EDVMS MEETING
DEC 10-12, 2003

PUBERTAL MALE AND FEMALE RAT ASSAYS

EDSTAC TIER 1 Endocrine Disrupter Screening and Battery 1998

- *In Vitro*
  - ER Binding and/or Transcriptional Activation
  - AR Binding and/or Transcriptional Activation
  - Steroid Hormone Synthesis
- *In Vivo*
  - Uterotropic Assay in Ovariectomized Adult Rat (3d)
  - Pubertal Female Rat Assay including Thyroid (20d)
  - Hershberger Assay with Castrate-immature-male rat (10d)
  - Frog Metamorphosis Assay
  - Short-term Assessment of the Fish Reproductive System

EDSTAC Screening Battery:

- *Detects Estrogens by sc and oral routes in vivo and in vitro*
- *Detects Androgens and Anti- in a senstive assay using simple endpoints (organ weights) and in vitro.*
- *Detects HPG/CNS alterations related to FSH, LH, prolactin, dopamine, GH with simple, precise developmental landmark without RIAs.*
- *Detects Inhibition of Steroidogenesis in vivo (in female) with simple, precise endpoints and in vitro*
- *Detects Thyroid effects in intact Female Rat (RIAs) and Frog Metamorphosis*
- *Uses extensively utlized in vivo endpoints/assays*

Questions?

- Given the number of chemicals run in more than one lab, is a formal interlab study still needed?
- Is the pubertal male assay sensitive enough to replace the Hershberger assay in T1S?
- Should we evaluate the adrenal or other endpoints in more detail?
- What mechanisms of action need further evaluation to establish the sensitivity of the assay?
- Should we be running concurrent dose-range finding studies?
- Can we reduce the sample size in these assays?

Comments

- Data analyses presented used SAS Proc GLM with BW21 as a covariate.
- Post hoc t-tests were used to compare means to control, by block, when F-value significant.
- We had several a priori hypotheses for many of the chemicals because they had been tested prior to the RTI and TI 2003 studies
- Analyses done by LEG at EPA
- Use of relative organ weights is not appropriate for EDC screening as the results can be very misleading. ANCOVA not much better when treatment affects body weight.
- Need MTD of 10%

Importance of Strain Effects in Screening Assays???

The OECD program to validate the rat uterotrophic bioassay: Phase Two - Dose Response Studies.
Jun Kanno, Lesley Onyon, Shyamal Peddada , John Ashby, Elard Jacob and William Owens.
doi:10.1289/ehp.5780 (available at http://dx.doi.org/). Online 23 January 2003

Abstract:

The Organisation for Economic Co-operation and Development has completed Phase 2 of an international validation program for the rodent uterotrophic bioassay. The purpose of the validation program was to demonstrate the performance of two versions of the uterotrophic bioassay, the immature female rat and the adult ovariectomized rat, in four standardized protocols.

. ..... It is noteworthy that these results were reproducible under a variety of different experimental conditions (e.g., *animal strain, diet*, housing, bedding, vehicle, animal age, and so on), indicating that the bioassay's performance as a screen is robust.

Pubertal Assessment of the LE versus the SD rats TI-2000. **For each sex there were 12 rats/sex/strain, run in two complete blocks to determine intralab variability using 6 treatment groups (a control and 5 chemicals at a single dose).**

Factorial design - 2 Strains x 6 Treatments x 2 Blocks for each sex

Endocrine-Disrupting Chemicals: Prepubertal Exposures and Effects on Sexual Maturation and Thyroid Activity in the Female Rat. A Focus on the EDSTAC Recommendations Jerome M. Goldman, Susan C. Laws, Sharon K. Balchak,Ralph L. Cooper, and Robert J. Kavlock. Critical Reviews in Toxicology, 30(2):135-196 (2000)

Modified Research Protocol for Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats.

Purpose and Applicability

The purpose of this protocol is to outline procedures to quantify the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile female rat. This assay detects agents that display antithyroid, estrogenic, antiestrogenic (estrogen receptor [ER] or steroid-enzyme-mediated) activity, or alter puberty via changes in luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) and growth hormone (GH) secretion, or via alterations in hypothalamic function.

Required Endpoints:

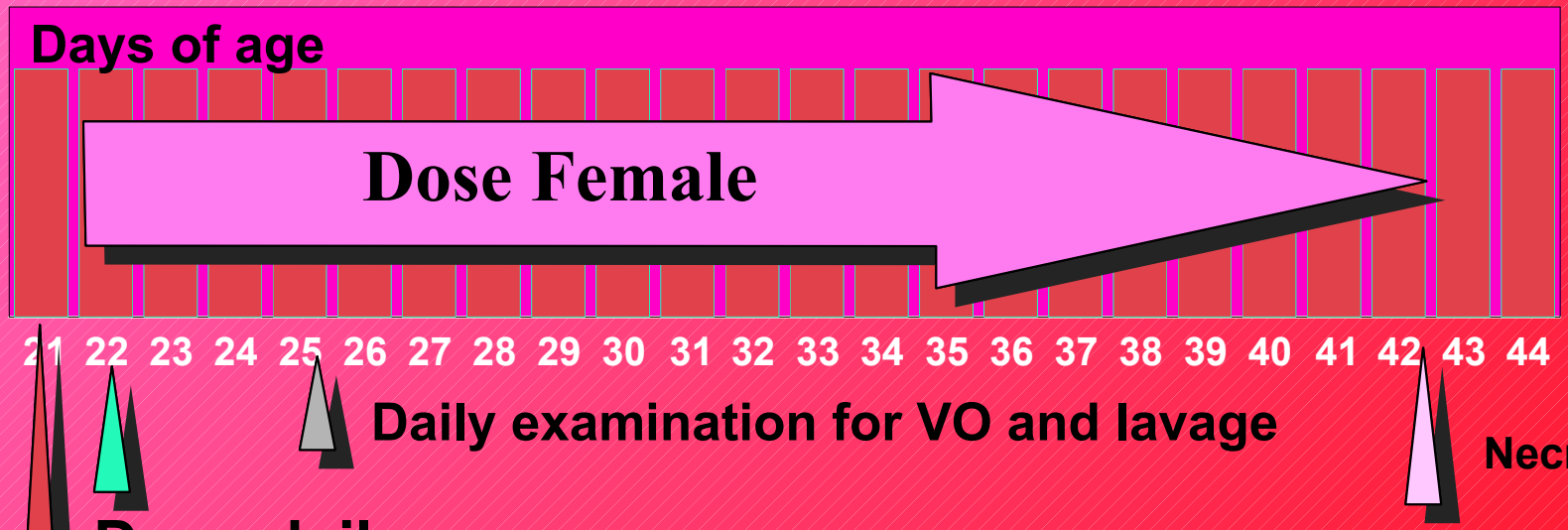
Growth (body weight). Liver, kidney, pituitary, and adrenal weights
Age and weight at vaginal opening
Serum thyroxine (T4), thyroid histology and thyroid stimulating hormone (TSH)
Uterine and ovarian weights and histology
Vaginal cytology

Optional Endpoints:

Serum tri-iodothyronine (T3), estradiol (E2), and prolactin
Thyroid weight
Liver, kidney, pituitary, adrenal and vaginal histology
Ex vivo ovarian and pituitary hormone production
Hypothalamic neurotransmitter concentrations
Estrous cycle length (requires extension of dosing)

Our Immature (21 - 43 Days of Age) Intact Female Rat Protocol to Evaluate Pubertal Development and Thyroid Function.

Detects inhibition of steroidogenesis, antithyroid and (anti)estrogenic activities and altered HPG maturation.



Assign to Treatments based upon body weight. 15/group

Endpoints:

- **Growth**
- **Age and weight at vaginal opening (VO)**
- **Vaginal cytology**
- **Serum thyroxine and thyroid-stimulating hormones**
- **Ovarian and uterine weights and histology**
- **Liver, kidney, pituitary and adrenal weights**

LEGJR

Published studies that have used the pubertal female assay

- Atrazine - 2 labs
- Nonyphenol
- DES - 2 labs
- Methoxychlor - VO and cycles
- Estradiol
- Octylphenol - VO and cycles
- Nonylphenol - VO and cycles
- Tamoxifen
- Fadrazole
- Ketoconazole
- Finasteride
- Testolactone
- Flutamide
- Perchlorate - in prep
- PBDE - submitted
- PTU
- MBC - VO and cycles
- DBP - VO and cycles

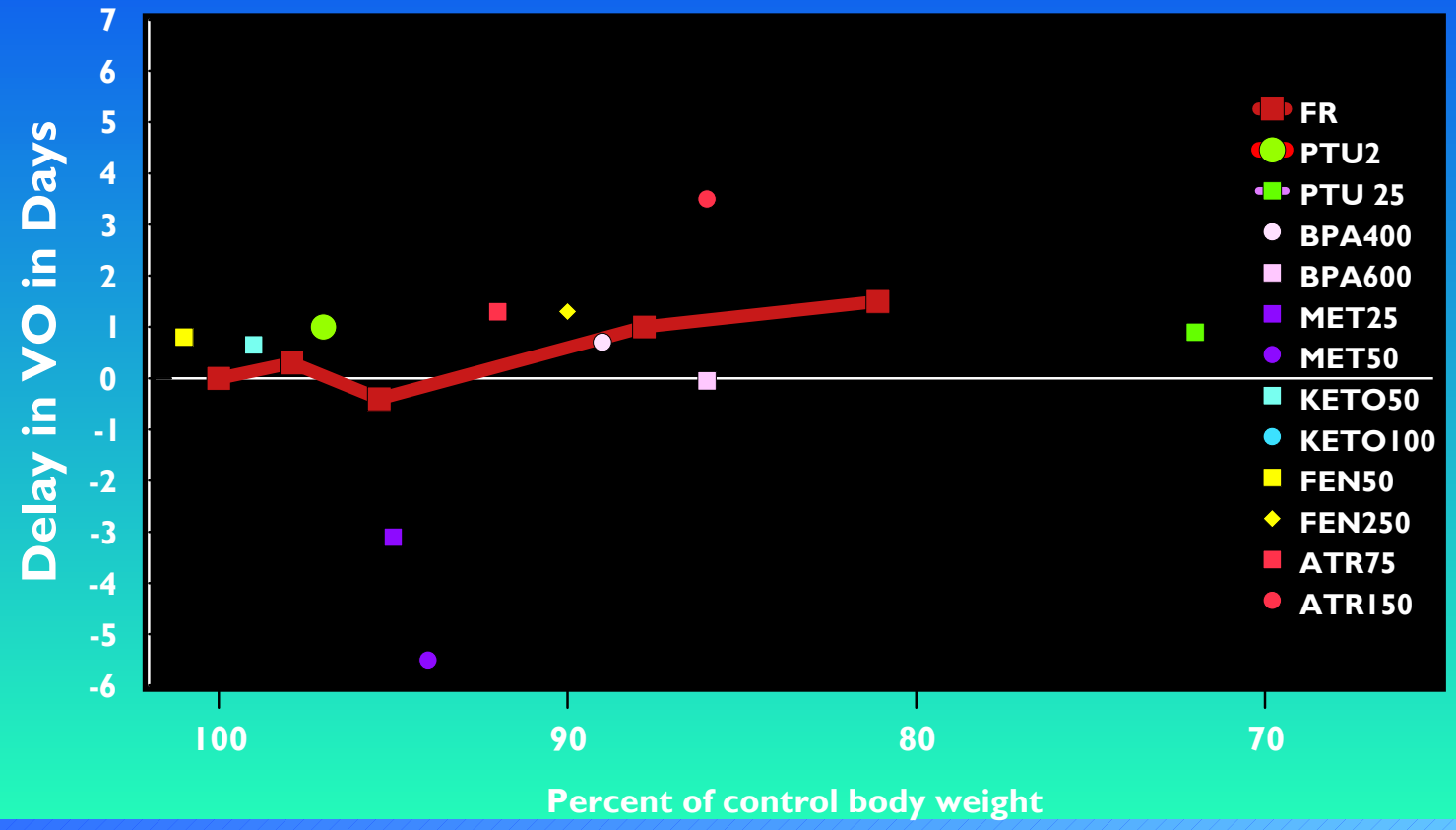
Chemicals studied this year in the pubertal female assay. New data

- RTI - completed
  - Atrazine 75 or 150 mg/kg/d
  - Fenarimol 50 or 250 mg/kg/d
  - Methoxychlor 25 and 50 mg/kg/d
  - Bisphenol A 400 or 600 mg/kg/d
  - Ketoconazole 50 and 100 mg/kg/d
  - PTU 2 and 25 mg/kg/d

- TherImmune 2000 (2x2x6 factorial. Blocks, LE versus SD and chemicals. n=6/strain/block) - completed
  - Ethynyl estradiol .005 mg/kg/d
  - Tamoxifen 10 mg/kg/d
  - PTU 240 mg/kg/d
  - Ketoconazole 100 mg/kg/d
  - Pimozide 30 mg/kg/d
  - Methoxychlor 100 mg/kg/d

- TherImmune 2003 - completed
  - Ethynyl estradiol 0.0025 and 0.005 mg/kg/d
  - Methoxychlor 12.5, 25 and 50 mg/kg/d
  - Phenobarbital 50 and 100 mg/kg/d

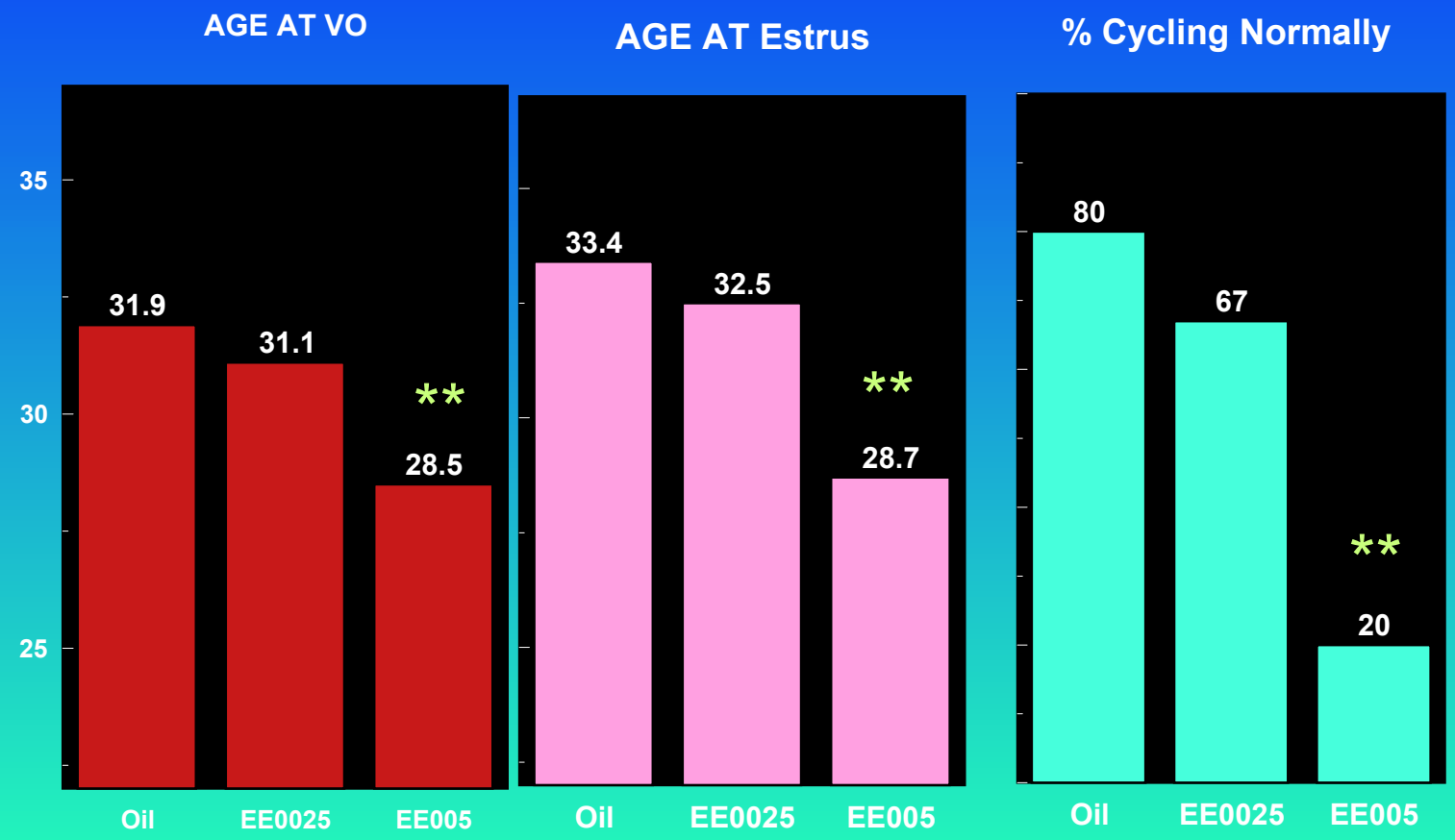
Relationship between reduction in body weight due to restricted feeding and delayed age at VO



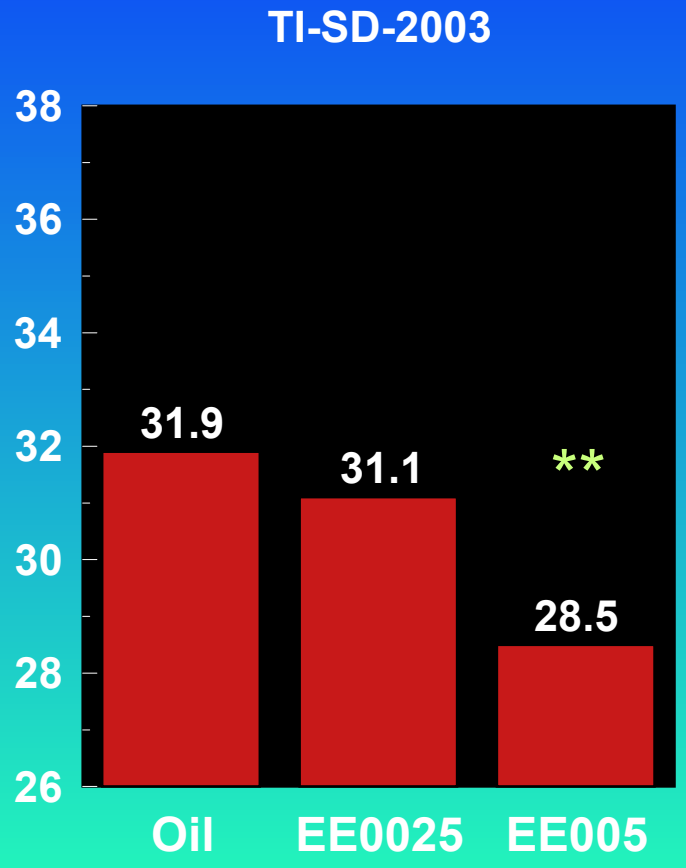
Estrogens in the pubertal female assay

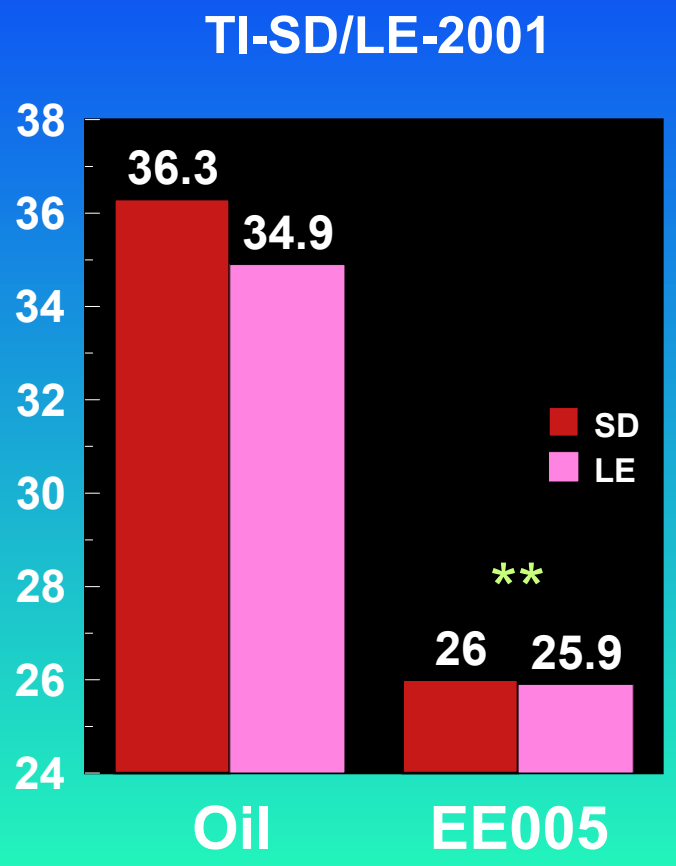
- Easily detected by accelerated VO if they are effective with oral administration in the rat

Effect of Ethynyl Estradiol on puberty in the SD female rat TI- SD-2003

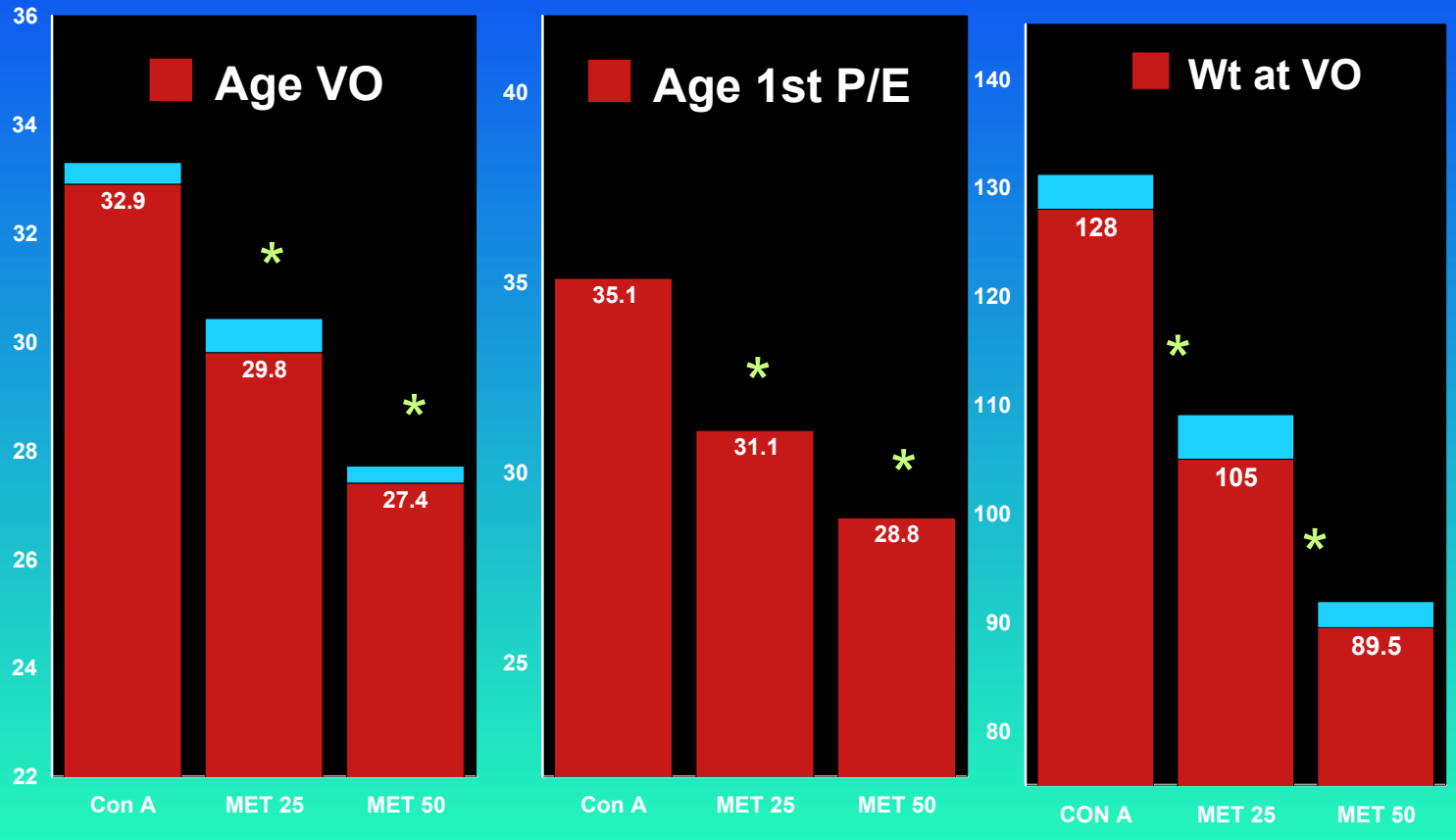


Effect of Ethynyl estradiol on AGE AT VO in the SD female rat TI- SD-2003. Note: Ovarian and Uterine histological changes noted in almost all females in TI-SD/LE-2000 (SD more affected) but no affects noted in TI-SD-2003 study.

