - Do changes in mammary gland differentiation make the animal more susceptible to tumors?
  - Does an advanced puberty and longer lifetime exposure to estrogens increase the risk of cancers?

Star Grant Presentations

- “Low Dose Effects of In Utero Exposure to Cadmium on Puberty”, Dr. Mary Beth Martin, Georgetown University
- “Study of Phthalates in Pregnant Women and Children”, Shanna Swan, University of Missouri