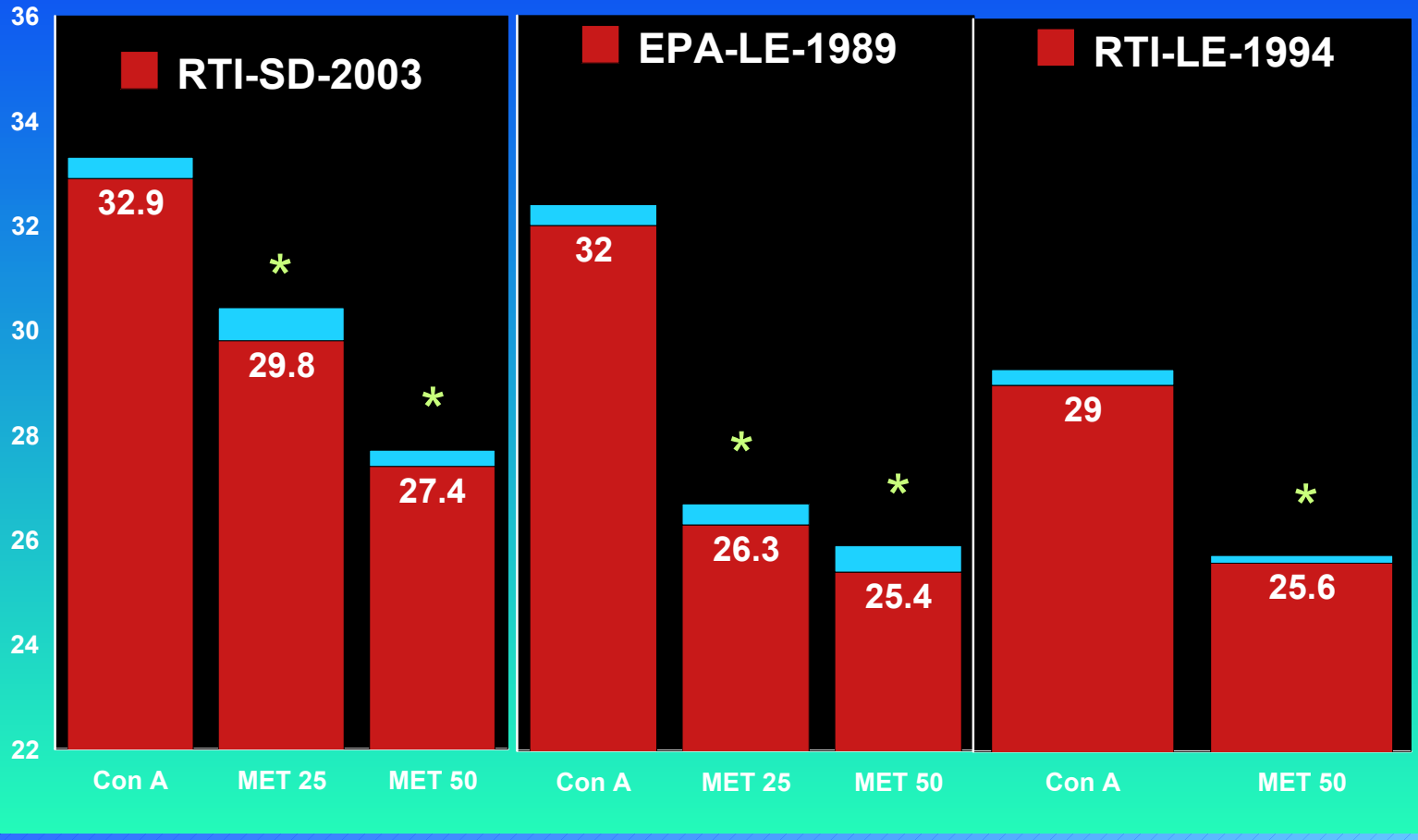




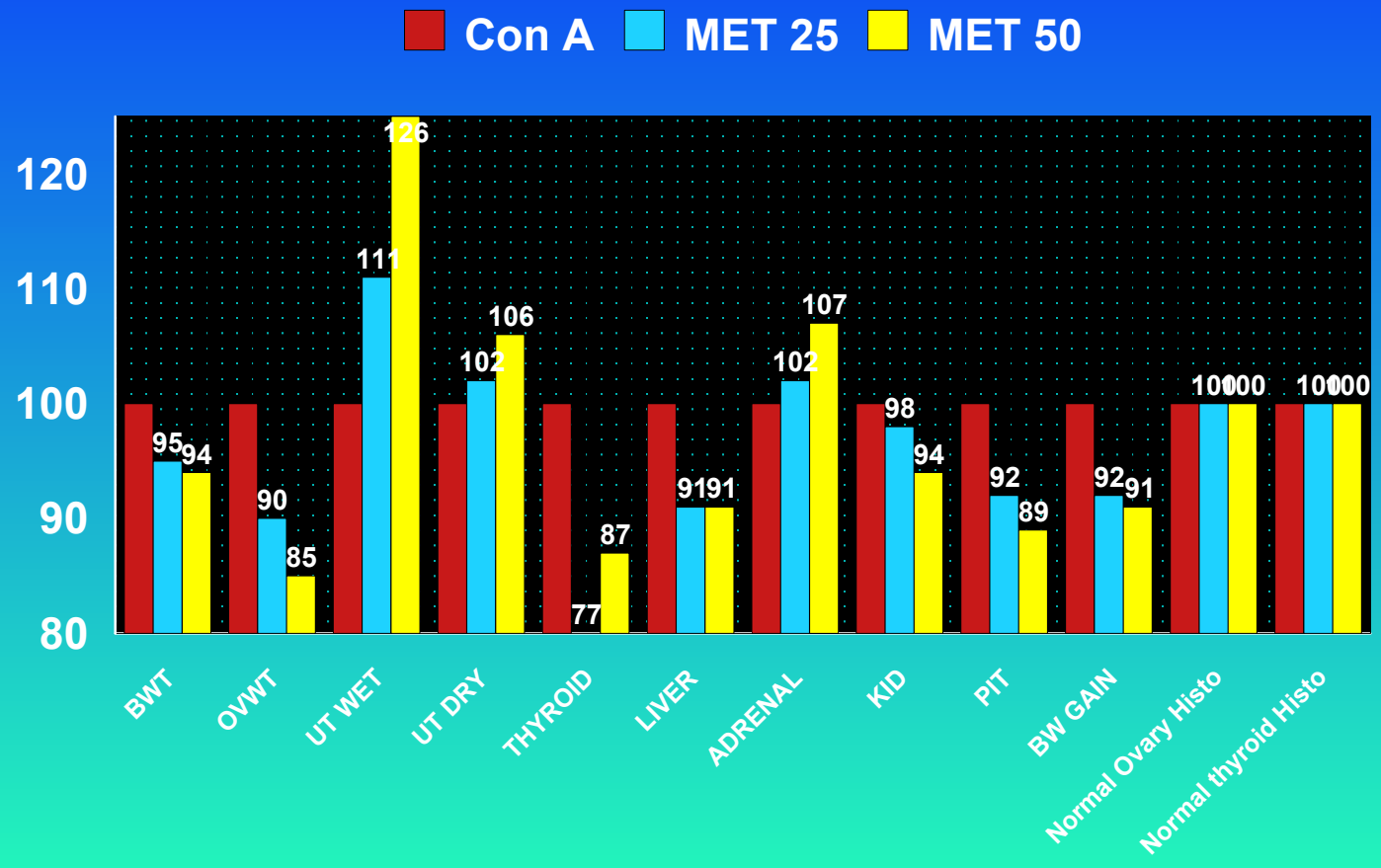
Effects of Methoxychlor in the RTI-SD-2003 Pubertal Female SD rat assay



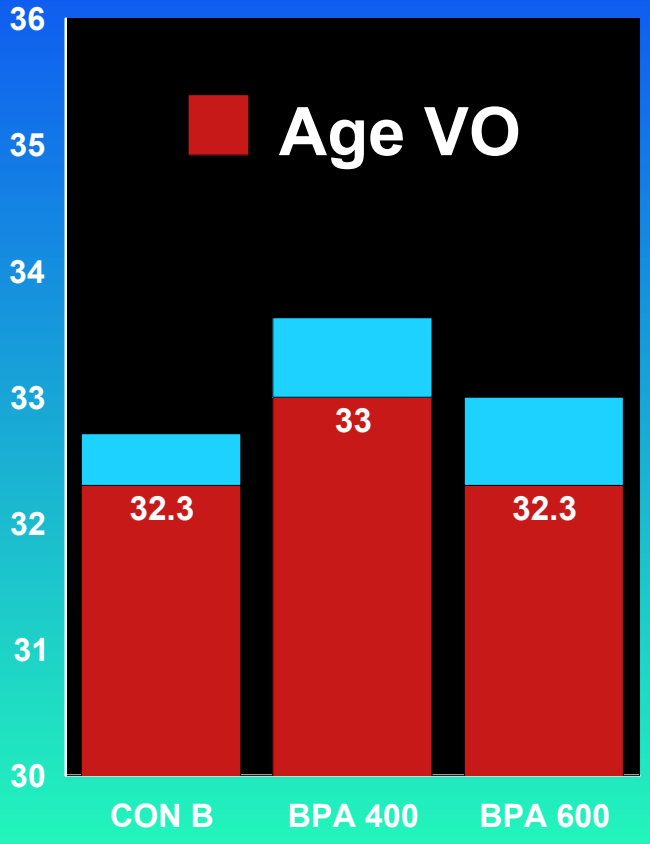
Effects of Methoxychlor ON AGE AT VO in the Female RTI-1994-LE, EPA-1989 and RTI-SD-2003 rat studies

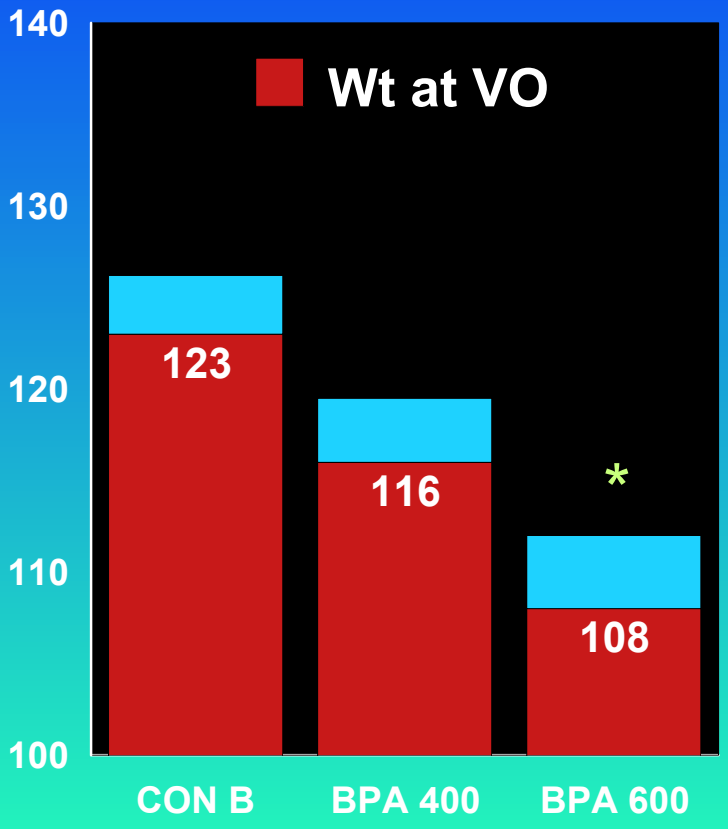


Effects of Methoxychlor in the Pubertal Female SD rat assay

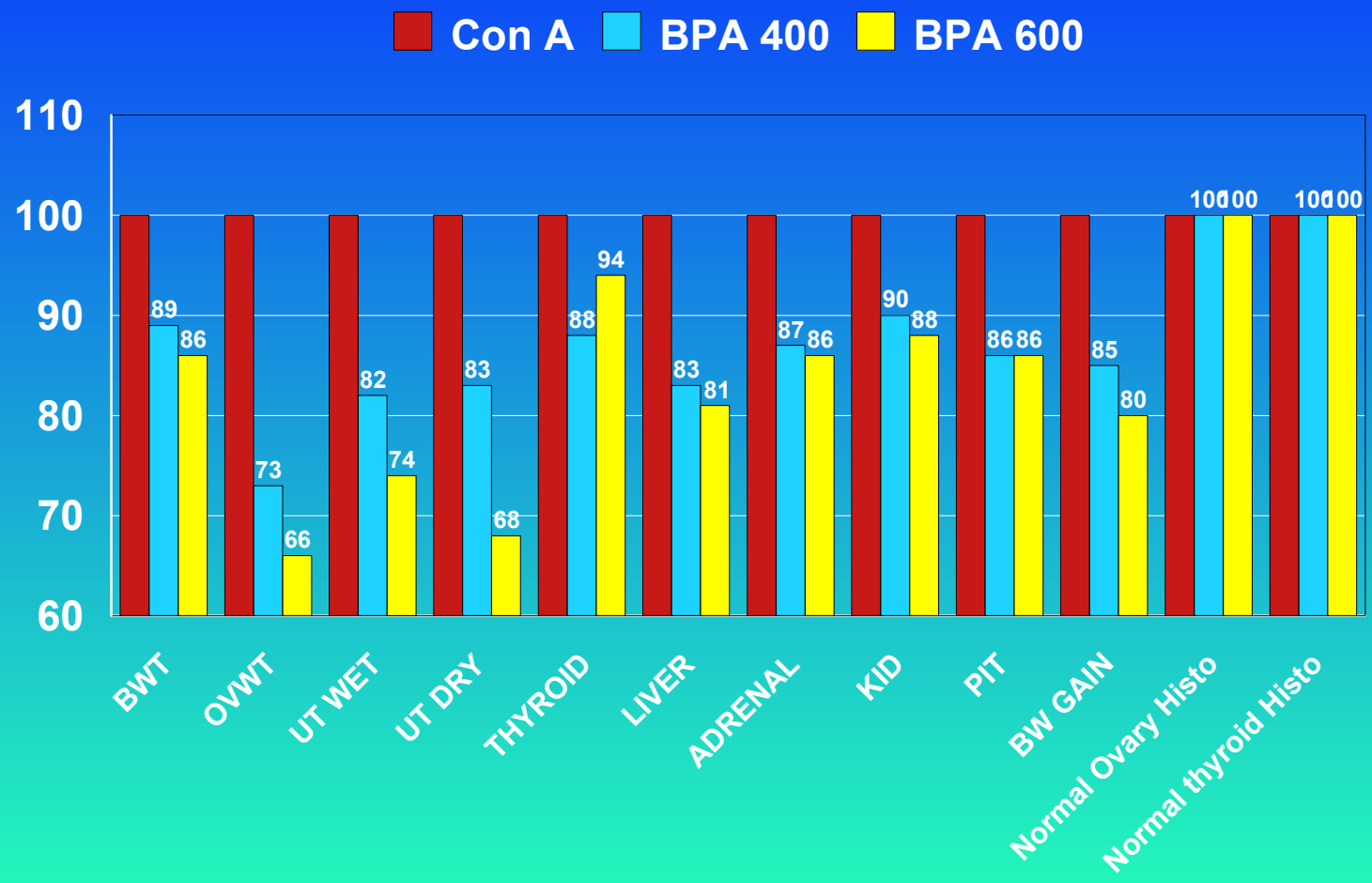


Lack of effect of BPA in the RTI-SD-2003 Pubertal Female SD rat assay on age and weight at VO. Octylphenol and Nonylphenol accelerate VO in this assay. BPA is positive in the in vitro and uterotropic assays but negative here

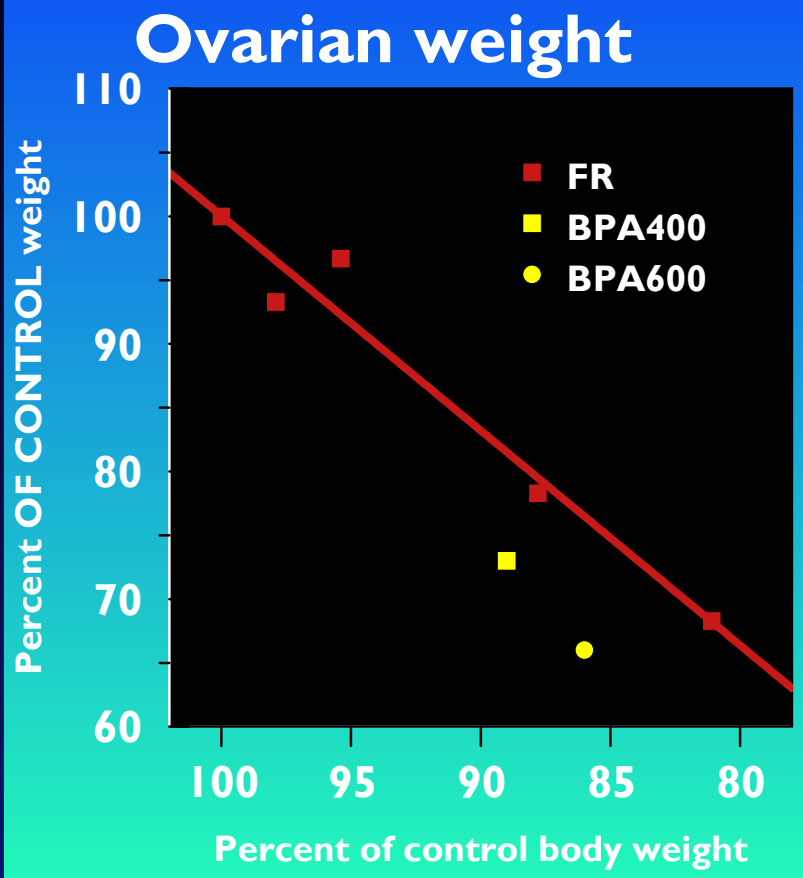


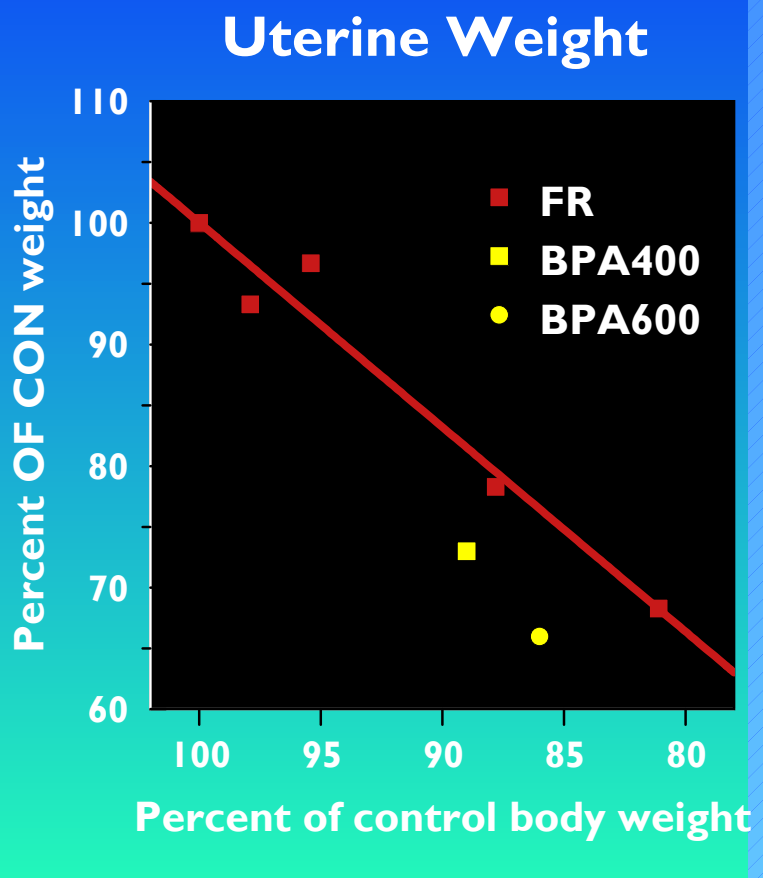


Effects of BPA in the RTI-SD-2003 Pubertal Female SD rat assay

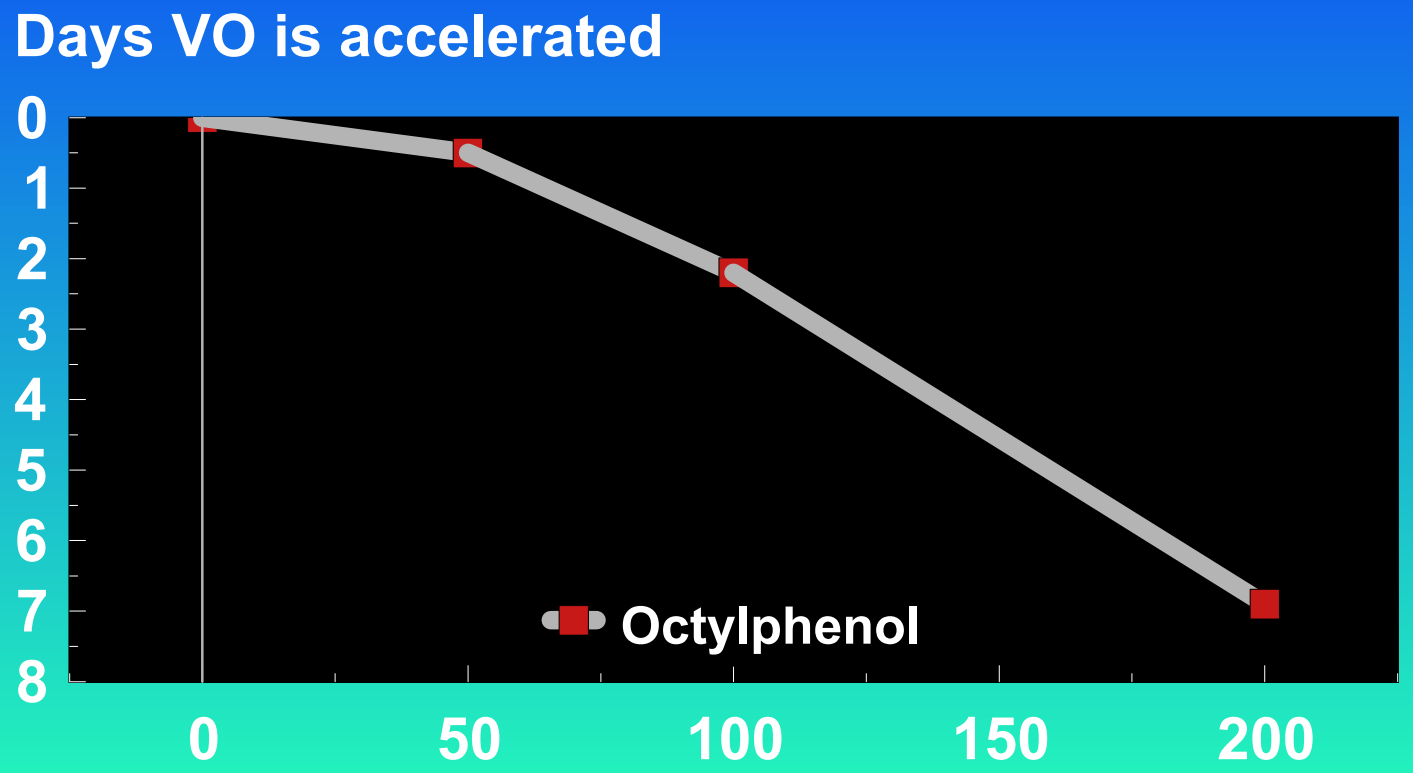


Relationship between reduction in body weight due to restricted feeding versus ovary and uterine weights





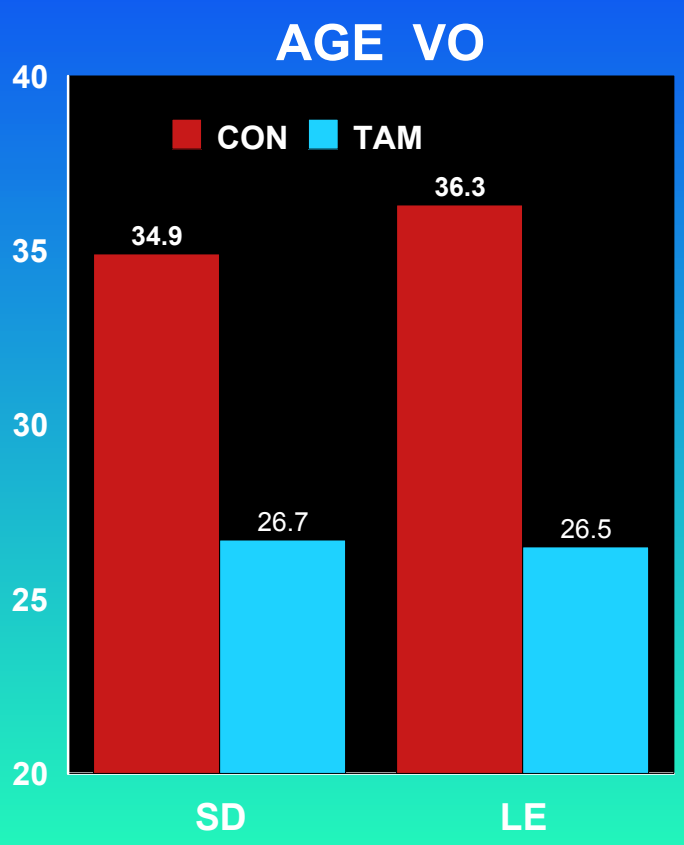
Daily oral (mg/kg/d) Octylphenol treatment induces pseudoprecocious puberty in the 21 day old female rat. Data are expressed as the number of days that VO was accelerated (Gray and Ostby, 1998). Also see Stoker et al. and Goldman et al., 2001.

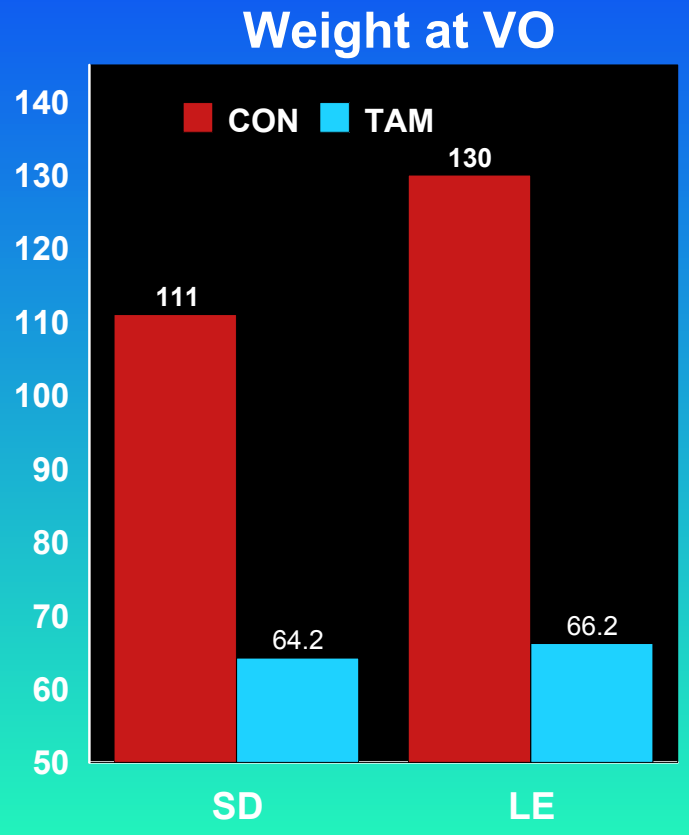


"Antiestrogens" and Inhibitors of Steroidogenesis

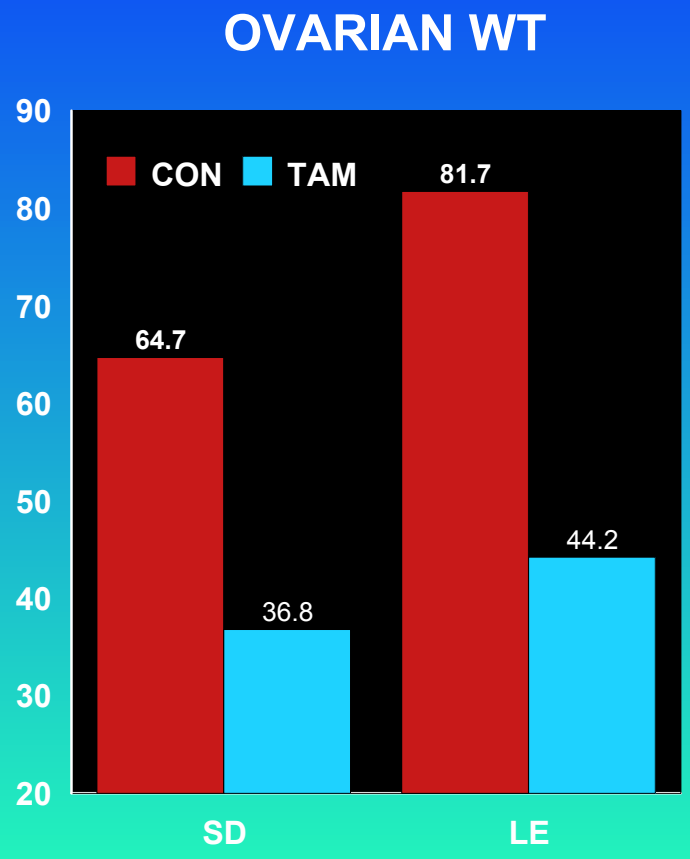
- Tamoxifen-mixed agonist/antagonist
- ICI and ZM - ER antagonists
- Antralex - GnRH antagonist
- Fadrazole - Aromatase inhibitor
- Fenarimol - fungicide that inhibits aromatase in vivo and in vitro
- Ketoconazole - like fenarimol, but less specific for P450 aromatase
- Results as expected, or better, except Fenarimol

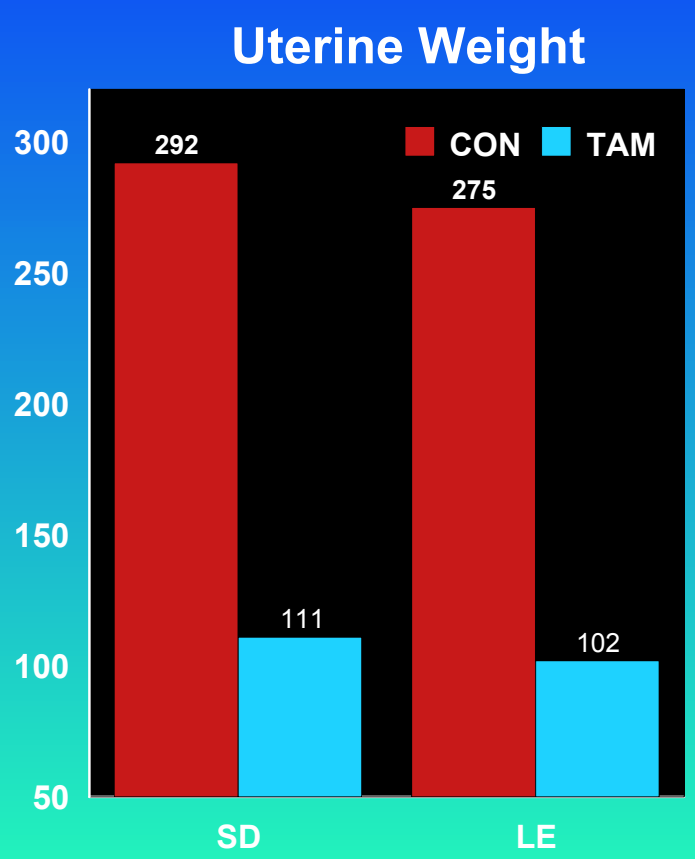
Effects of TAMOXIFEN 10 MG/KG in the SD AND LE Pubertal Female rat from TI 2000



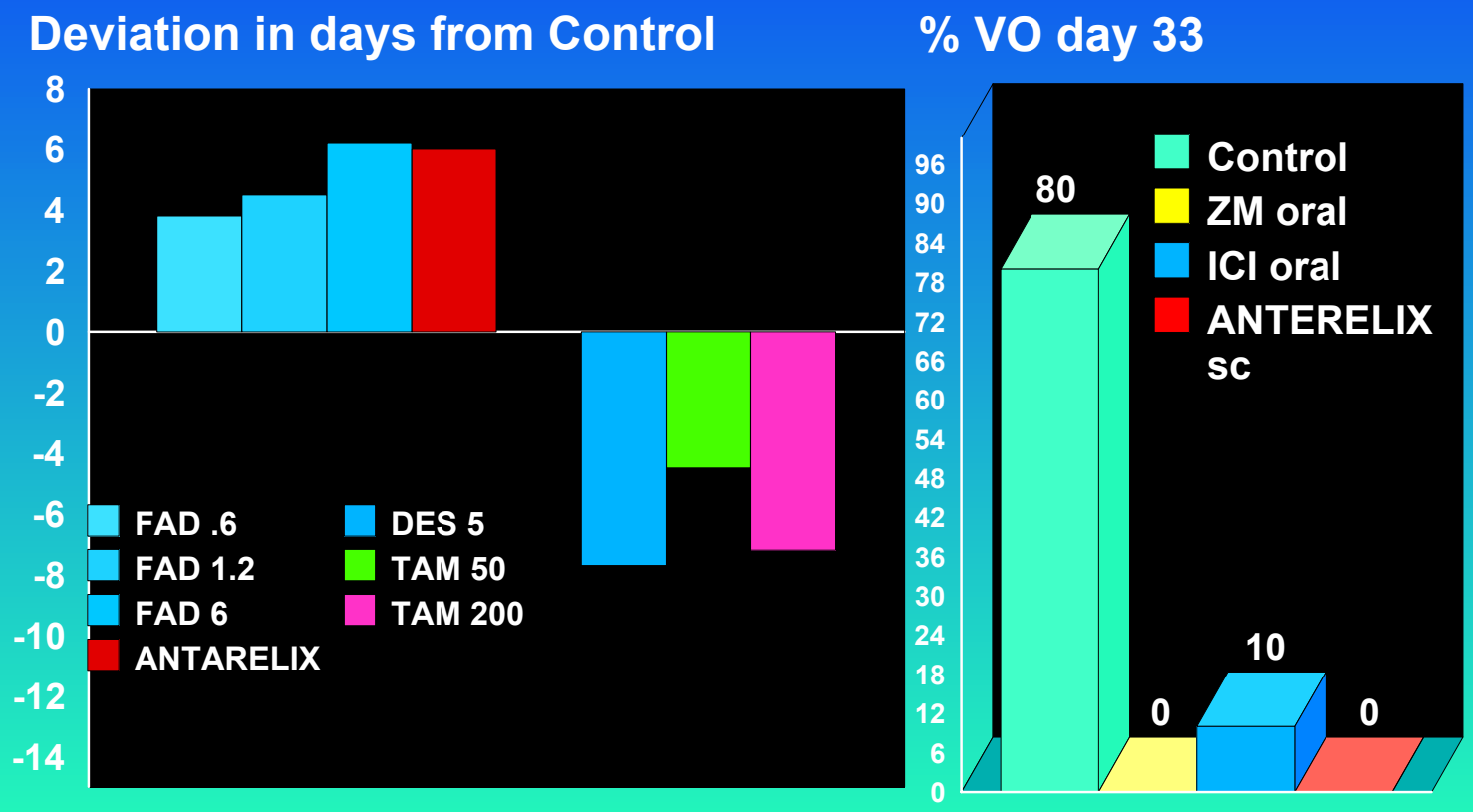


Effects of TAMOXIFEN 10 MG/KG in the SD and LE Pubertal Female rat from TI 2000

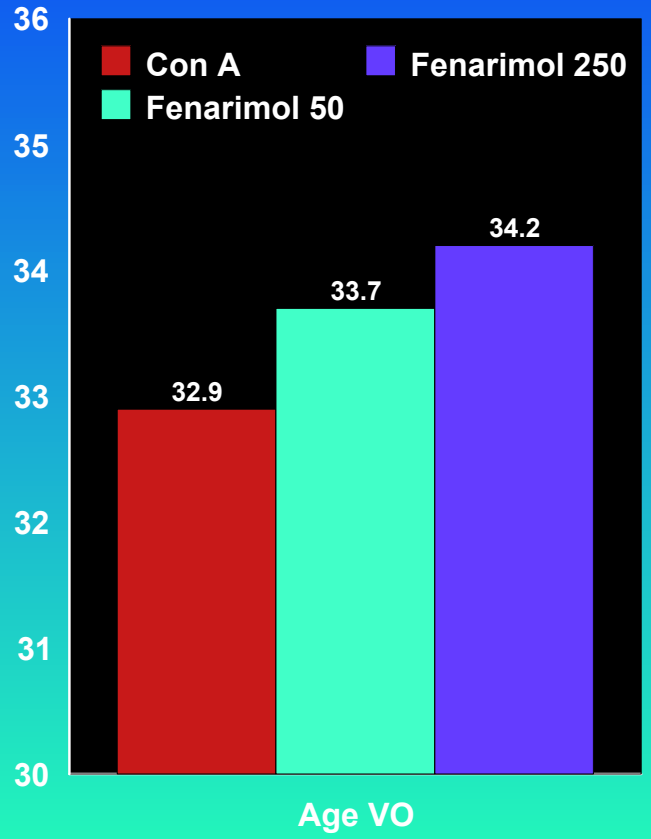


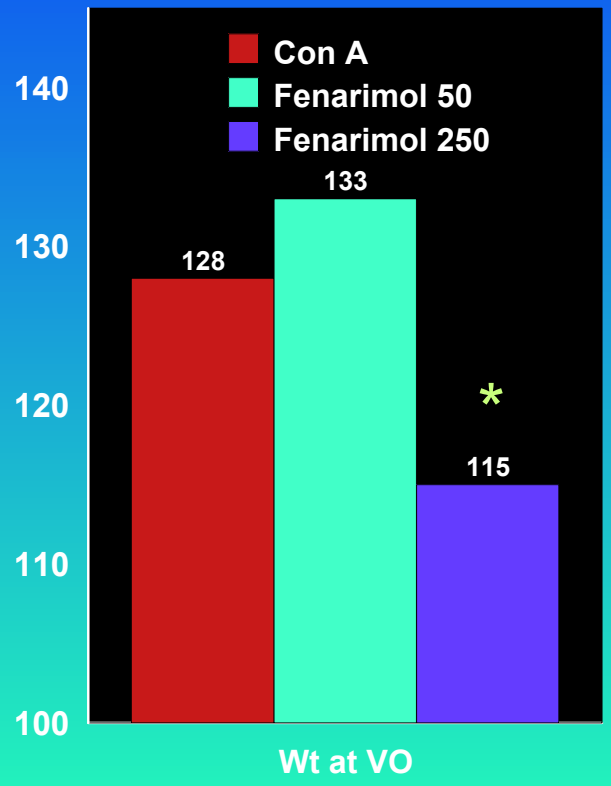


Effect of "(anti)estrogens" on age at VO in the Female rat. Data from Ashby et al., 2002; Marty et al., 1999; and Kim et al., 2002. ZM 189,154 and ICI 182,780 are ER antagonists. Tamoxifen is a mixed ER agonist and antagonist. Antralex is a GnRH antagonist.

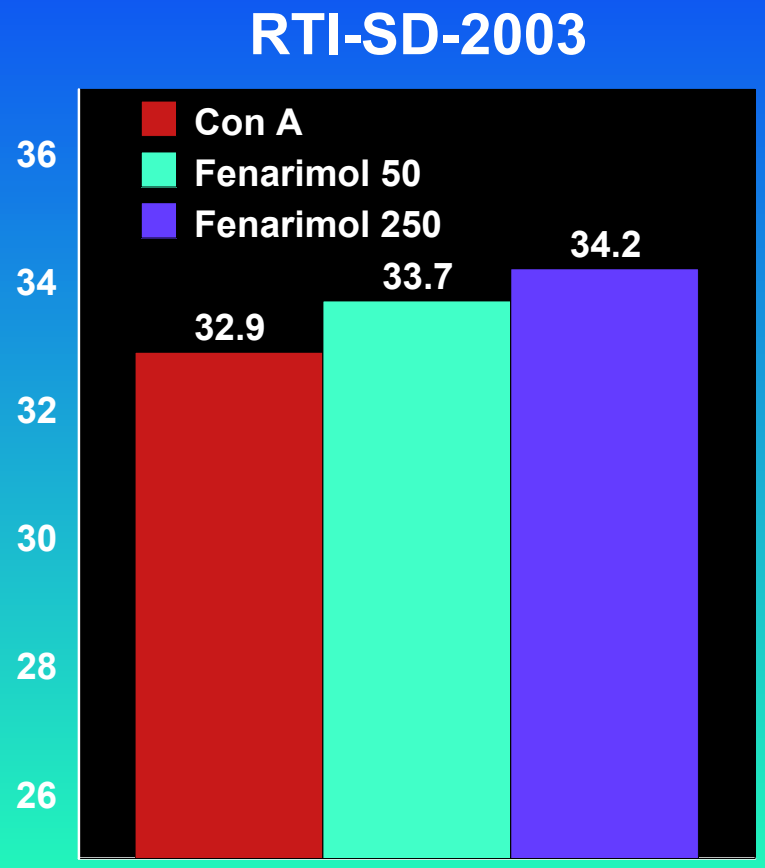


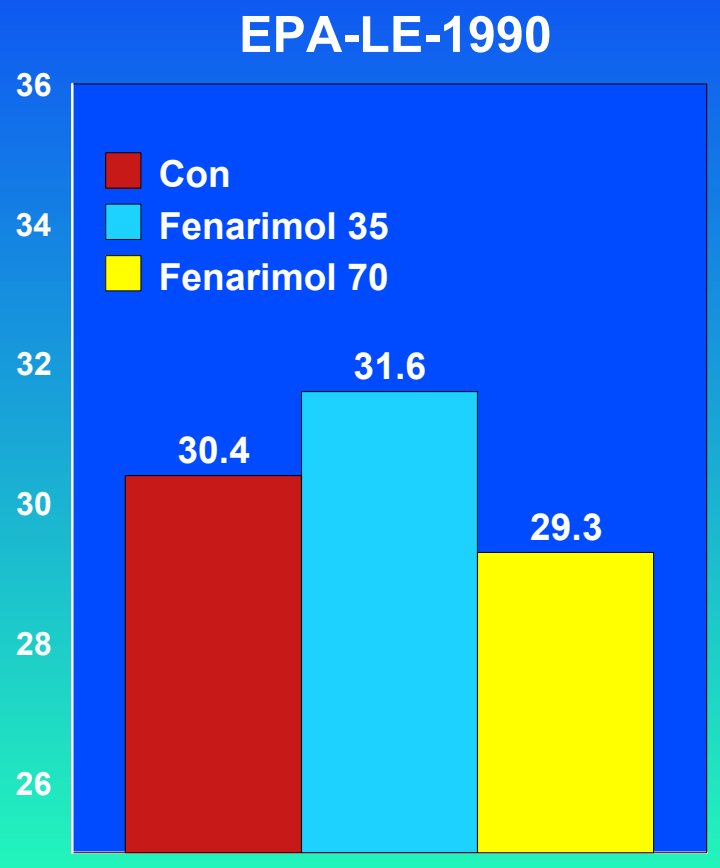
Effects of FENARIMOL in the Pubertal Female SD rat assay RTI-SD-2003



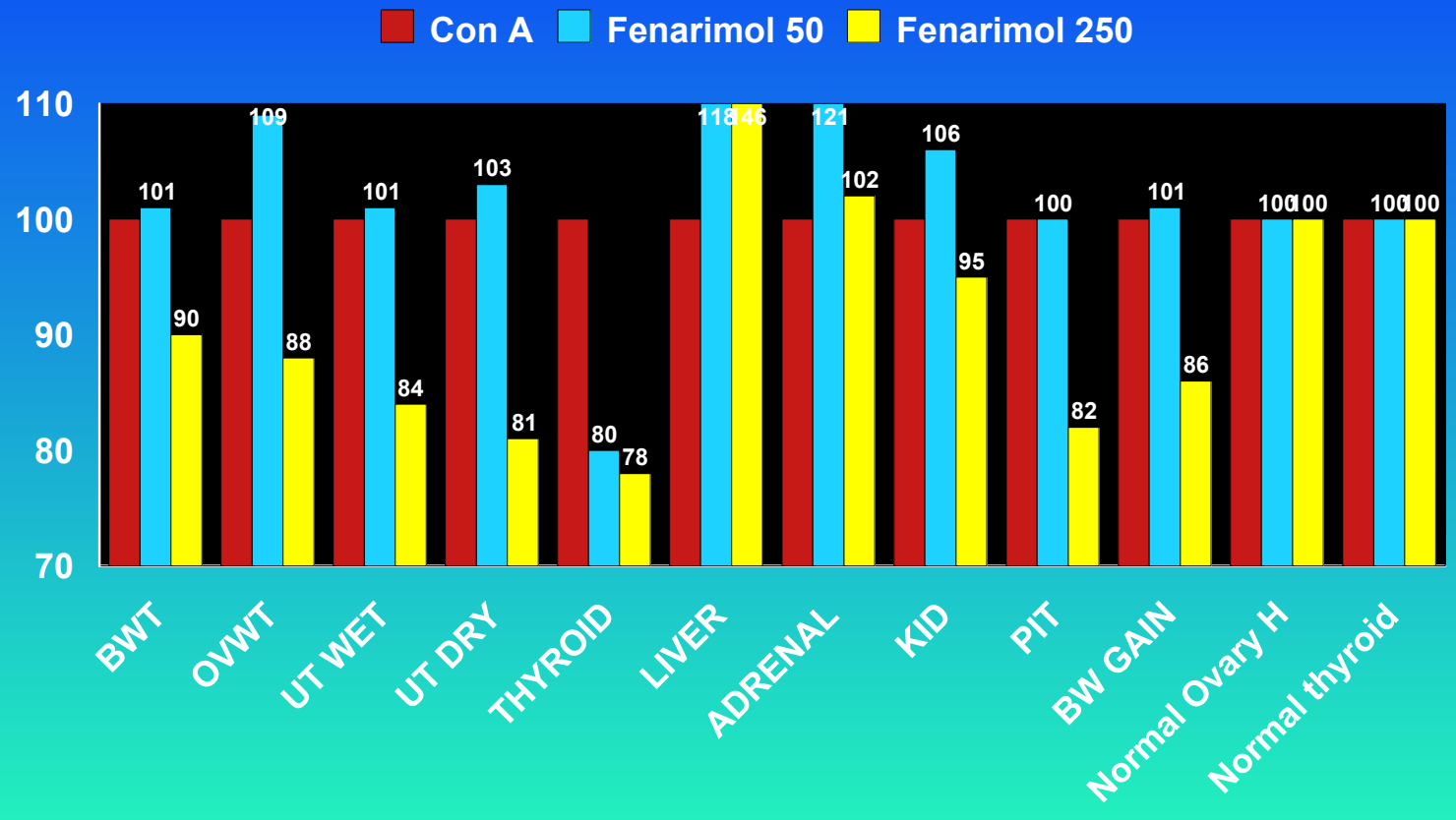


Lack of effect of FENARIMOL on VO in the Female rat from RTI-SD-2003 and EPA-LE-1990 studies

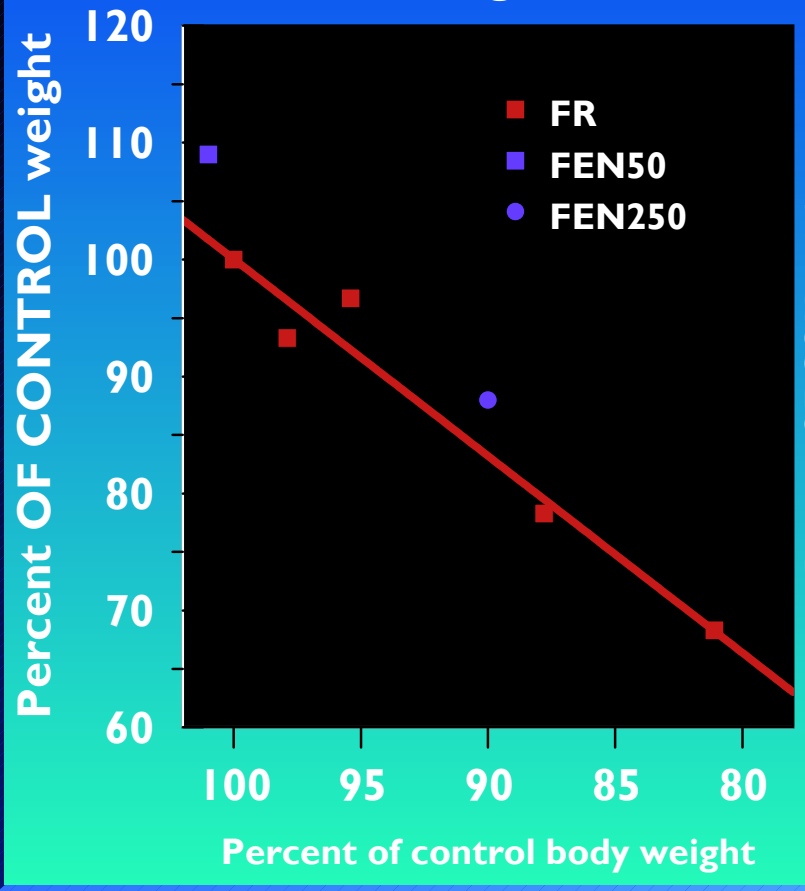


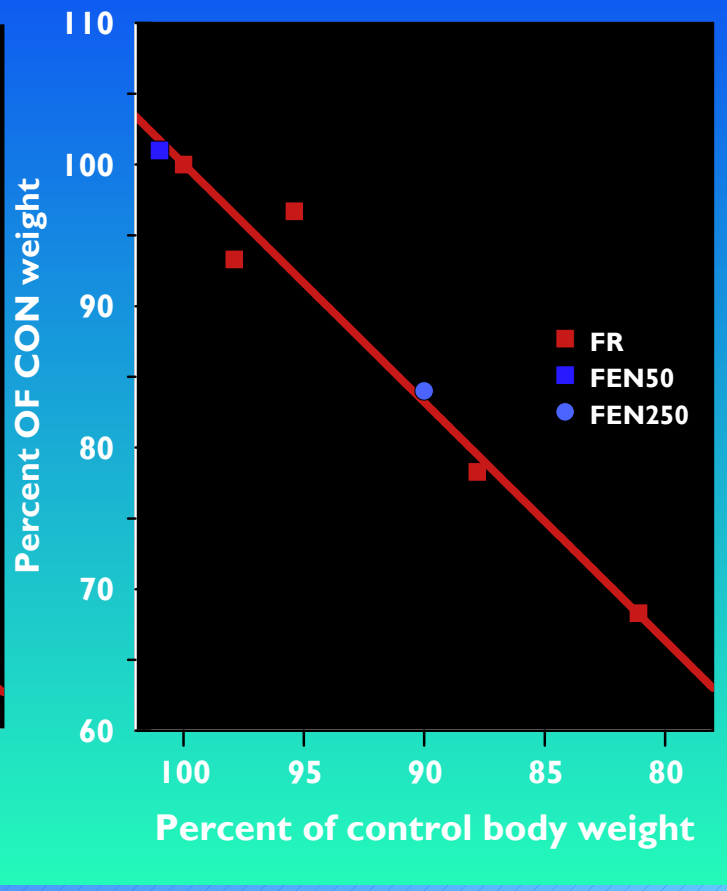


Effects of FENARIMOL in the Pubertal Female SD rat assay RTI-SDS-2003

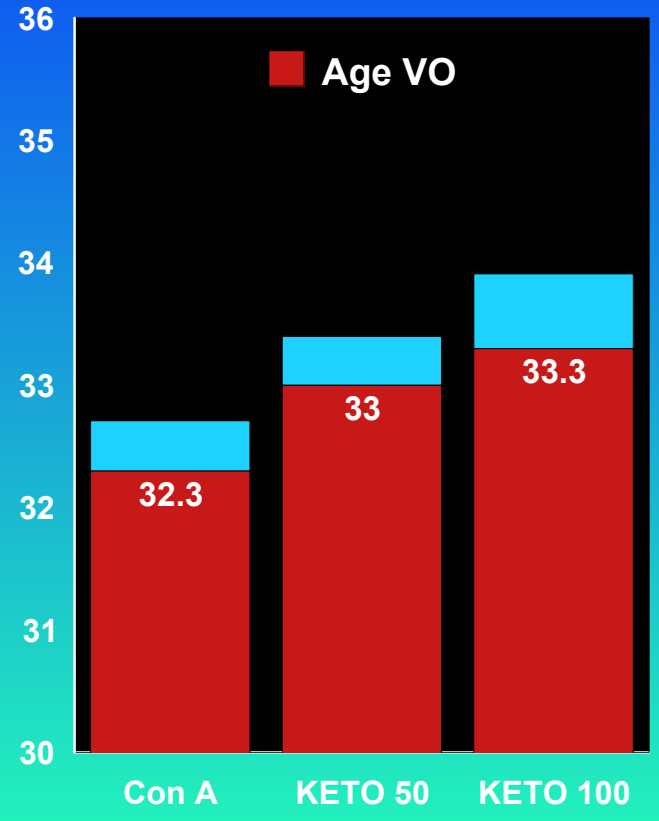


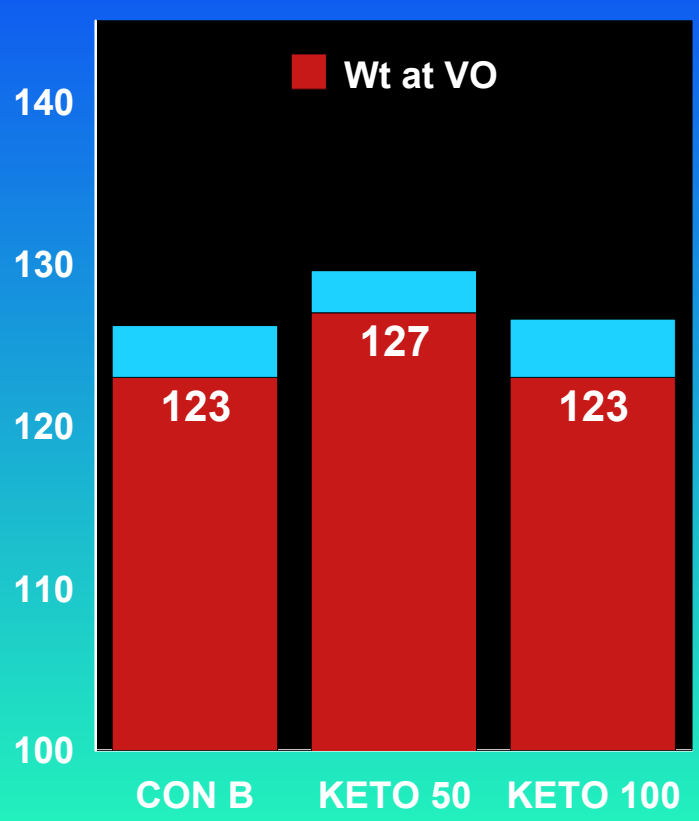
Relationship between reduction in body weight due to restricted feeding versus ovary and uterine weights



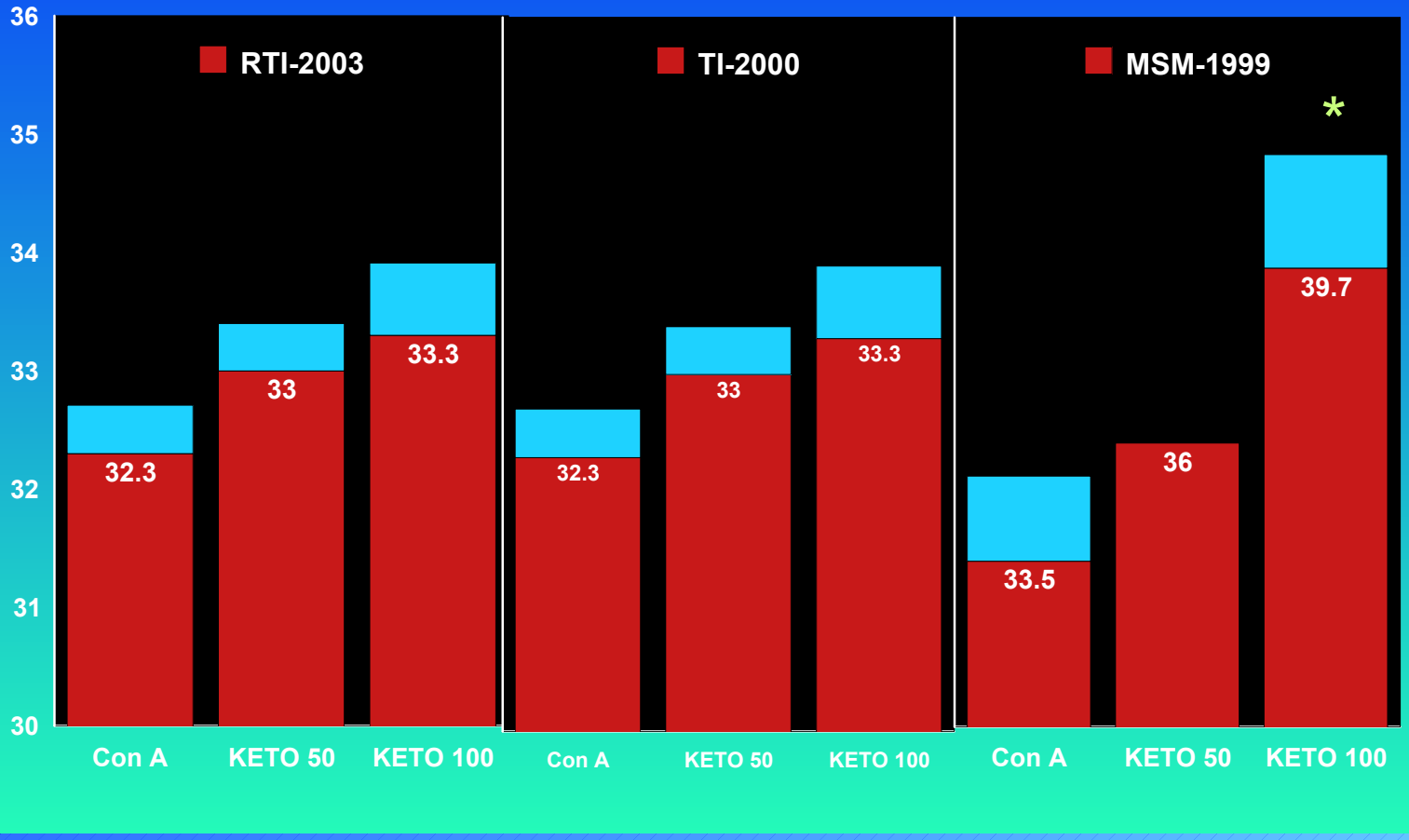


Effects of KETOCONAZOLE in the RTI-SD-2003 Pubertal Female SD rat assay. OVARIAN HISTOPATH IS KEY FOR THIS CHEMICAL

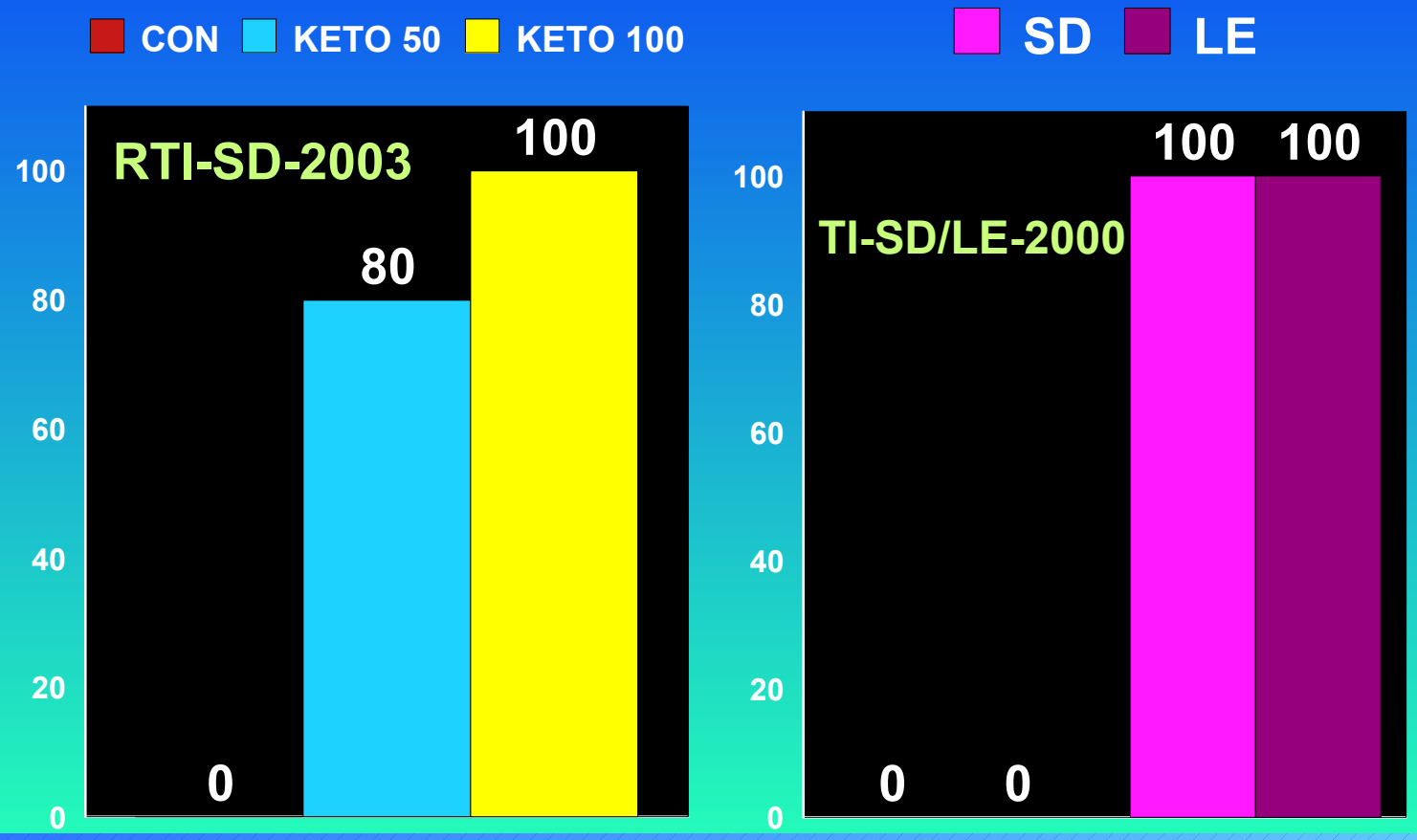




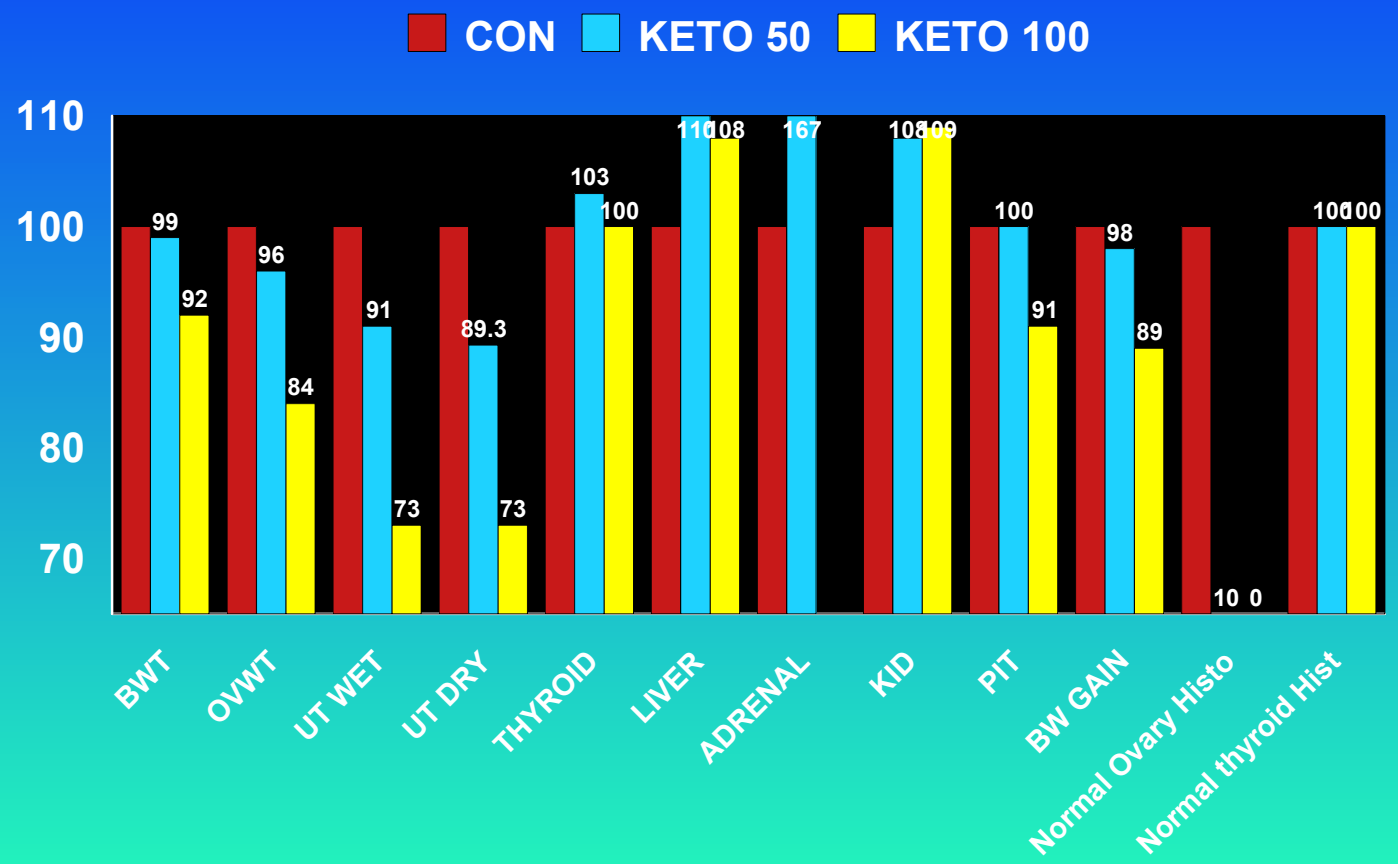
Effects of KETOCONAZOLE on age at VO in the Female rat RTI-SD-2003, TI-SD/LE-2000 and MSM-1999



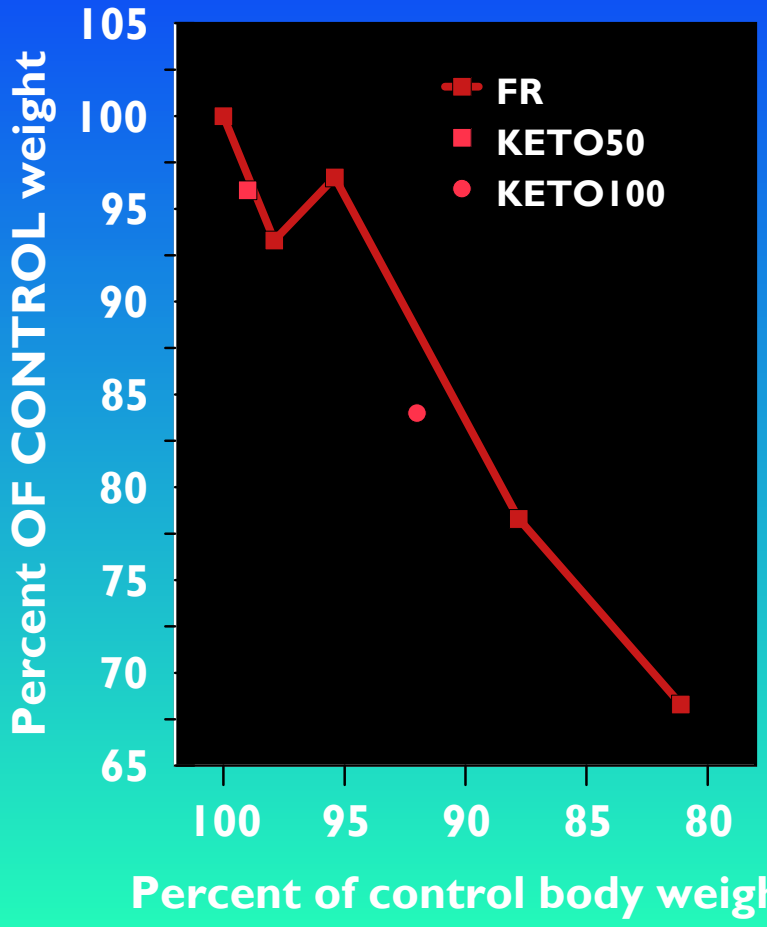
Effects of KETOCONAZOLE in the RTI-SD-2003 and TI-SD/LE-2000 Pubertal Female SD rat assay. Percent of females with ovarian histologic lesions

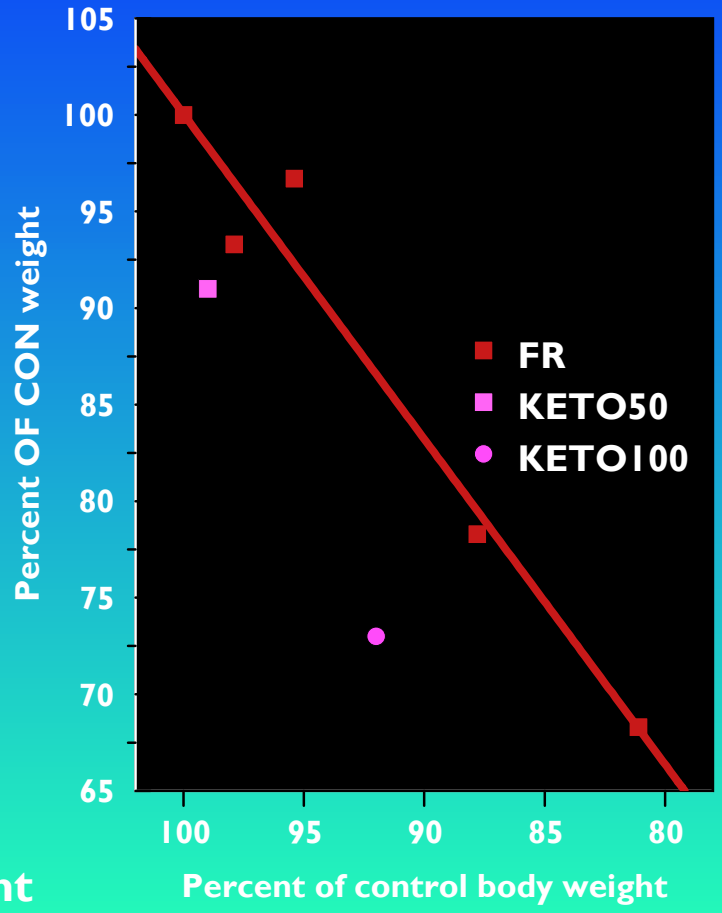


Effects of KETOCONAZOLE in the RTI-SD-2003 Pubertal Female SD rat assay



Relationship between reduction in body weight due to restricted feeding versus ovary and uterine weights

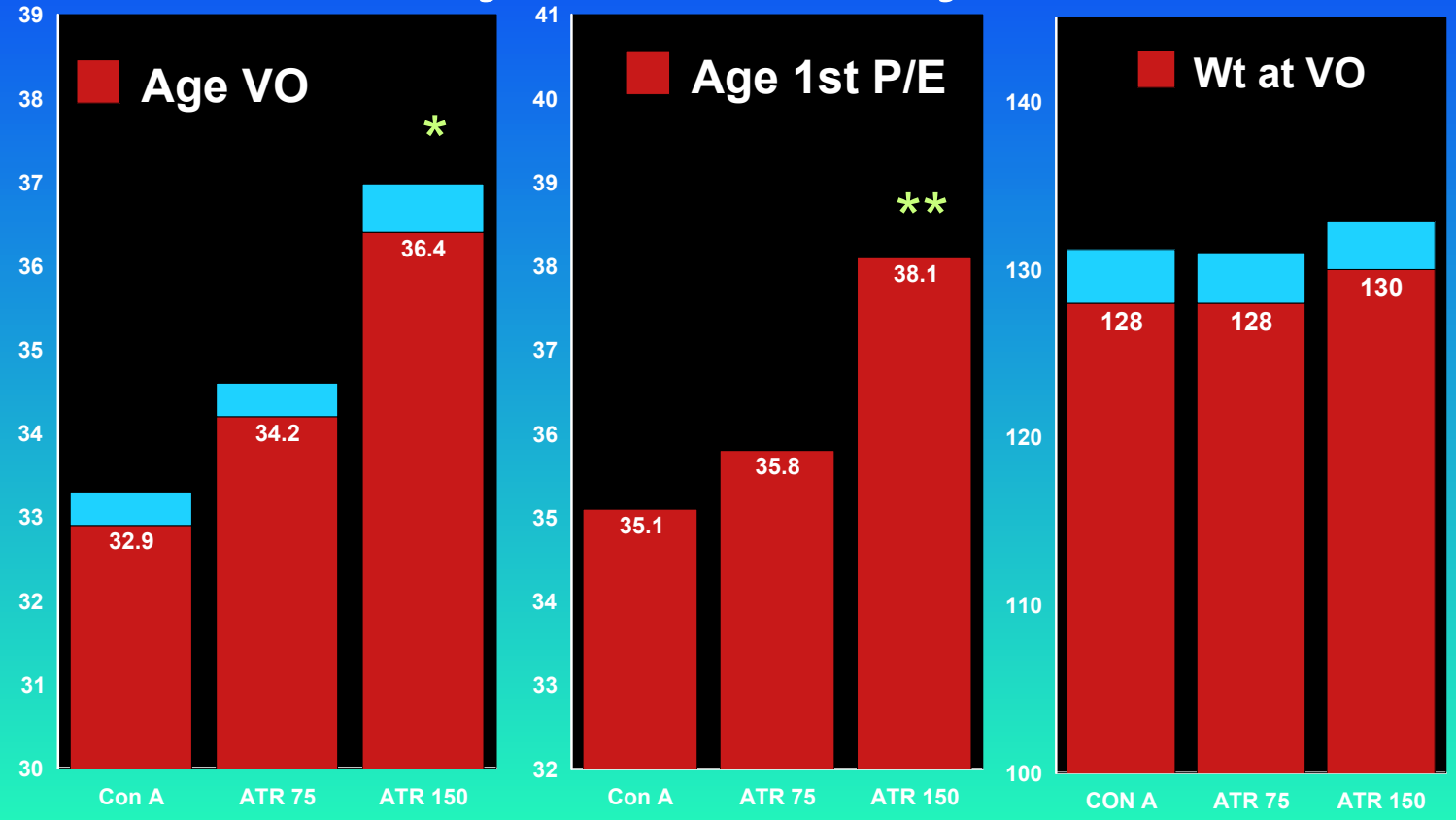




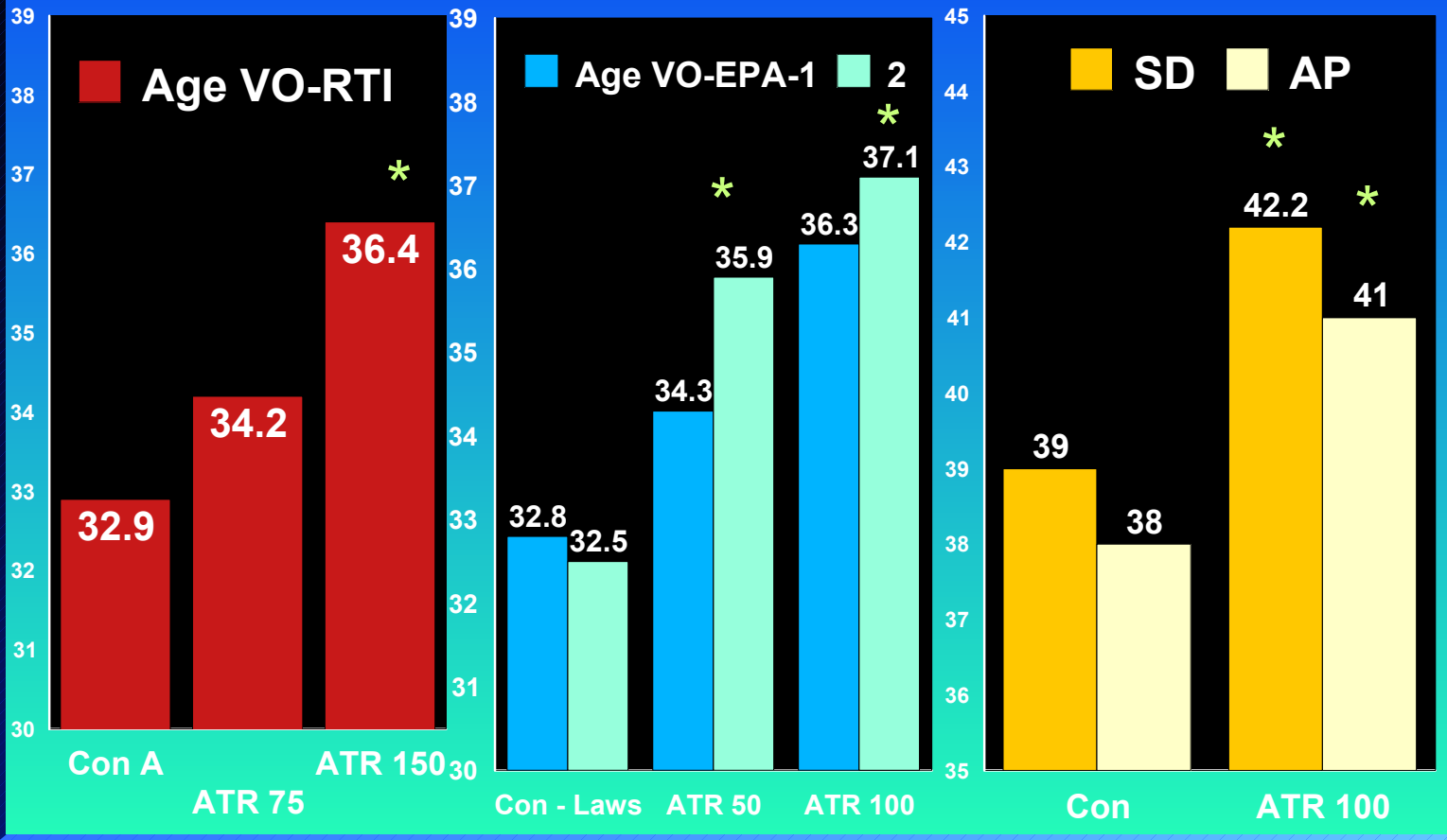
Effects of CNS active chemicals on puberty in the female

- Atrazine - alters CNS dopamine, prolactin and LH surges, also may affect aromatase and etc. Results reproducible
- Phenobarbital - alters CNS, pituitary and liver function, enhancing hormone metabolism and secretion. Results robust and reproducible

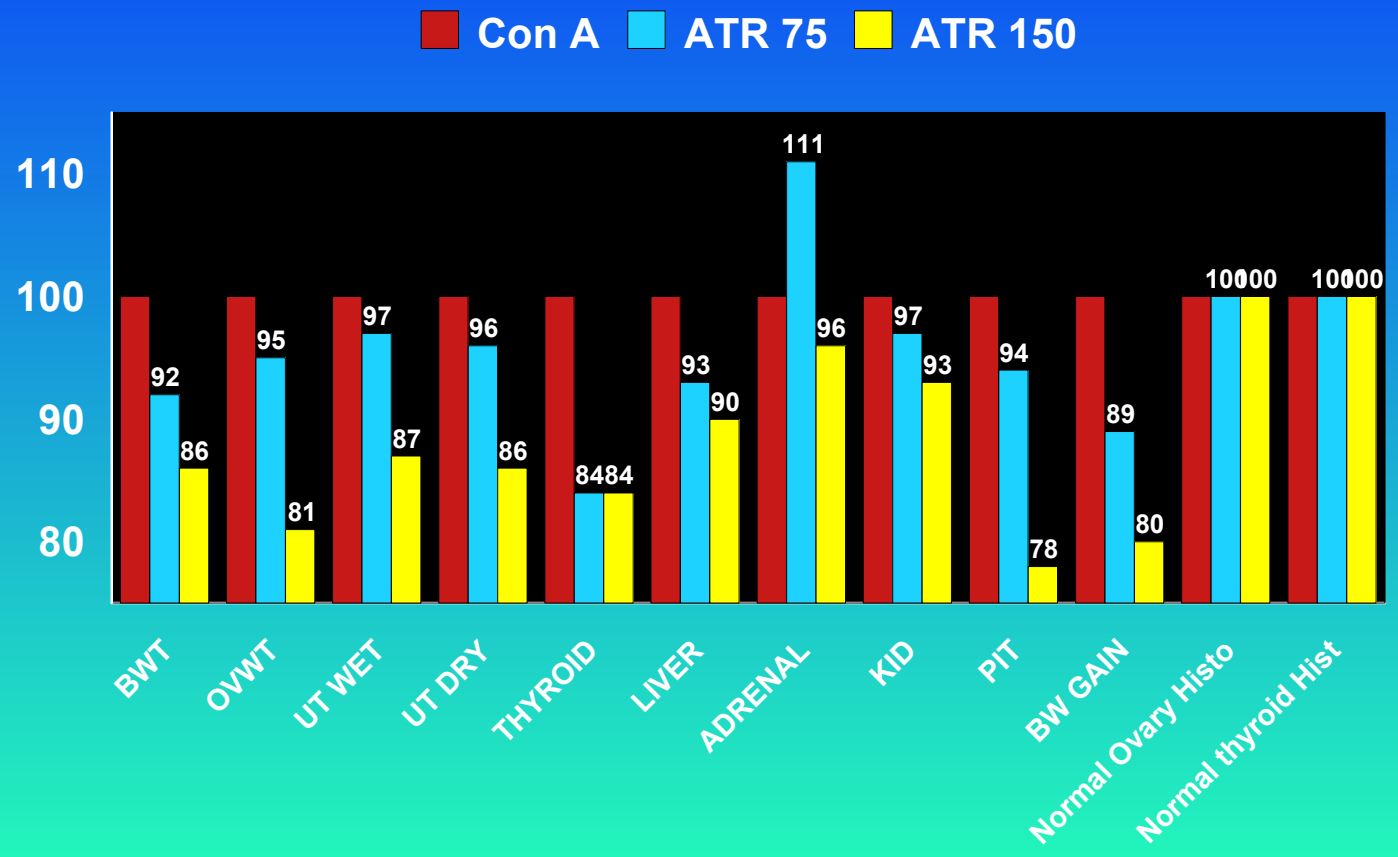
Effects of ATRAZINE in the Pubertal RTI-SD-2003 Female rat age and weight at VO and age at first estrus. Note: EPA needs to provide more guidance on analysis of estrous cycle data



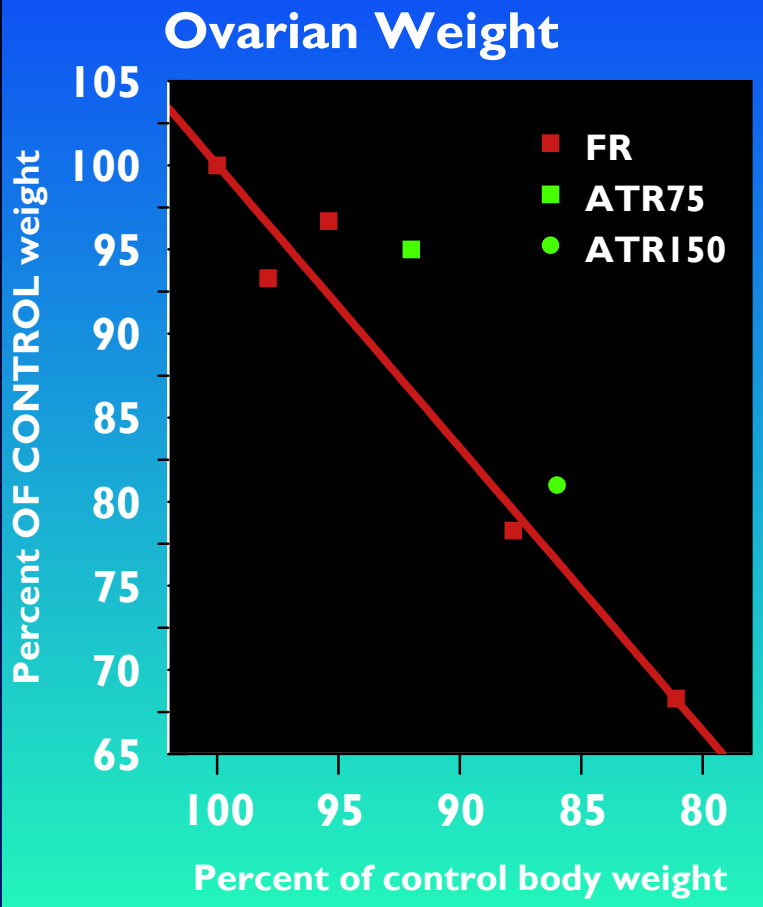
Effects of ATRAZINE at 100-150 mg in the Pubertal Female rat assay. RTI (SD) versus Laws (Wistar) and Ashby et al (SD and AP strains).

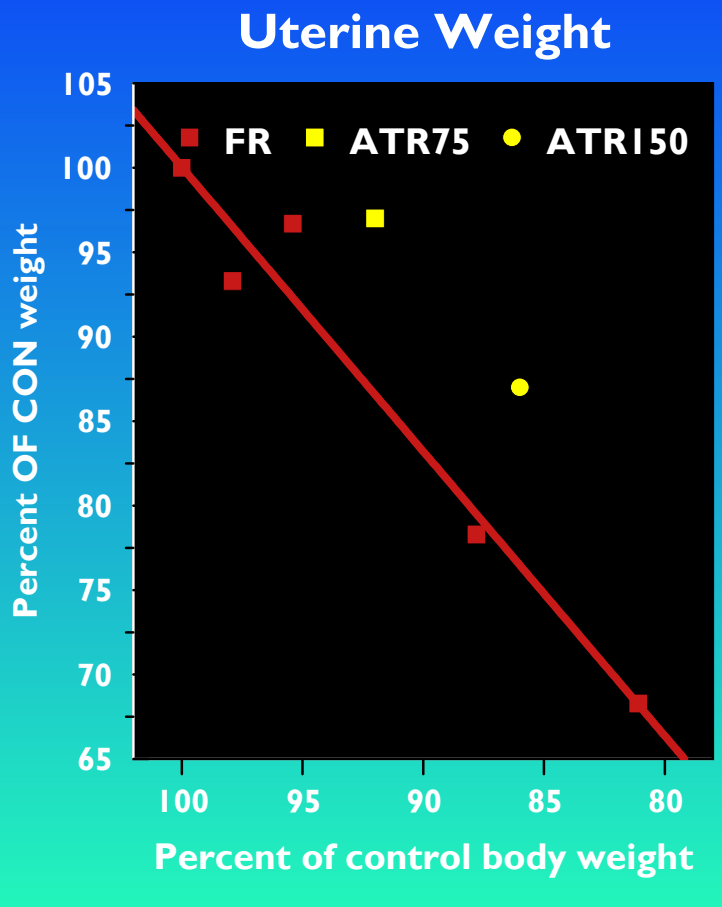


Effects of ATRAZINE in the RTI-SD-2003 Pubertal Female SD rat assay

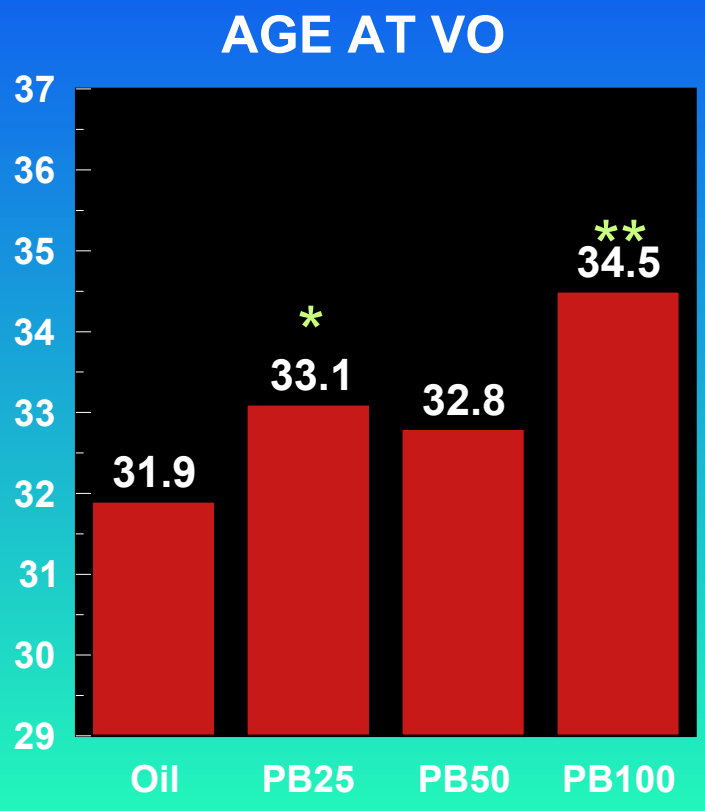


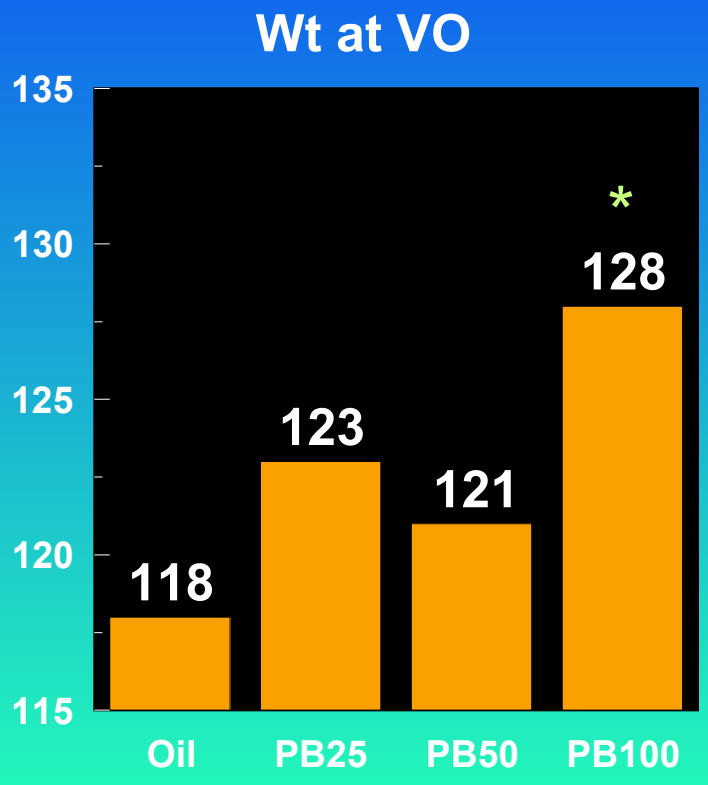
Relationship between reduction in body weight due to restricted feeding versus ovary and uterine weight



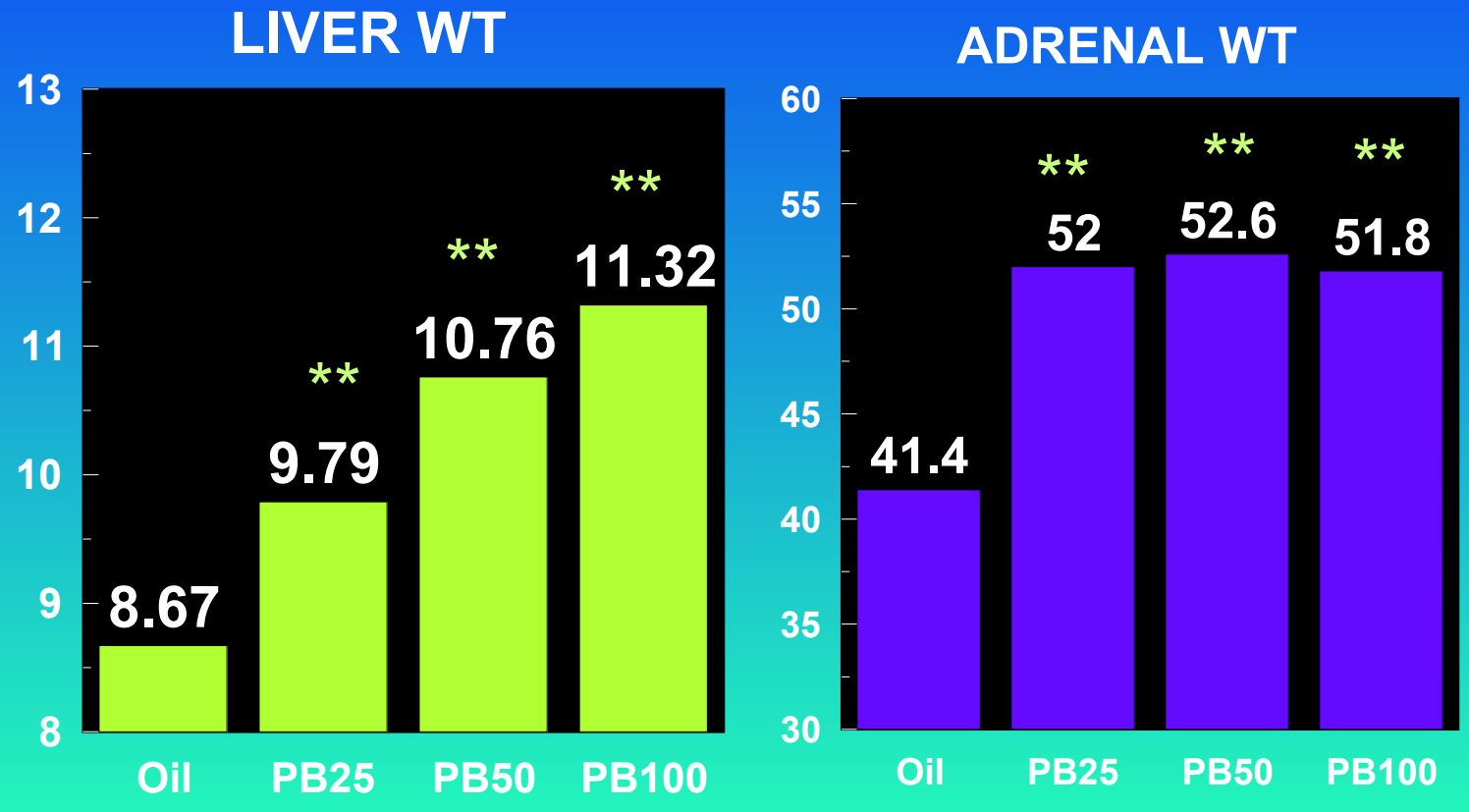


Effects of Phenobarbital on female rat puberty TI-SD-2003





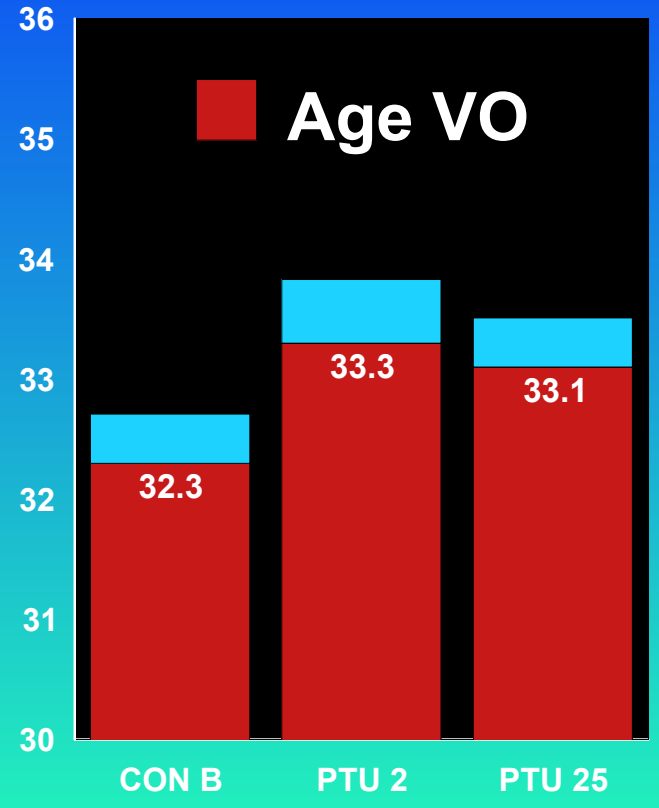
Effects of Phenobarbital on female rat puberty. TI-SD-2003

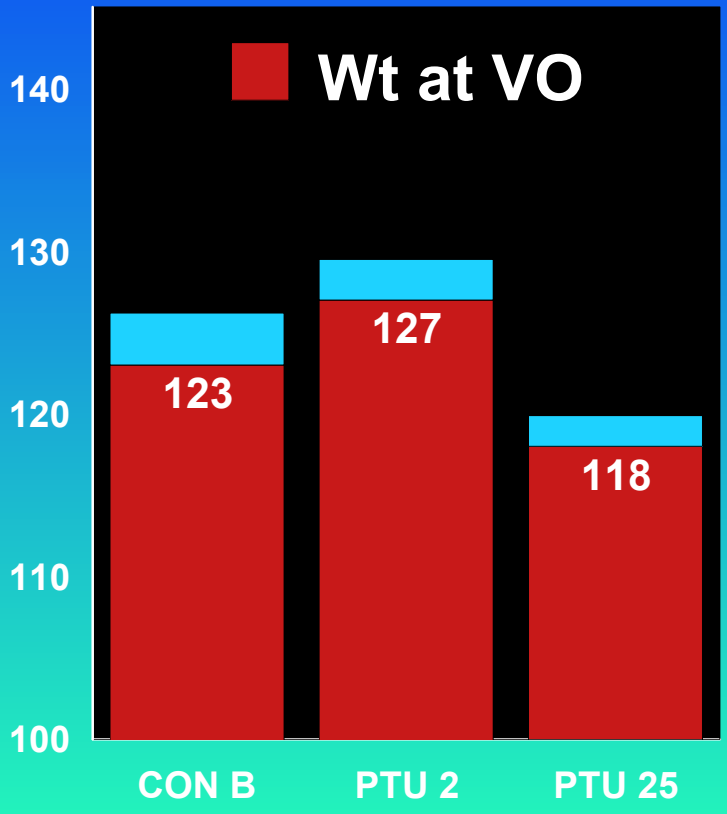


Antithyroid effects on puberty in the female

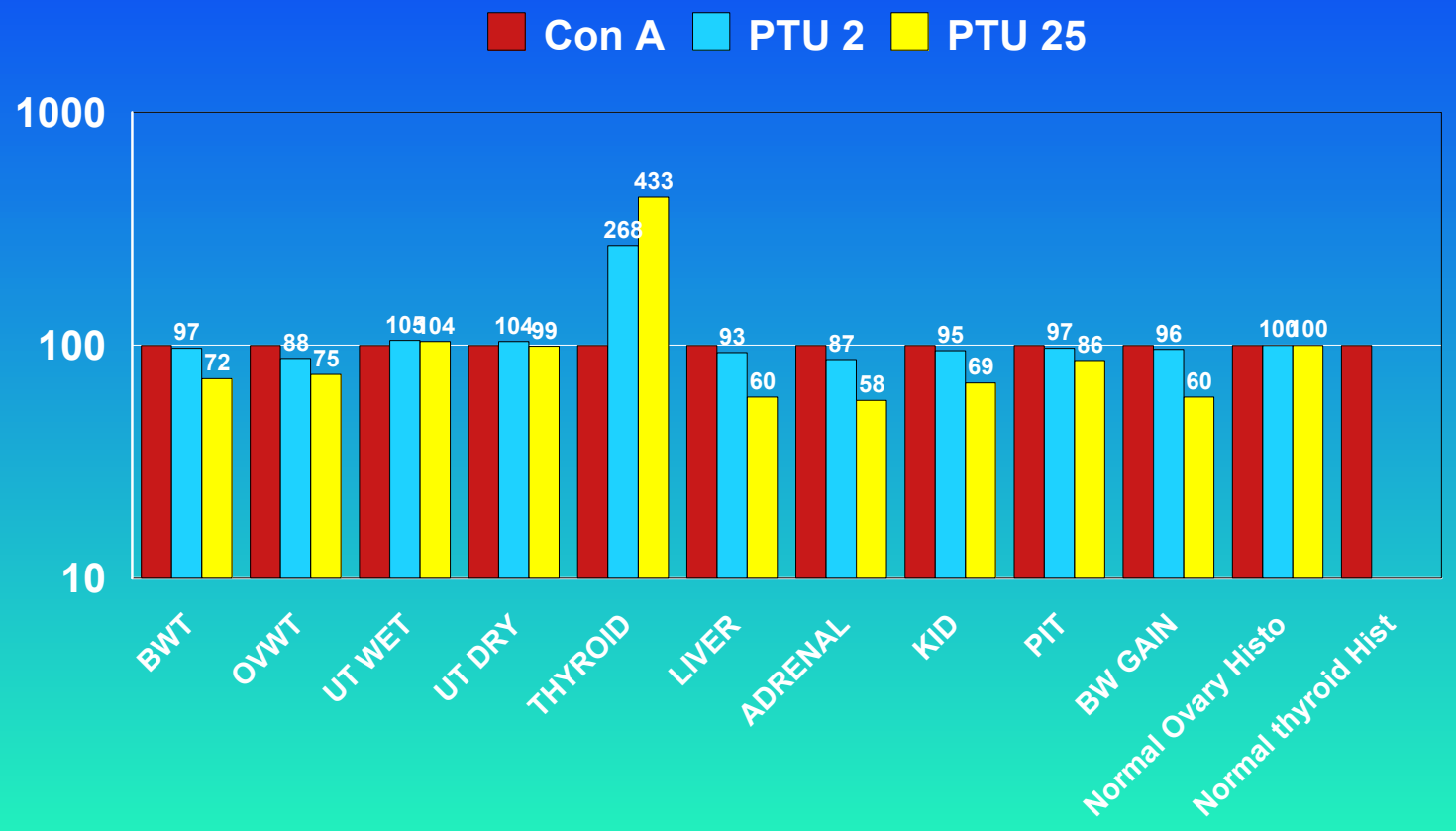
- PTU and the corresponding growth retardation have surprisingly little affect on pubertal landmarks

Effects of PTU in the RTI-SD-2003 Pubertal Female SD rat assay





Effects of PTU in the RTI-SD-2003 Pubertal Female SD rat assay



OBSERVATIONS ON THE PUBERTAL FEMALE ASSAY

- Responds very well to estrogens
- Responds very well to GnRH antagonist and potent aromatase inhibitors
- Responds well to ketoconazole, but effects not the pubertal landmarks
- Reproducible responses to Atrazine
- Reproducible responses to Phenobarbital
- Negative for Fenarimol at doses tested. Need a study at the MTD with doses from dose-range finding, not guessing

EDVMS MEETING
DEC 10-12, 2003

PUBERTAL MALE AND FEMALE RAT ASSAYS

Endocrine-Disrupting Chemicals: Prepubertal Exposures and Effects on Sexual Maturation and Thyroid Function in the Male Rat. A Focus on the EDSTAC Recommendations. Tammy E. Stoker, Louise G. Parks, L. Earl Gray,and Ralph L. Cooper. Critical Reviews in Toxicology, 30(2):197-252 (2000)

Research Protocol for Assessment of Pubertal Development and Thyroid Function in Immature (23- to 53-Day-Old) Male Rats

Purpose and applicability

The purpose of this protocol is to outline procedures to quantify the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile/peripubertal male rat. This assay detects compounds that display antithryoid, estrogenic, androgenic, antiandrogenic [androgen receptor (AR) or steroid enzyme mediated] activity, or alters puberty via changes in follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, growth hormone (GH) or hypothalamic function.

Required endpoints

Growth (body weight)

Age and weight at preputial separation

Serum thyroxin (T4), thyroid histology and TSH

Seminal vesicle plus coagulating gland weight (with and without fluid), Ventral prostate weight, **Levator ani plus bulbocavernosus weight**, Epididymal and testis weights and histology

Optional measures

Serum testosterone, estradiol, LH, prolactin and tri-iodothyronine (T3)

Liver, kidney, adrenal and pituitary weights and histology

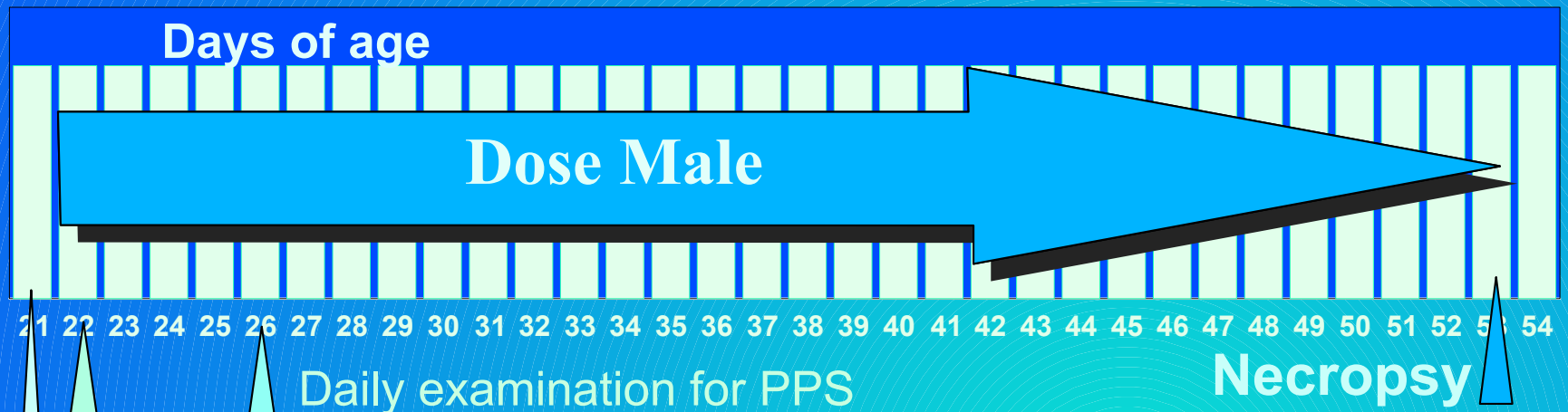
ex vivo testis and pituitary hormone production, Hypothalamic neurotransmitter levels

Alternative Assays for Possible Inclusion in T1S

- *the* ***Pubertal Male*** **assay was an Alternative assay that EDSTAC wanted developed and evaluated for screening**

- **If *standardized and validated* and found to be functionally equivalent, or better, then an alternative assay could be included in T1S.**

Our Immature (21 - 52 Days of Age) Intact Male Rat Protocol to Evaluate Pubertal Development and Thyroid Function. Detects inhibition of steroidogenesis, antithyroid and (anti)androgenic activities and altered HPG maturation.



Assign to Treatments based upon body weight. 15/group

Required endpoints:

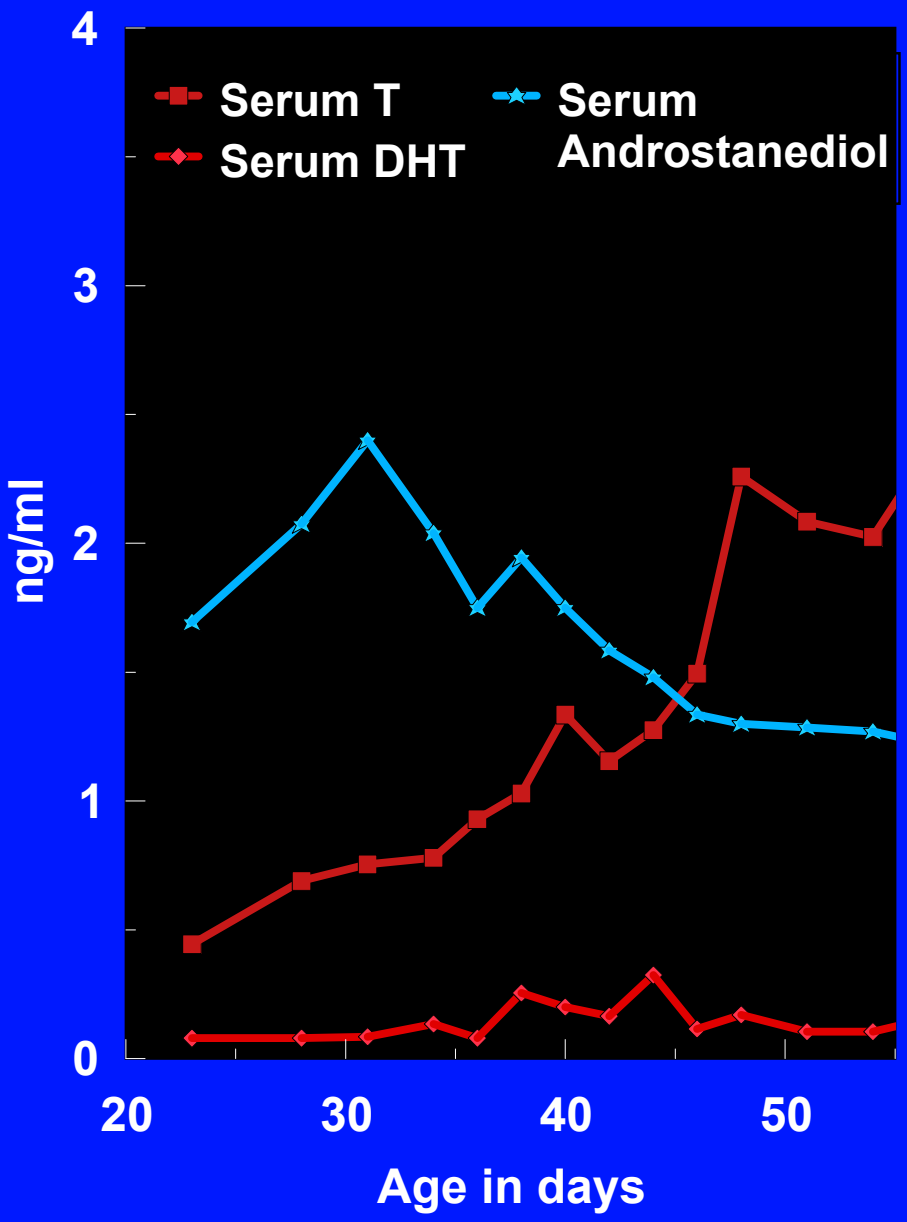
- **Growth**
  - **Age and weight at preputial separation (PPS)**
  - **Serum thyroxine and thyroid-stimulating hormones**
- **Thyroid Histology**
- **Seminal vesicle plus coagulating gland weight (with fluid)**
- **Ventral prostate weight, Levator ani/bulbocavernosus muscle weight**
- **Testis and epididymal weights and histology**
- **Liver, kidney, adrenal and pituitary weights**

Optional endpoints

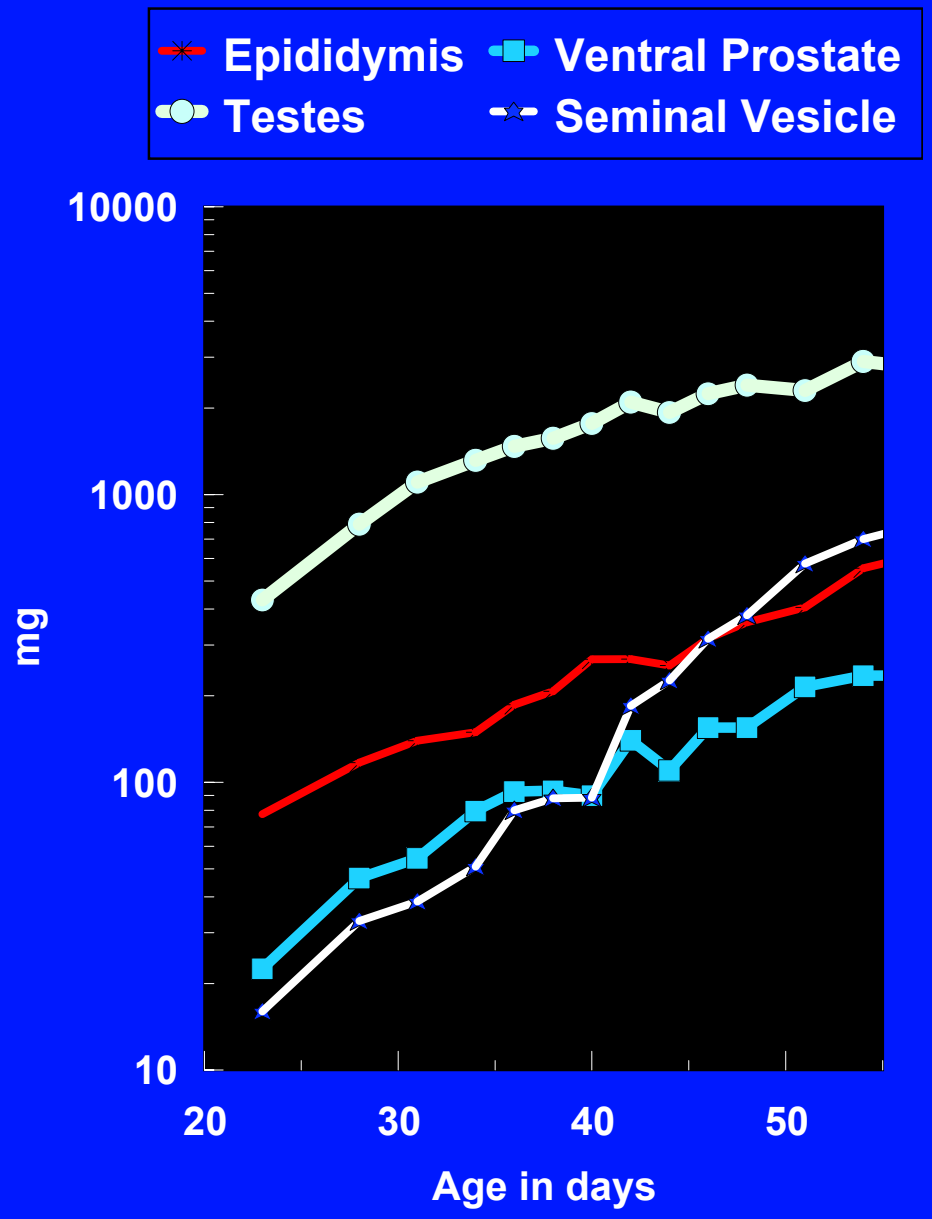
- **Serum hormones and ex vivo testis and pituitary hormone production**

LEGJR

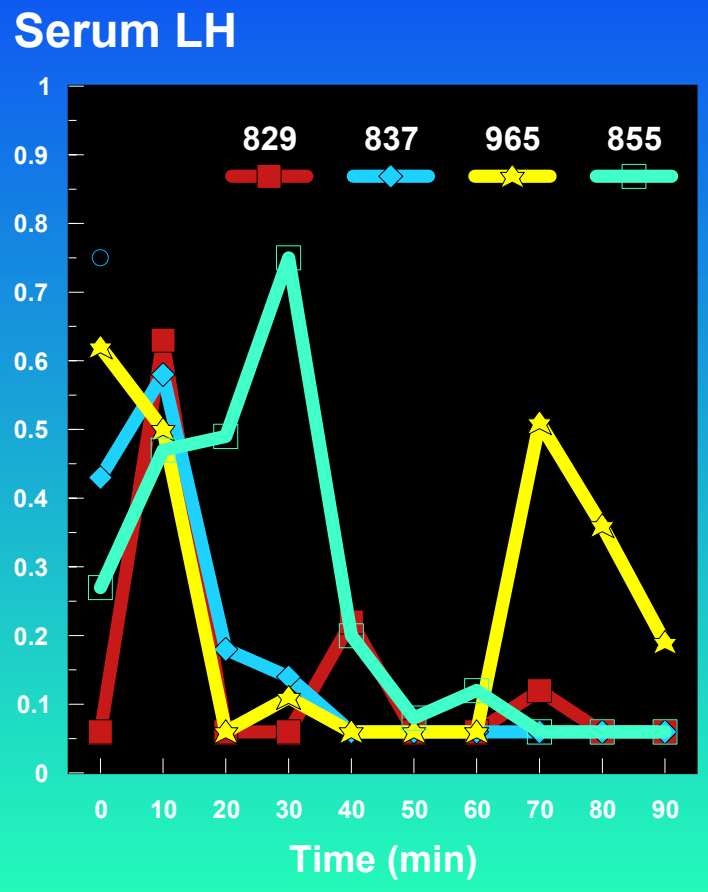
The serum androgen profile changes dramatically during puberty

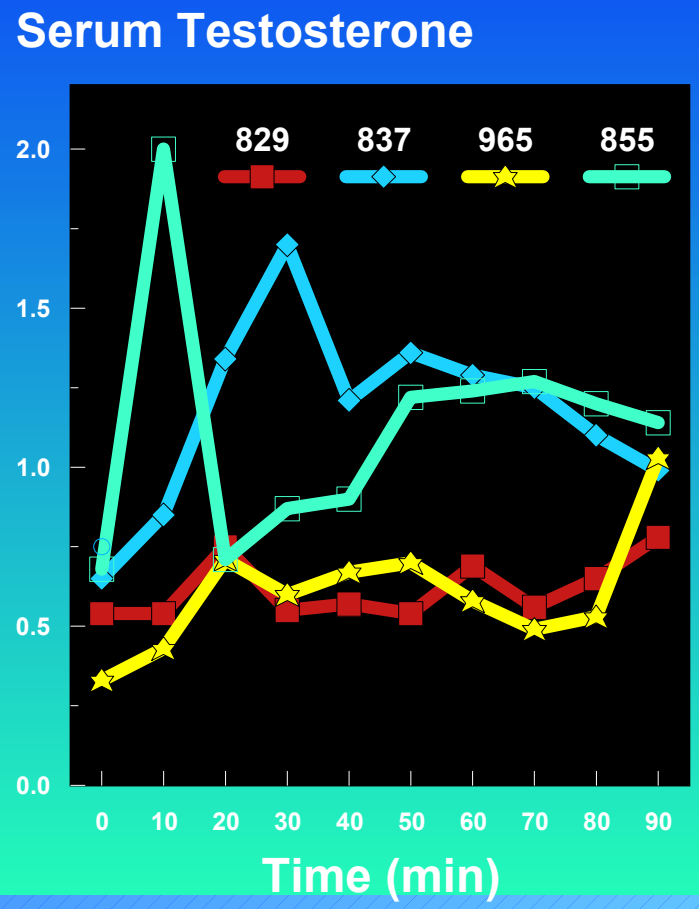


Reproductive organ weights grow rapidly during puberty



Basal Serum LH and Testosterone levels (ng/ml) in Control LE Adult Male rats measured repeatedly at ten minute intervals. Large CVs require large effects of large sample sizes (Fail et al., 1996)





Modified USEPA Endocrine Disrupter Screening and Testing Battery with the *pubertal male* assay replacing the Hershberger assay

- *In Vitro*
  - ER Binding and/or Transcriptional Activation
  - AR Binding and/or Transcriptional Activation
  - Steroid Hormone Synthesis
- *In Vivo*
  - Uterotropic Assay in Ovariectomized Adult Rat (3d)
  - Pubertal Female Rat Assay including Thyroid (20d)
  - ***Pubertal Male Rat Assay including Thyroid (30d)***
  - Frog Metamorphosis Assay
  - Short-term Assessment of the Fish Reproductive System

EDSTAC Screening Battery:

- *Detects Estrogens by sc and oral routes and in vitro*
- *Detects Androgens and Anti- in the pubertal male assay using simple endpoints (PPS and organ weights) and in vitro.*
- *Detects HPG/CNS alterations related to FSH, LH, prolactin, dopamine, GH with simple, precise developmental landmark without RIAs in pubertal female and male.*
- *Detects Inhibition of Steroidogenesis in vivo (in male and female) with simple, precise endpoints and in vitro*
- *Detects Thyroid effects in intact pubertal Male and Female Rat using RIAs and Frog Metamorphosis*
- *Uses extensively utlized in vivo endpoints/assays*

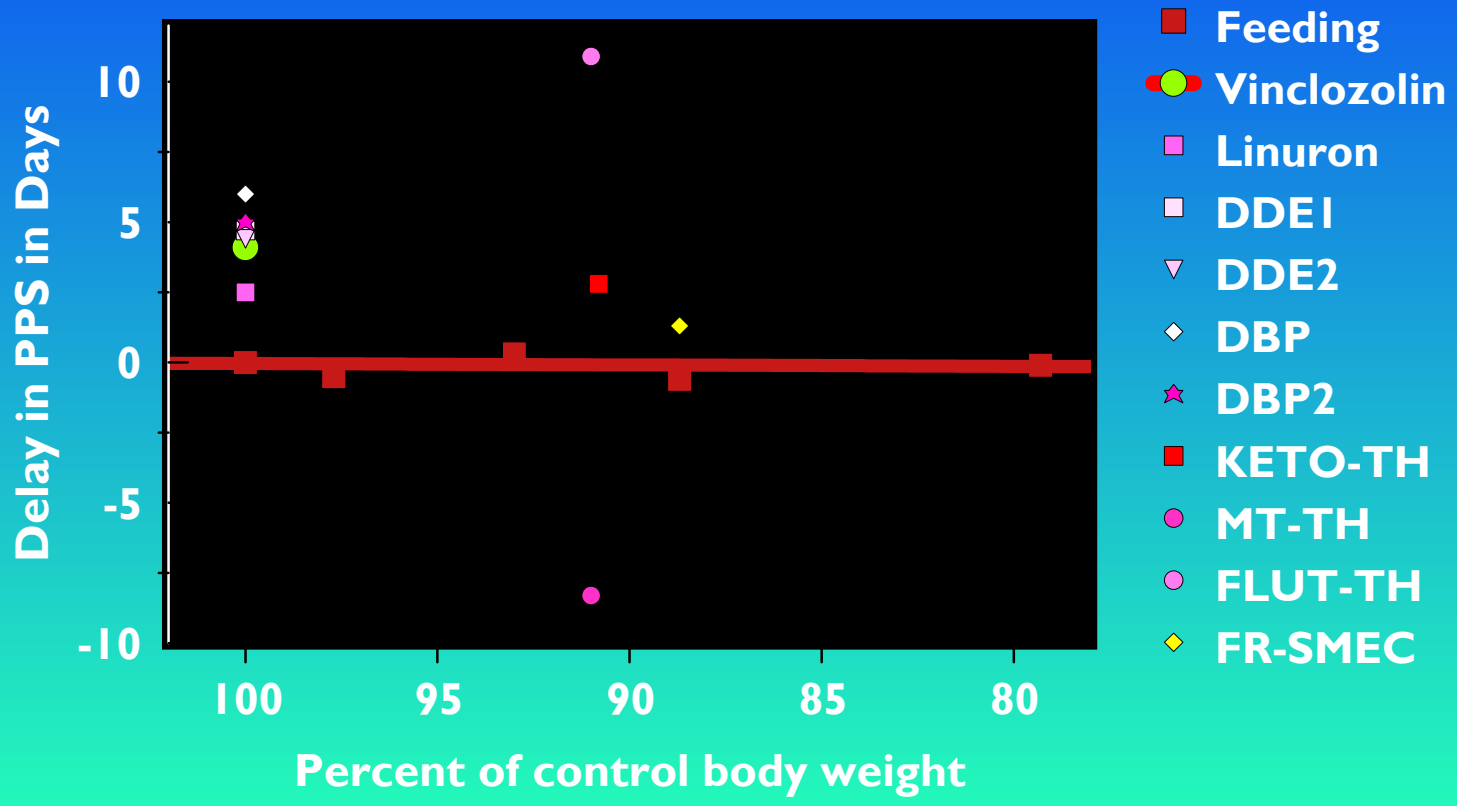
Chemicals investigated in the pubertal male assay at RTI and TI this year

- RTI - completed
  - Atrazine 75 and 150 mg/kg/d
  - p,p' DDE 50 and 100 mg/kg/d
  - Vinclozolin 30 and 100 mg/kg/d
  - Methoxychlor 25 and 50 mg/kg/d
  - PTU 2 and 25 mg/kg/d
  - Ketoconazole 50 and 100 mg/kg/d
  - Linuron 50 and 100 mg/kg/d
  - Phenobarbital 50 and 100 mg/kg/d
- TherImmune 2000 (2x2x6 factorial. Blocks, LE versus SD and chemicals, n=6/strain/block/chemical) - completed
  - Flutamide 50 mg/kg/d
  - Methyl Testosterone 80 mg/kg/d
  - PTU 240 mg/kg/d
  - Ketoconazole 100 mg/kg/d
  - Pimozide 30 mg/kg/d
  - DBP 1000 mg/kg/d
- TherImmune 2003 - completed
  - Phenobarbital 25, 50 and 100 mg/kg/d
  - Vinclozolin 10, 30 and 100 mg/kg/d
  - Flutamide 25 and 50 mg/kg/d

Published studies using the pubertal male assay

- p,p' DDE - several labs
- Vinclozolin - 2 labs
- Linuron - PPS
- Cyproterone acetate
- Flutamide - 3 labs
- Finasteride - 2 labs
- Ketoconazole - 2 labs
- Testolactone
- TP
- BBP
- DBP - 2 labs
- Methoxychlor - 2 labs
- BPA
- DES
- PTU
- Perchlorate - in prep
- PBDE - submitted
- Phenobarbital
- Fenitrothion
- MBC - PPS
- Atrazine - 2 labs

Relationship between reduction in body weight due to restricted feeding versus and delay in age at PPS

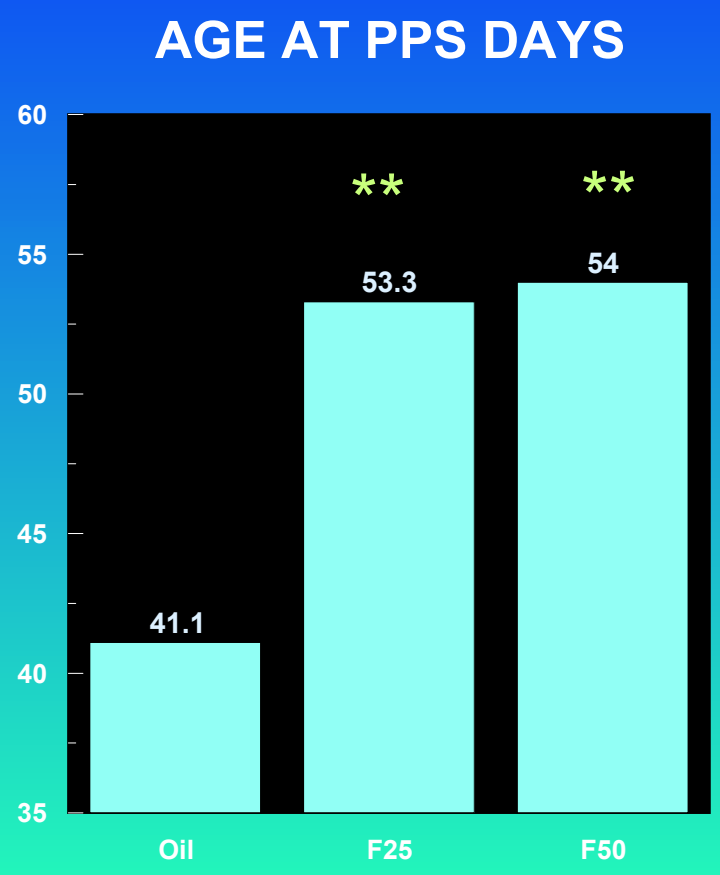


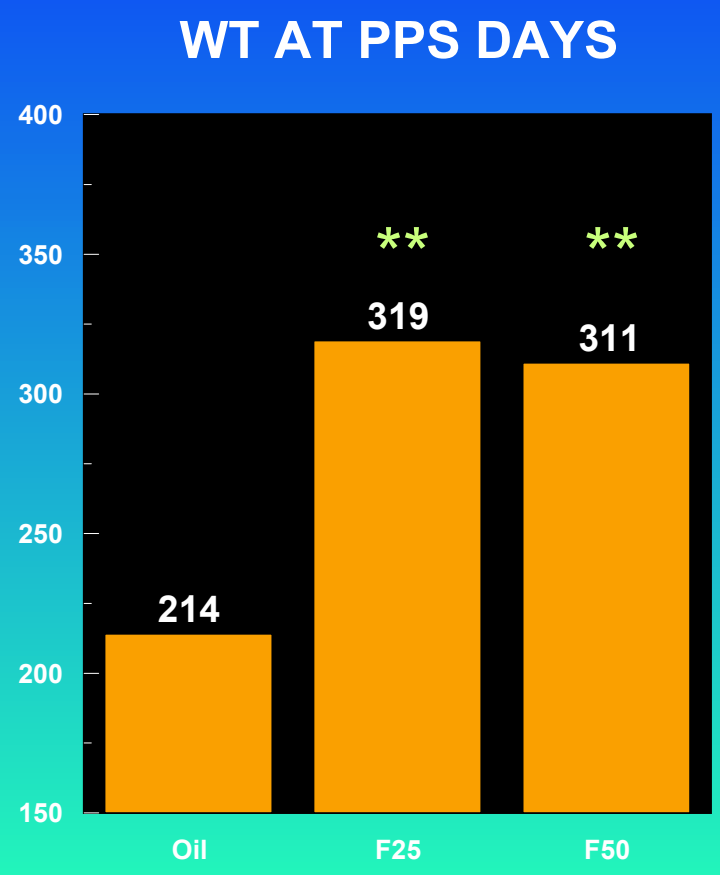
AR antagonists

- Flutamide
- Vinclozolin
- p,p' DDE
- Linuron

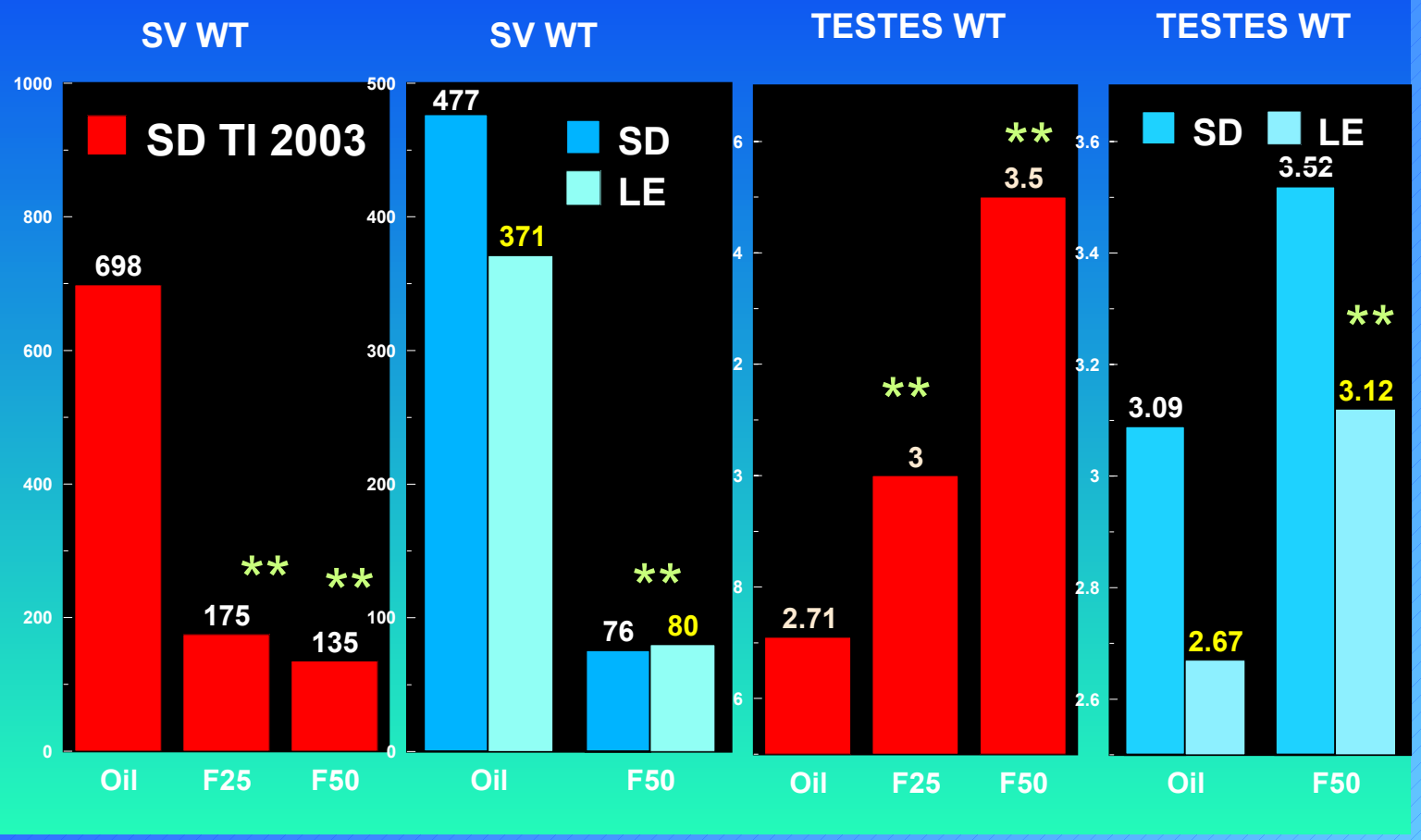
- Results -
  - all chemicals produced expected robust responses, easily detected all of the above.
  - Reproducible

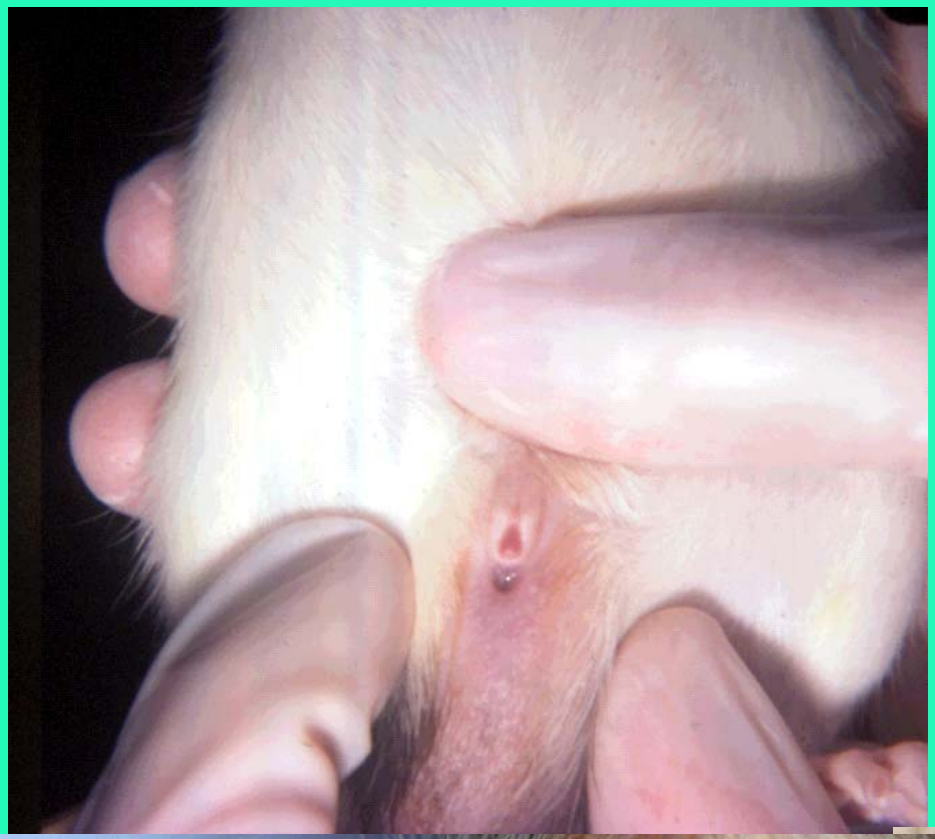
Effects of flutamide at 25 and 50 mg/kg on puberty in the male rat TI-SD-2003

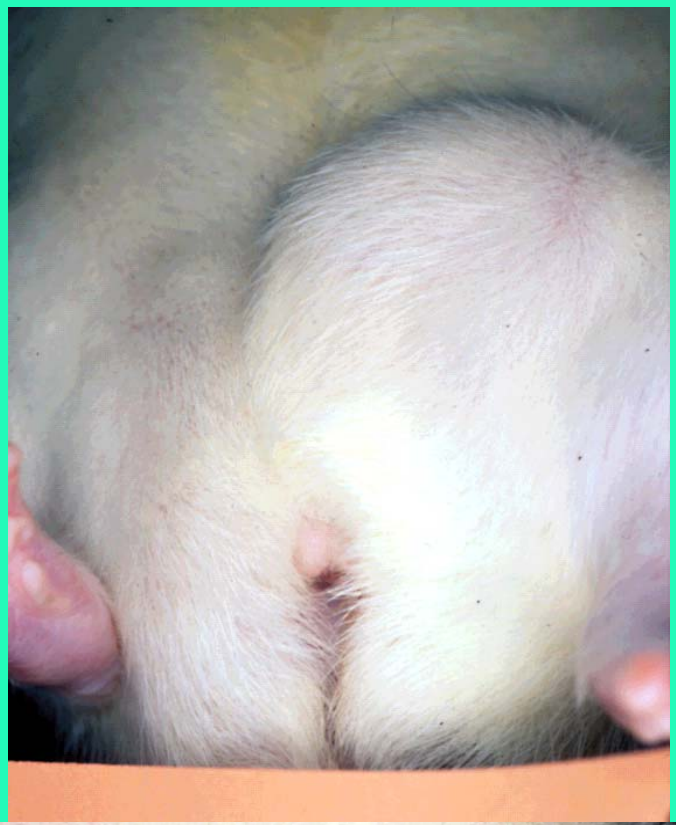


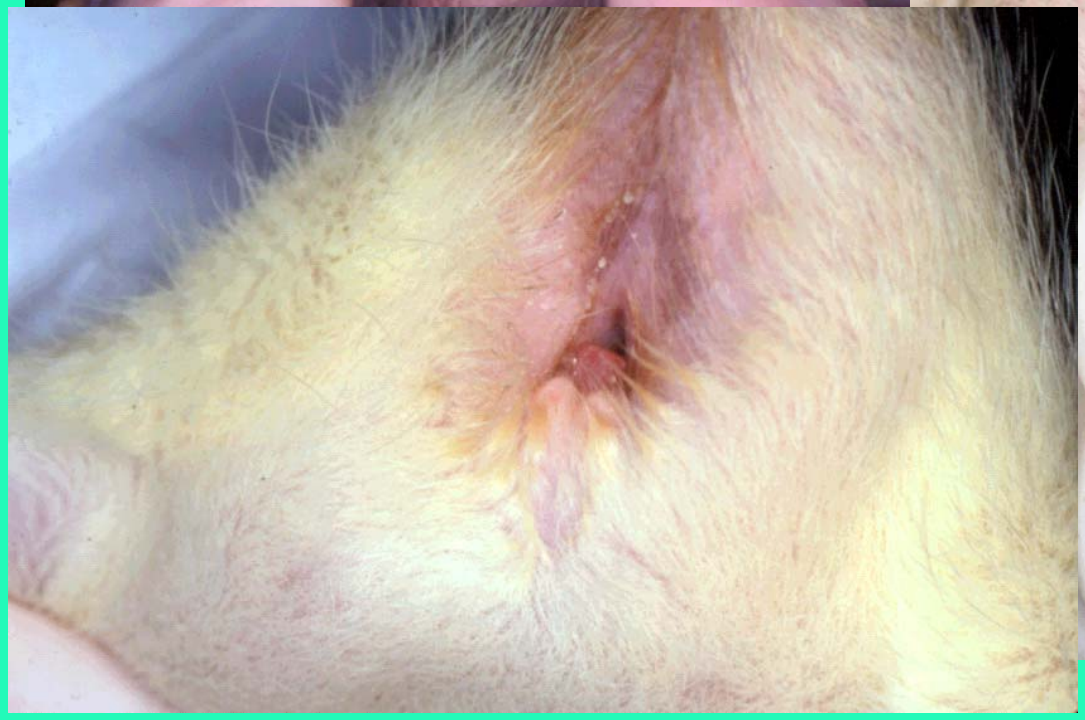


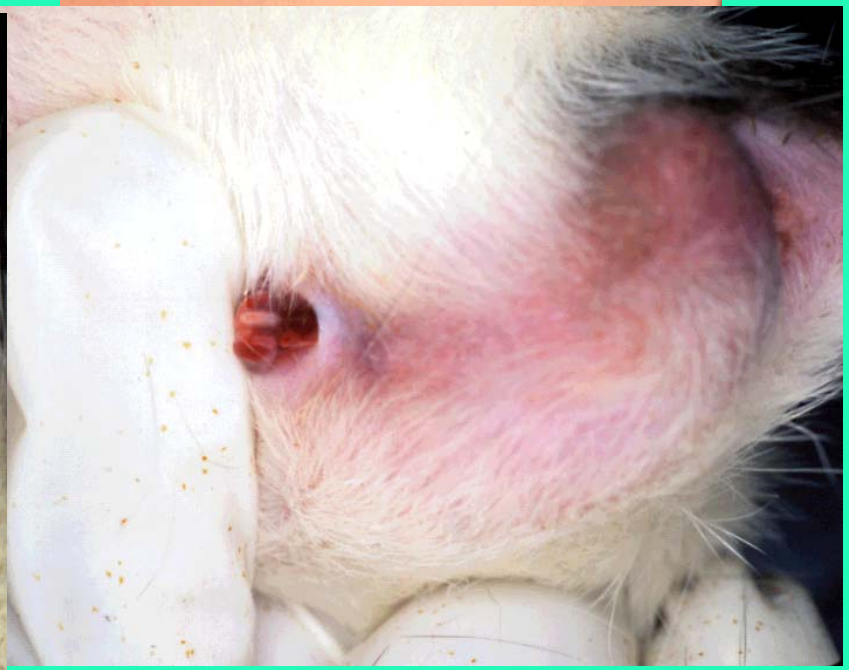
Effects of flutamide at 25 and 50 mg/kg In the male rat TI-SD-2003 (RED) and TI-SD/LE-2000



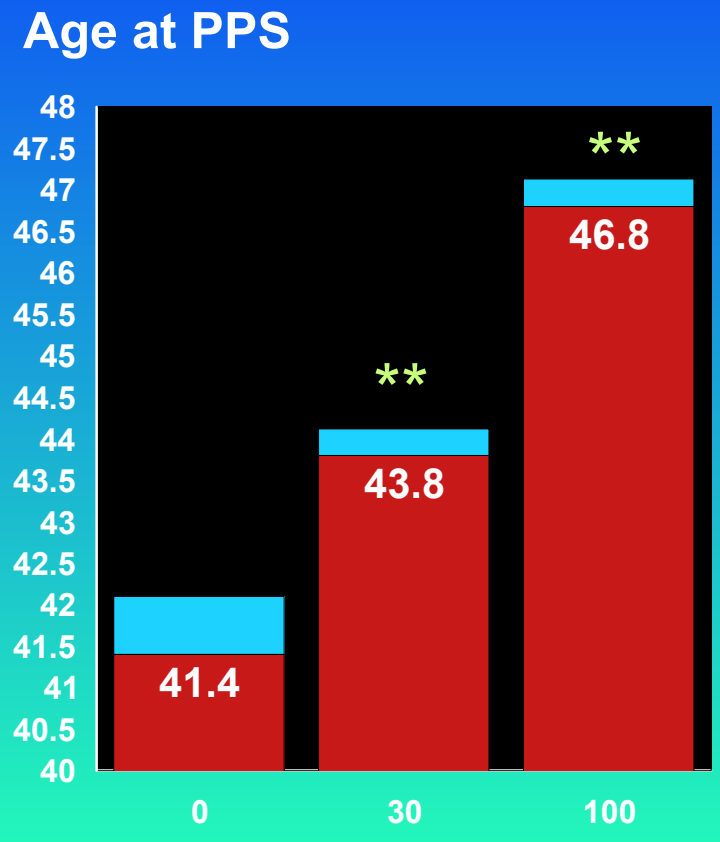


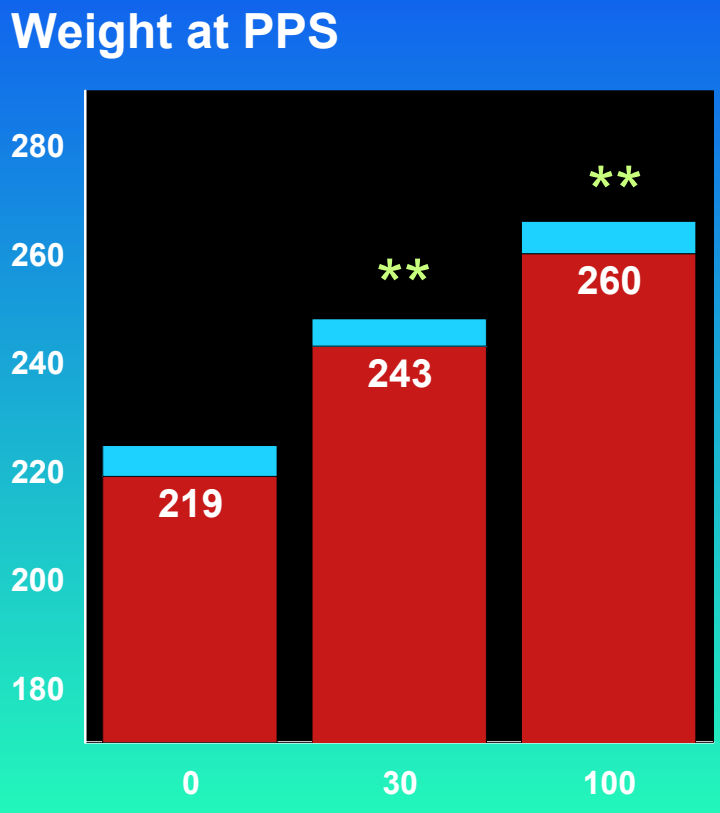




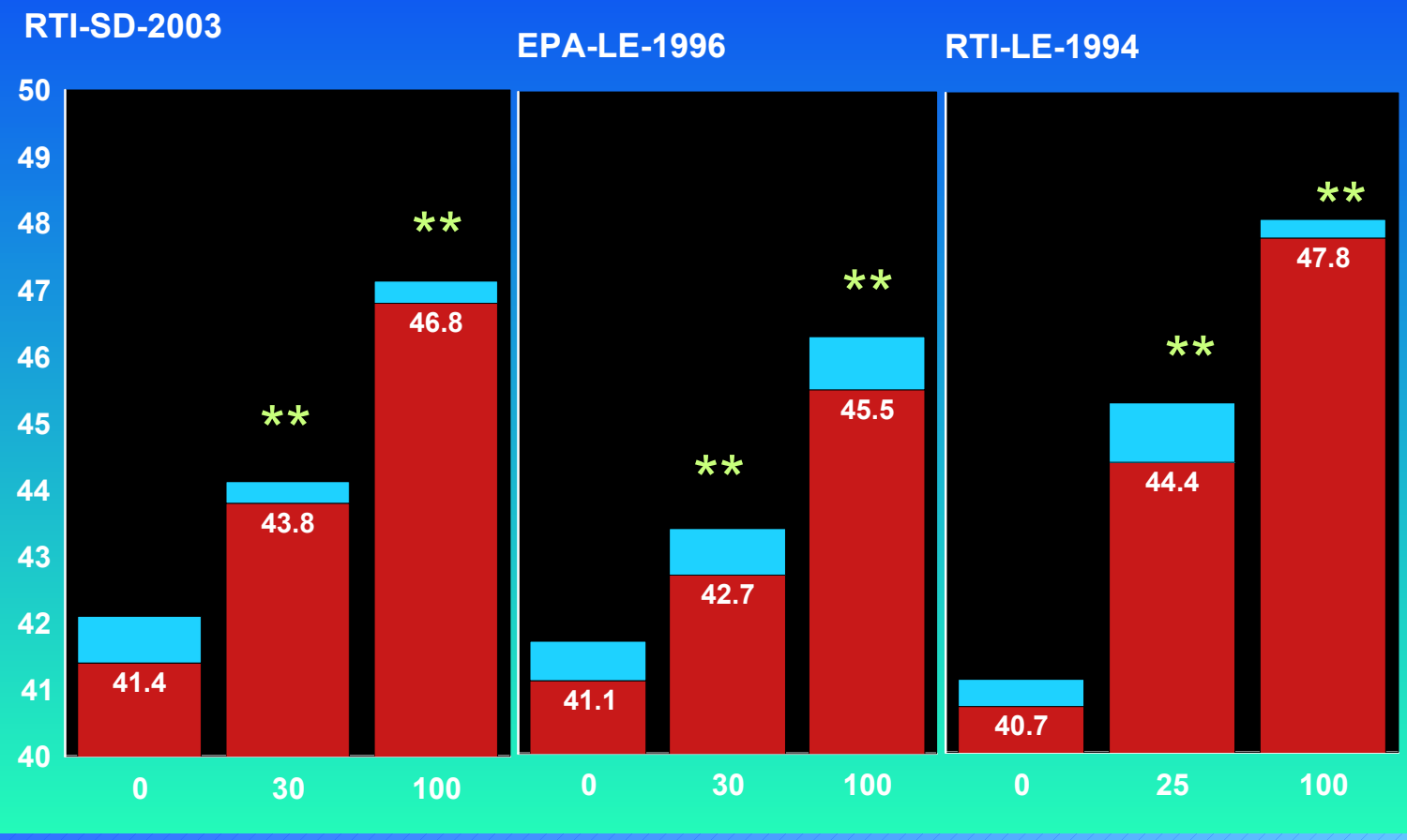


Effects of Vinclozolin in male pubertal SD rat assay-RTI 2003

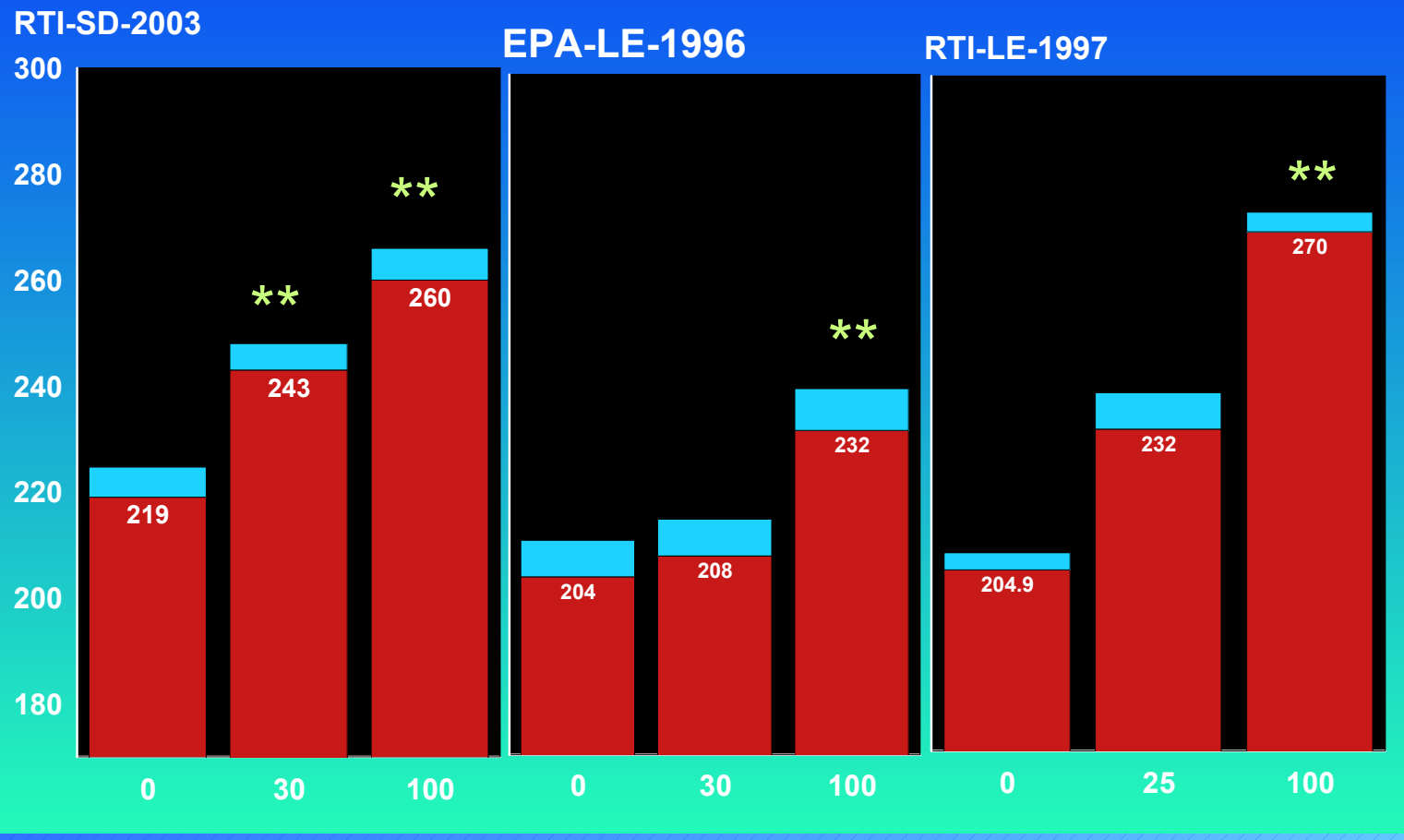




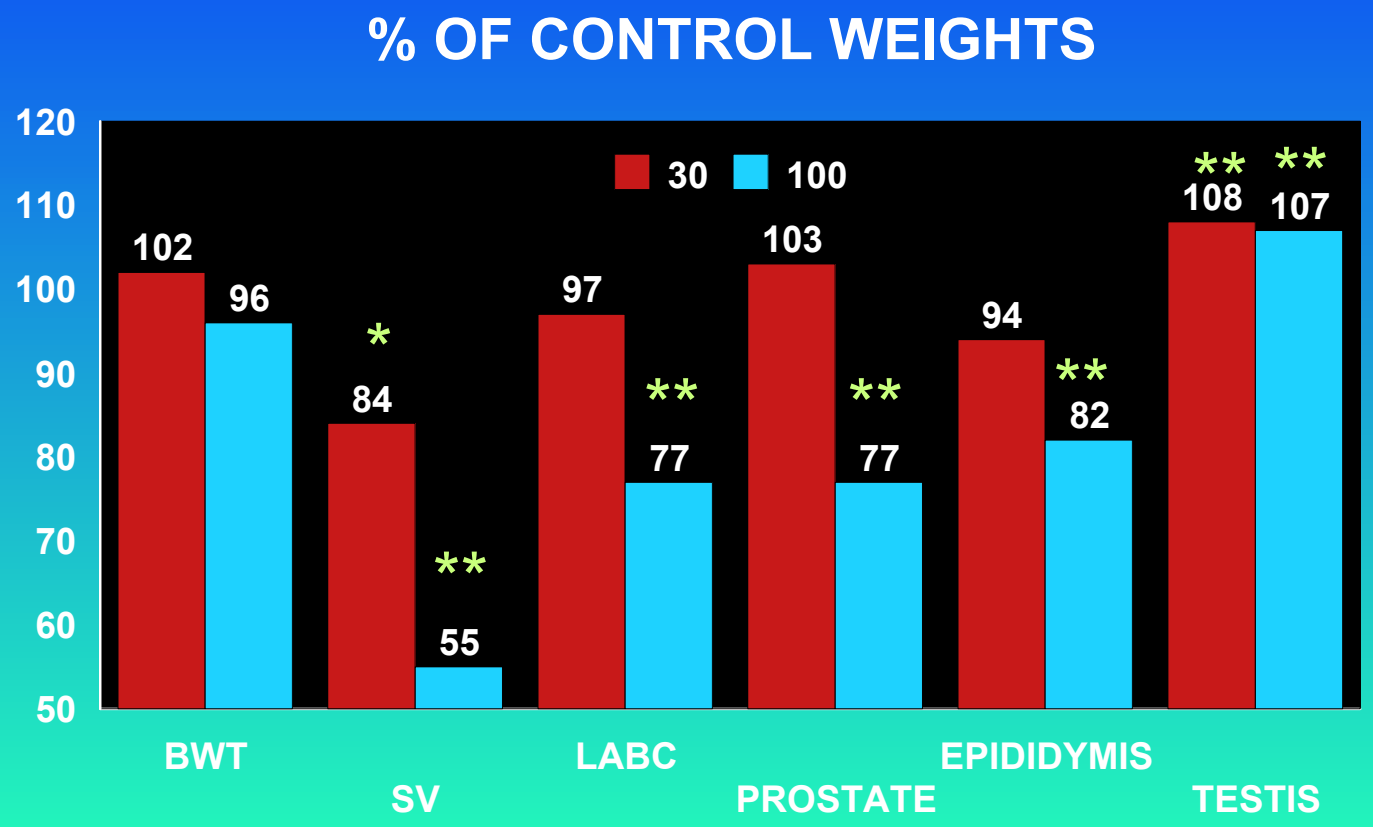
Effects of Vinclozolin on AGE AT PPS in male SD and LE rats



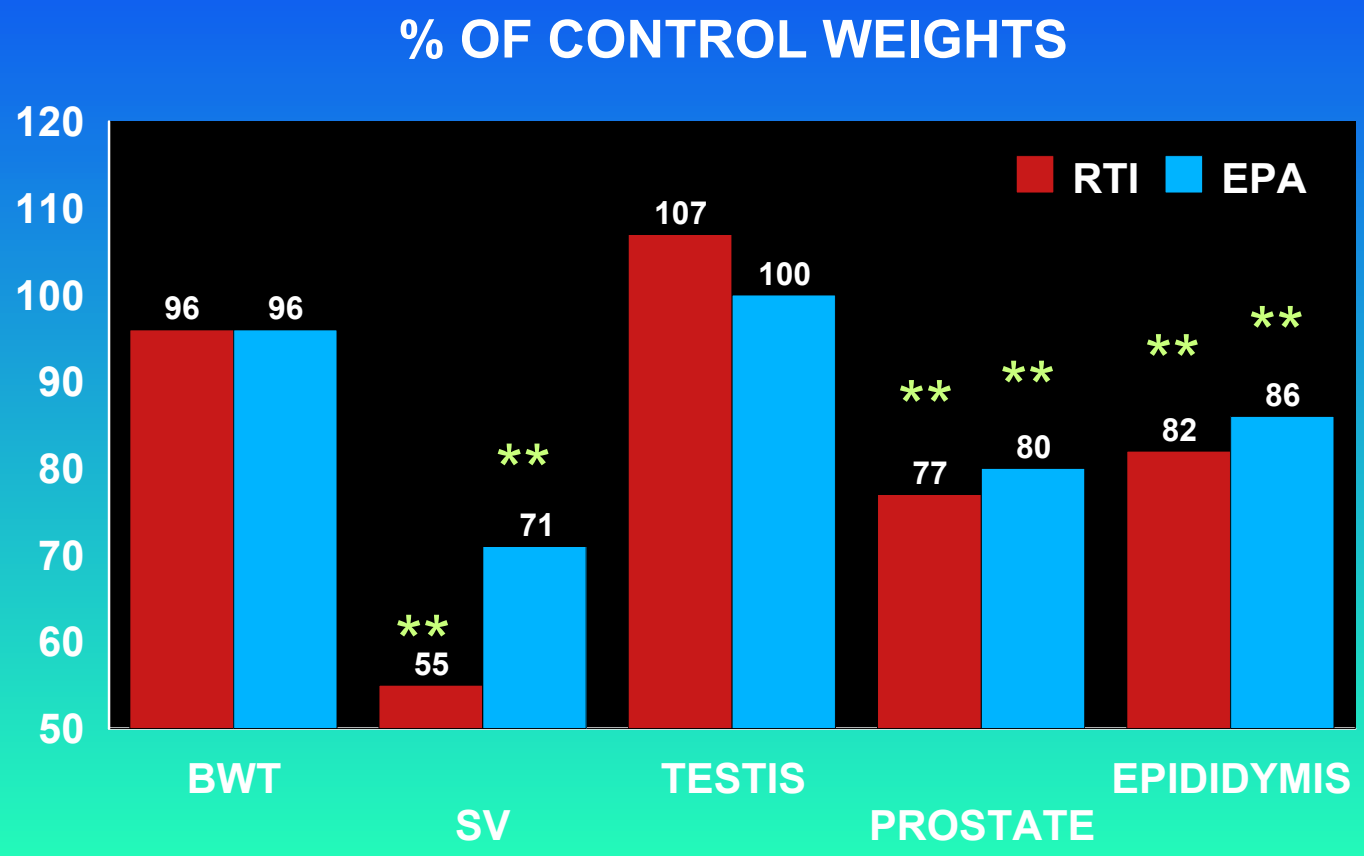
Effects of Vinclozolin in Weight at PPS in male LE and SD rats



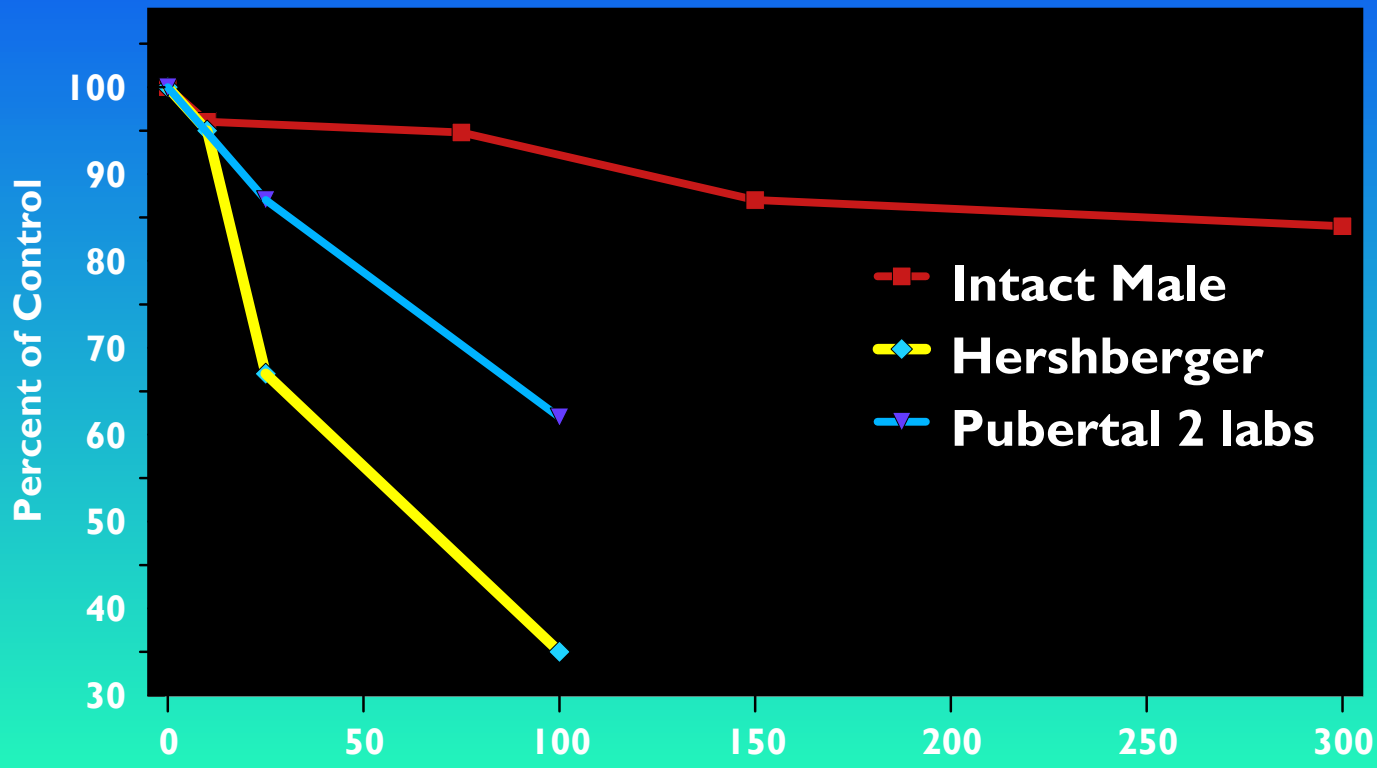
Effects of Vinclozolin in male RTI 2003 pubertal rat assay



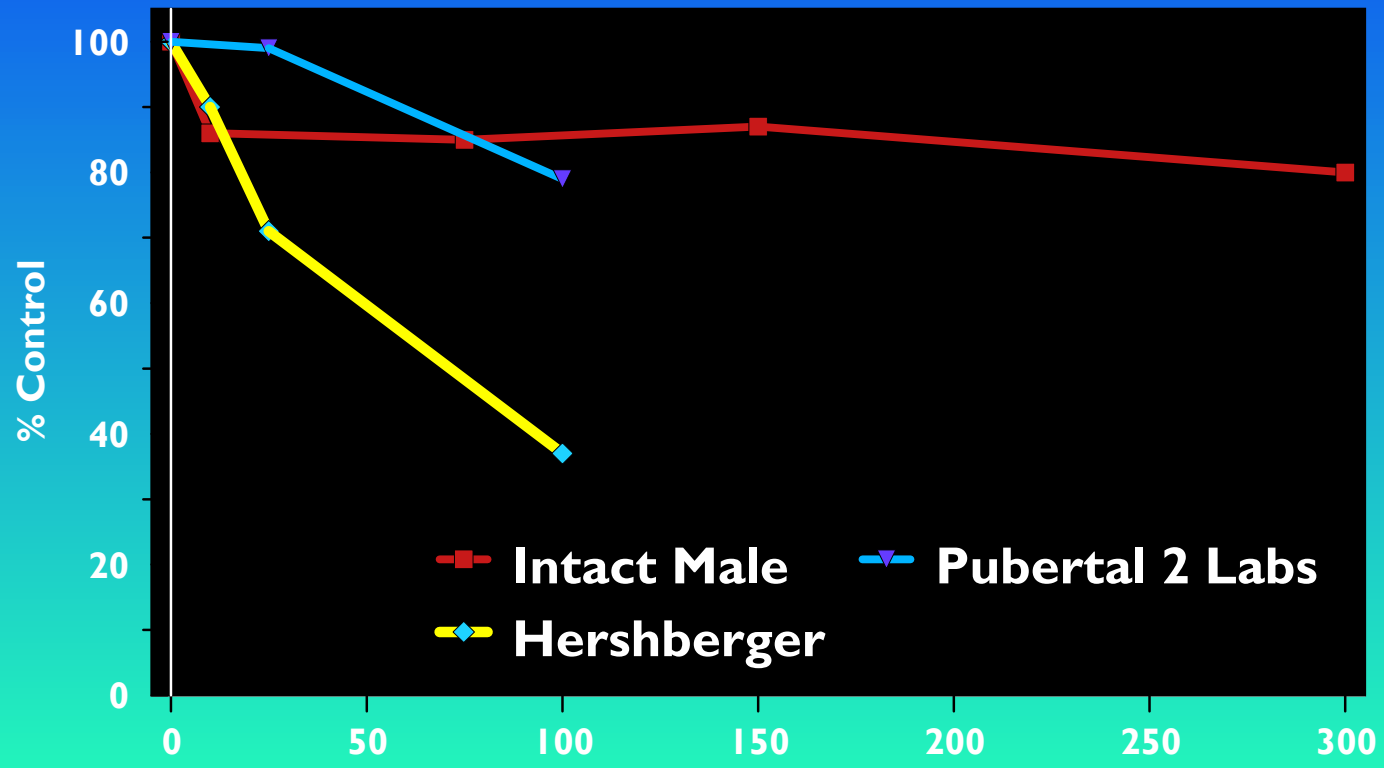
Effects of Vinclozolin on male reproductive organ weights in the RTI-SD-2003 study compared to EPA-LE-1996 pubertal rat assay at 100 mg/kg/d



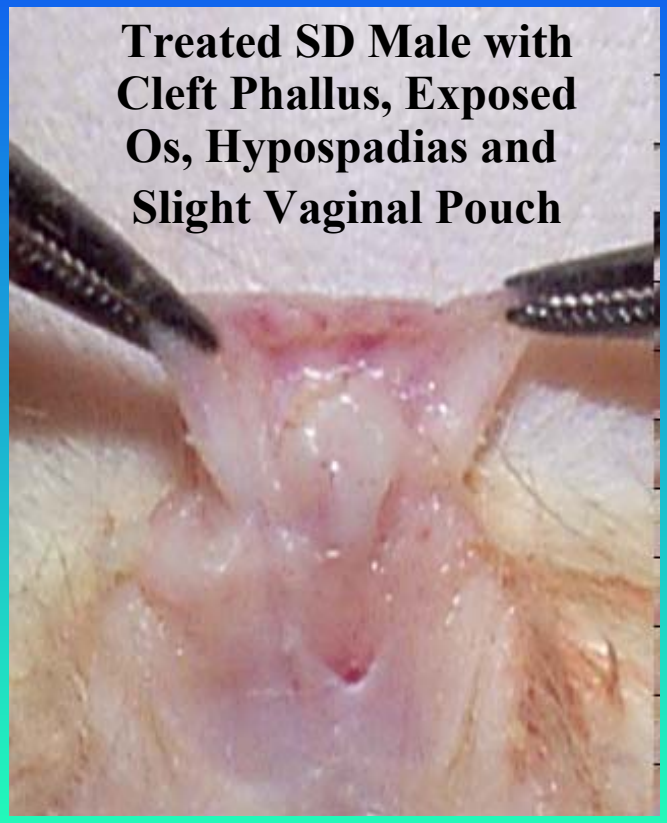
The intact male assay is much less sensitive to the effects of vinclozolin on ASO than are seminal vesicle weights in our Hershberger or pubertal male assays.



The Adult Intact Male assay is much less sensitive to the Effects of vinclozolin on ventral prostate weight. Males treated with TP in the Hershberger Assay.



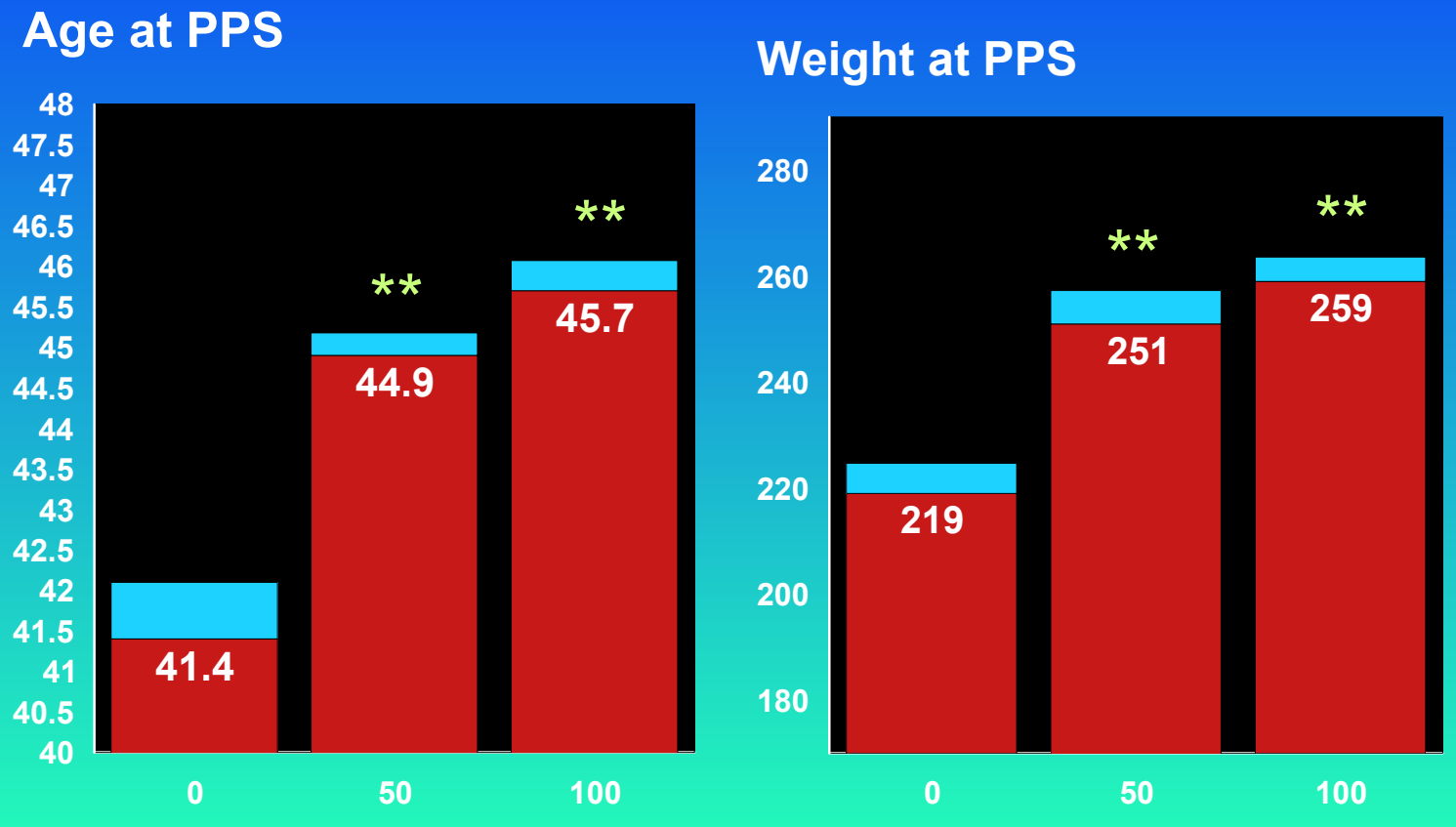
It is critical that the T1 Screening assay be able to detect weak antiandrogens like DDE and linuron because they cause reproductive tract malformation in utero. **Exposure to 100 mg p,p' DDE/kg-dam's body weight on gestational days 14-18 alters development of the External Genitalia. Gray et al, 1999.**



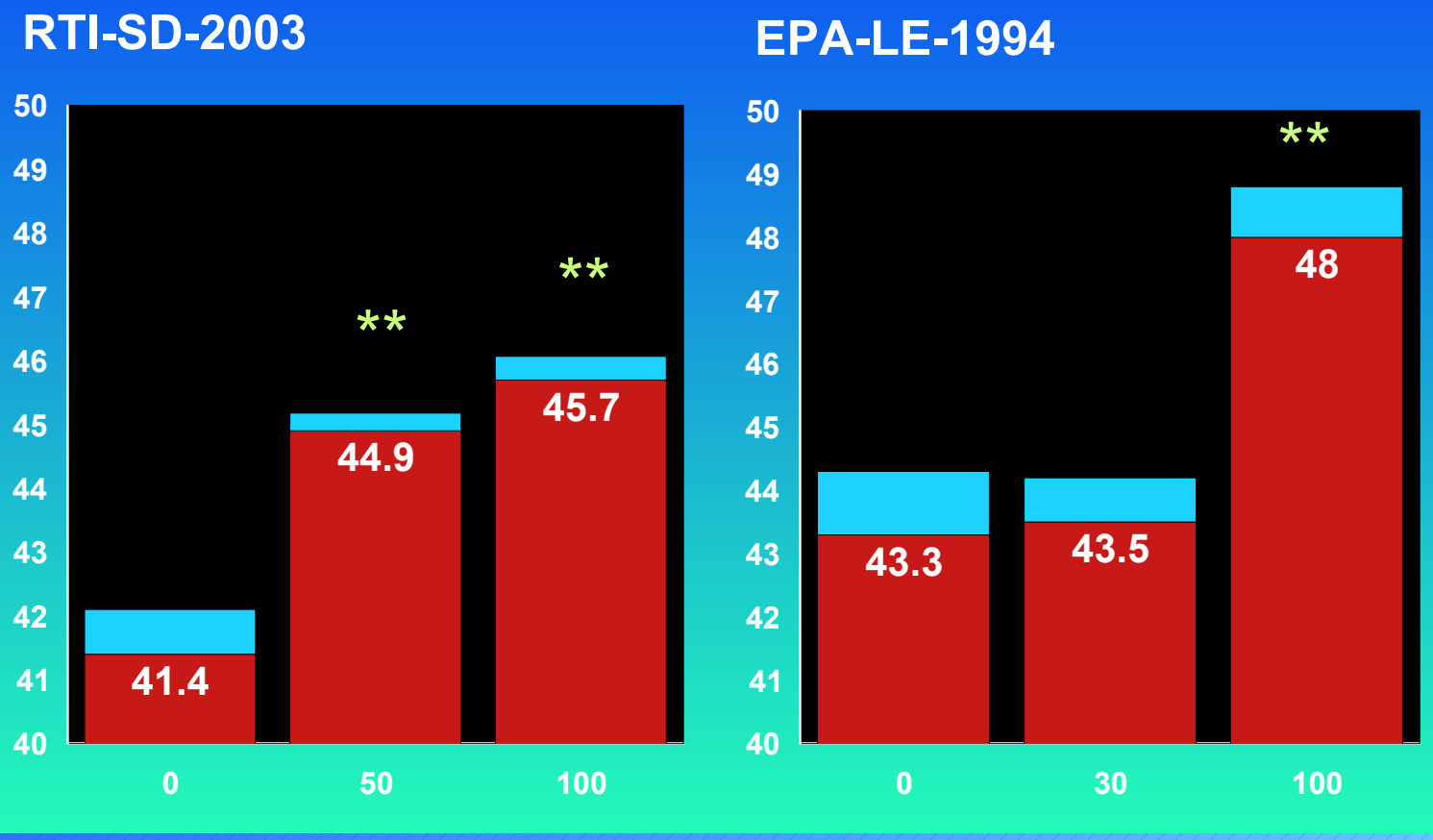
Effects of p,p' DDT and p,p' DDE treatment during sexual differentiation in Dutch Belted rabbits. (Veeramachaneni et al., 1996; 2000; Palmer et al., 2000)

- Does exposed weekly to p,p' DDT at 25 mg/kg from GD 1 through 6 weeks postpartum, followed by 10 mg/kg directly to male up to 12 weeks of age.
- Effects
  - Cryptorchidism
  - Atypical germ cells in undescended testes inclduing
  - Carcinoma-in-situ like cells
- At 8 and 12 weeks
  - serum p,p' DDT at 231 and 38 ppb
  - serum p,p' DDE at 187 and 37 ppb

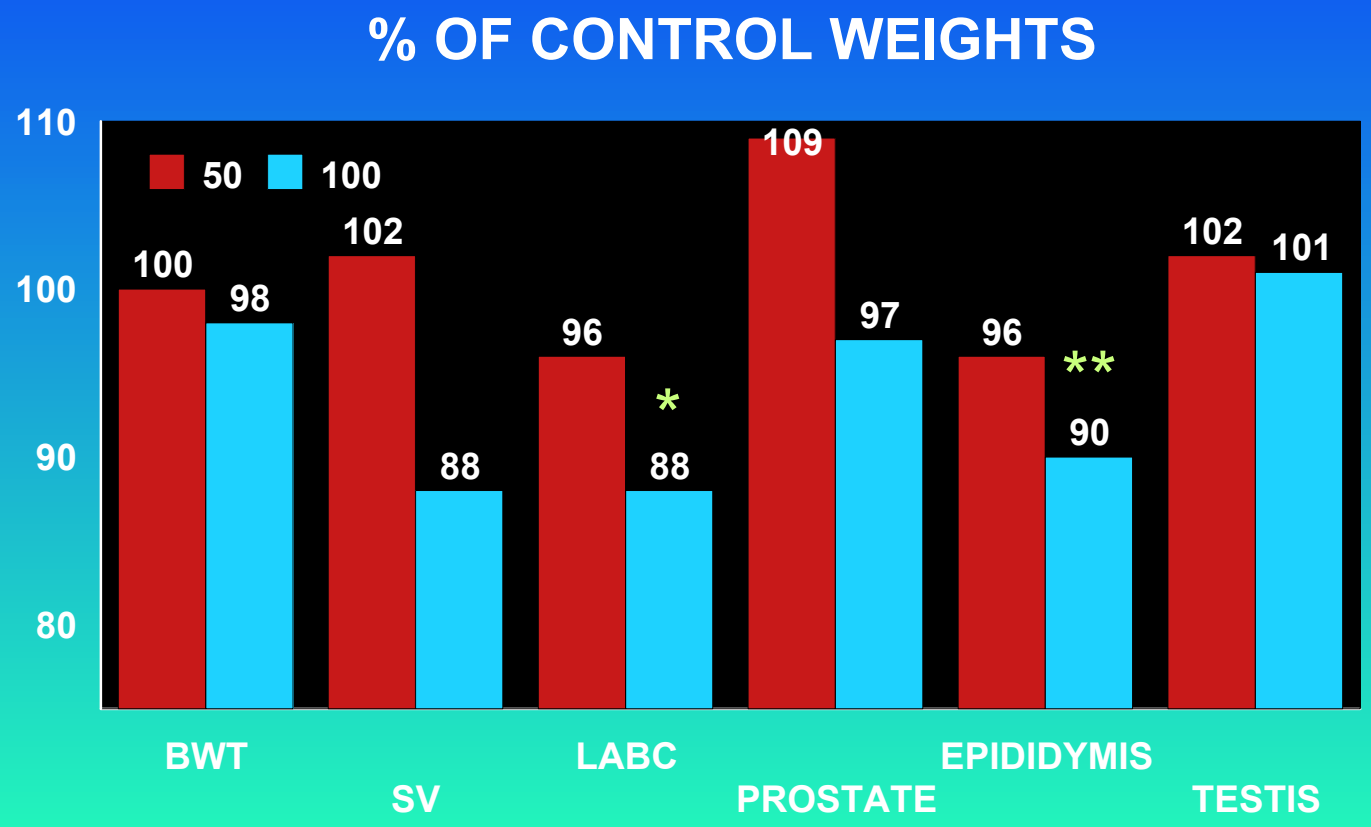
Effects of p,p' DDE in male pubertal rat assay RTI-SD-2003



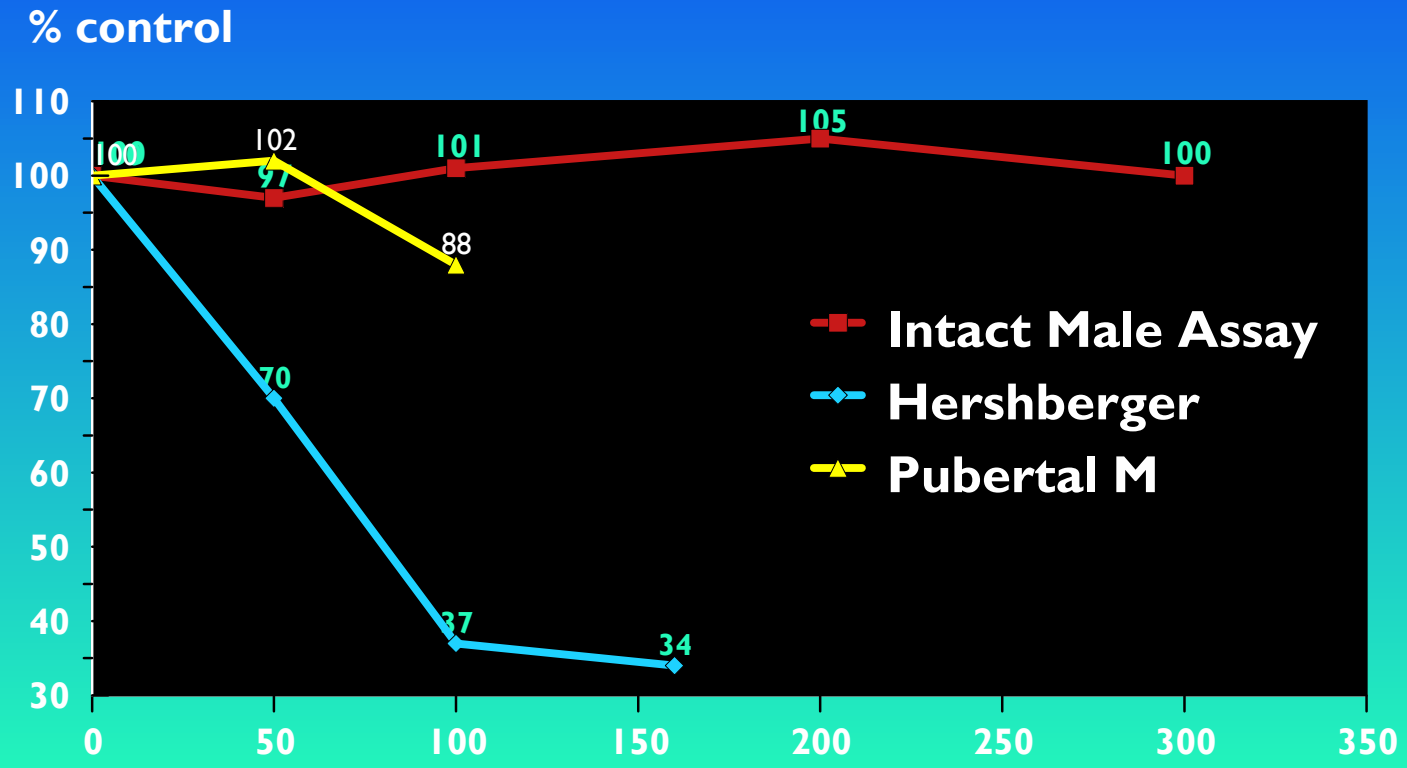
Effects of p,p' DDE in male pubertal rat assay



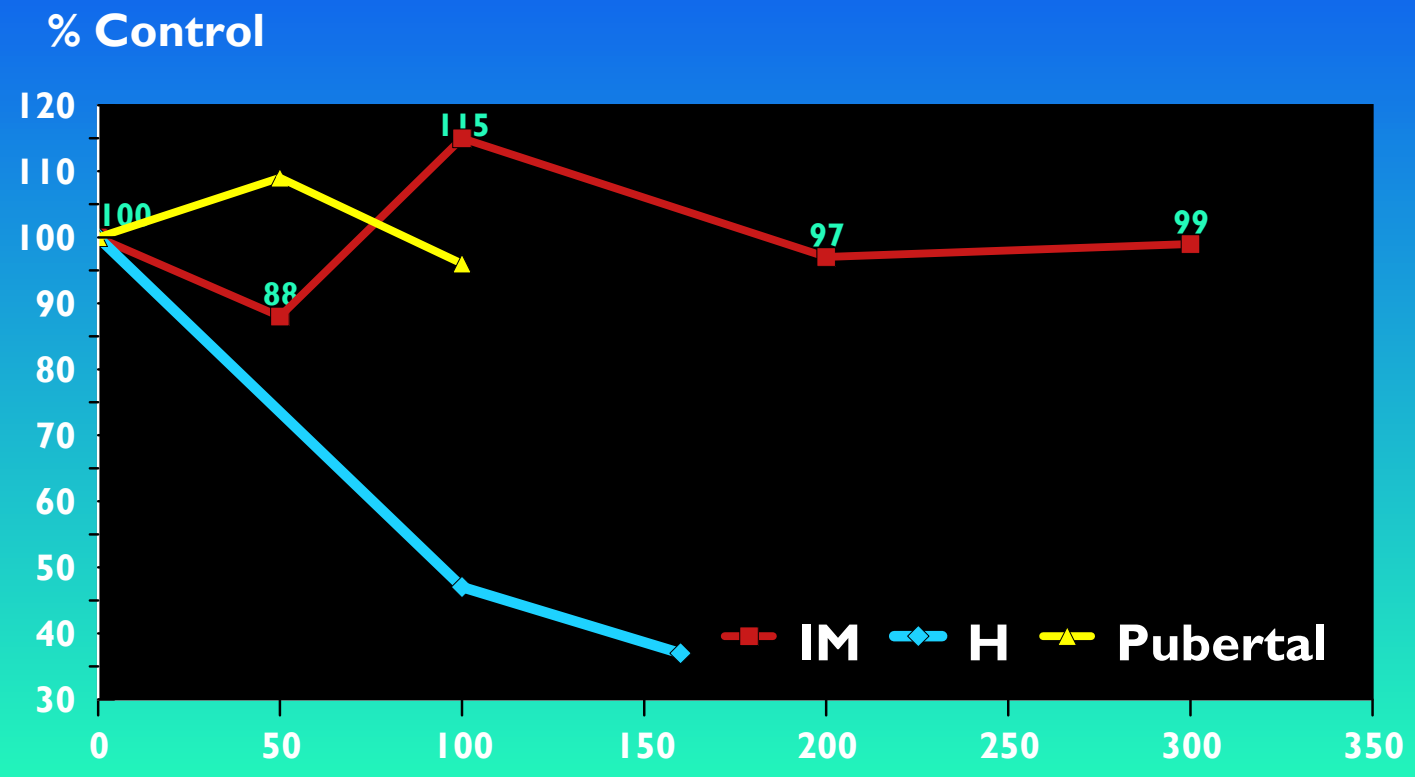
Effects of p,p' DDE in male pubertal rat assay RTI-SD-2003

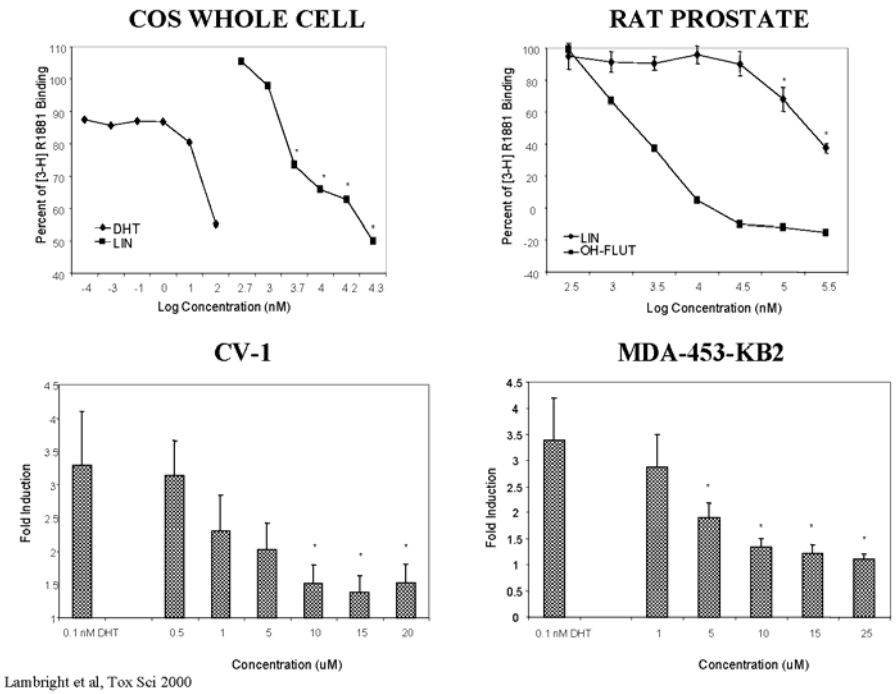


The Adult intact male assay is insensitive to the Effects of p,p' DDE at 100 mg/kg/d on SV versus ASO weight. Males treated with TP in the Hershberger Assay.



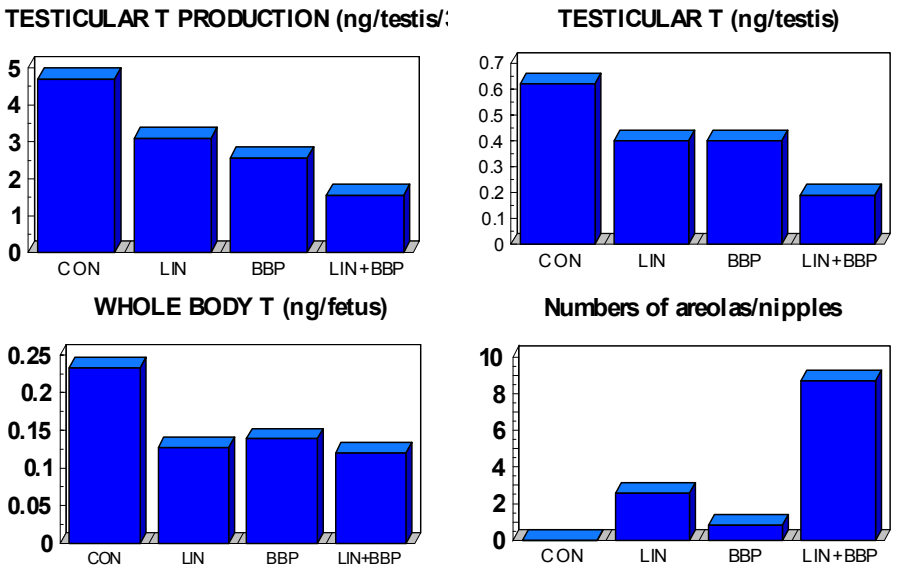
The intact male assay is insensitive to the Effects of p,p' DDE at 100 mg/kg/d on ventral prostate weight. Males treated with TP in the Hershberger Assay.





Lambright et al, Tox Sci 2000

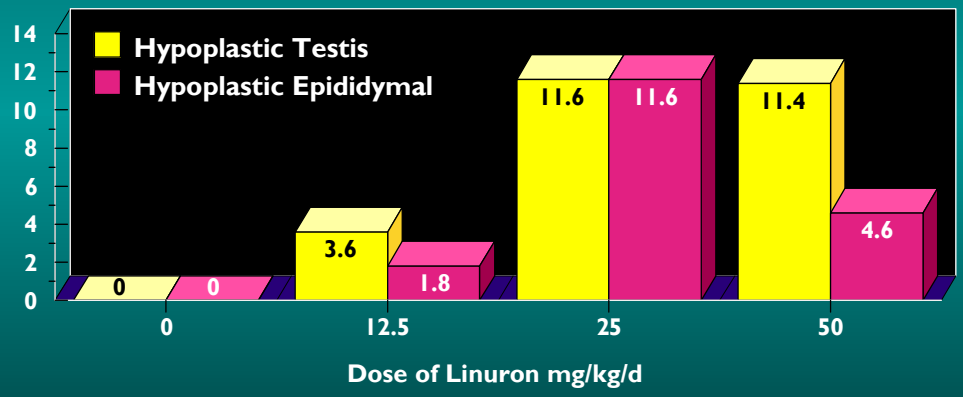
Effects of gestational day 14-18 treatment with linuron (75 mg/kg/d) and/or benzyl butyl phthalate (BBP) on fetal male rat testicular testosterone production in 3 h, fetal testis and whole body testosterone levels, and nipple/areola induction. All values differ significantly from control (Hotchkiss in prep)



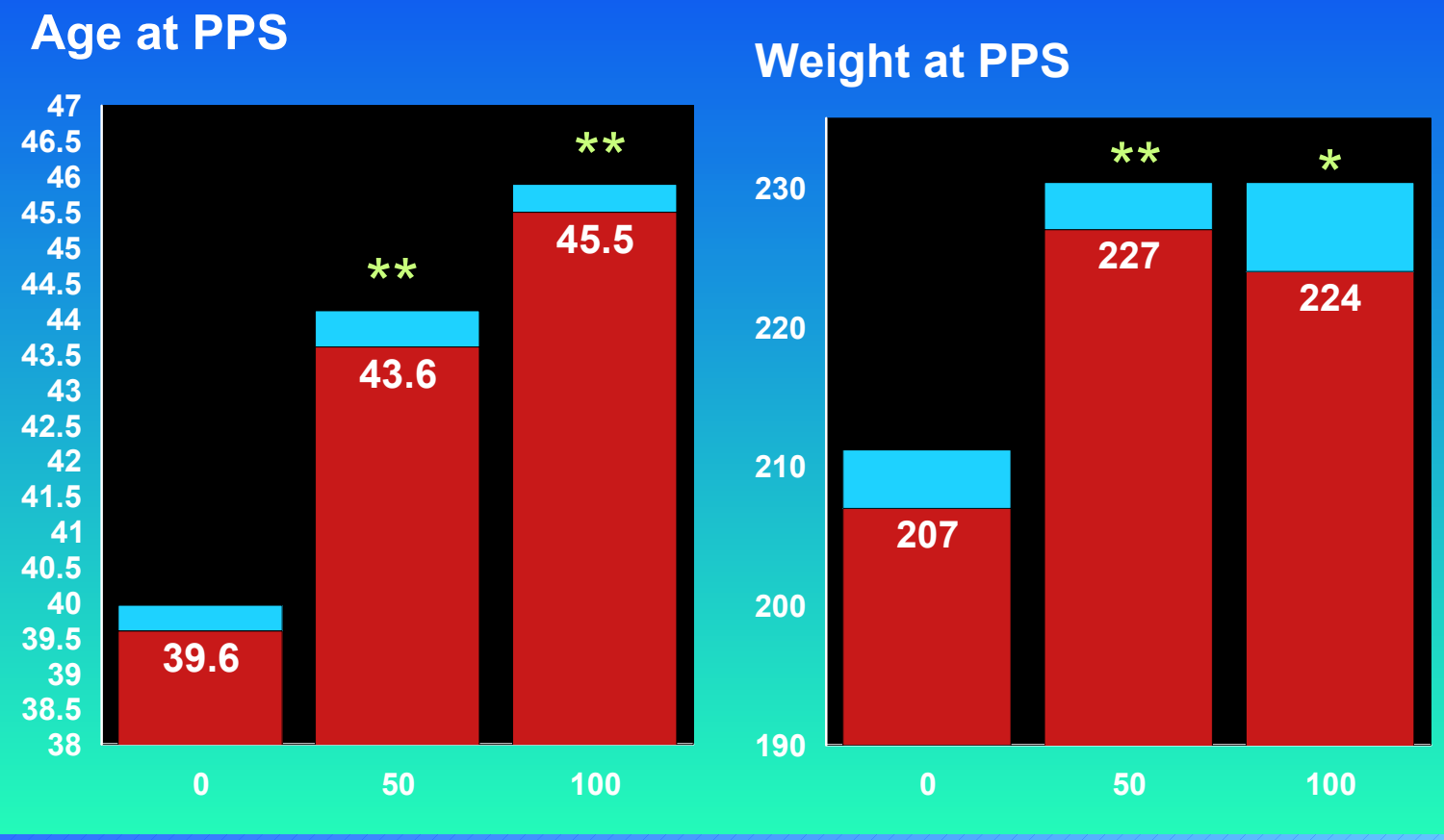
100 mg Linuron/kg/d Produces Epididymal Agenesis and Fluid-Filled Testes in F1 Males Exposed *In Utero* from Gestational Days 14 - 18

Low-dose Effects of Linuron on Sexual Differentiation of the male rat (McIntyre et al, 2000, Toxicol Appl Pharm 167, 87-99). Dams were dosed with linuron at 12.5, 25 or 50 mg/kg/d from GD 12 to 21. No direct exposure to pups. This pesticide is negative in published teratology and multigenerational tests. Could the current tests detect these malformations in only 20 F1 males/group?

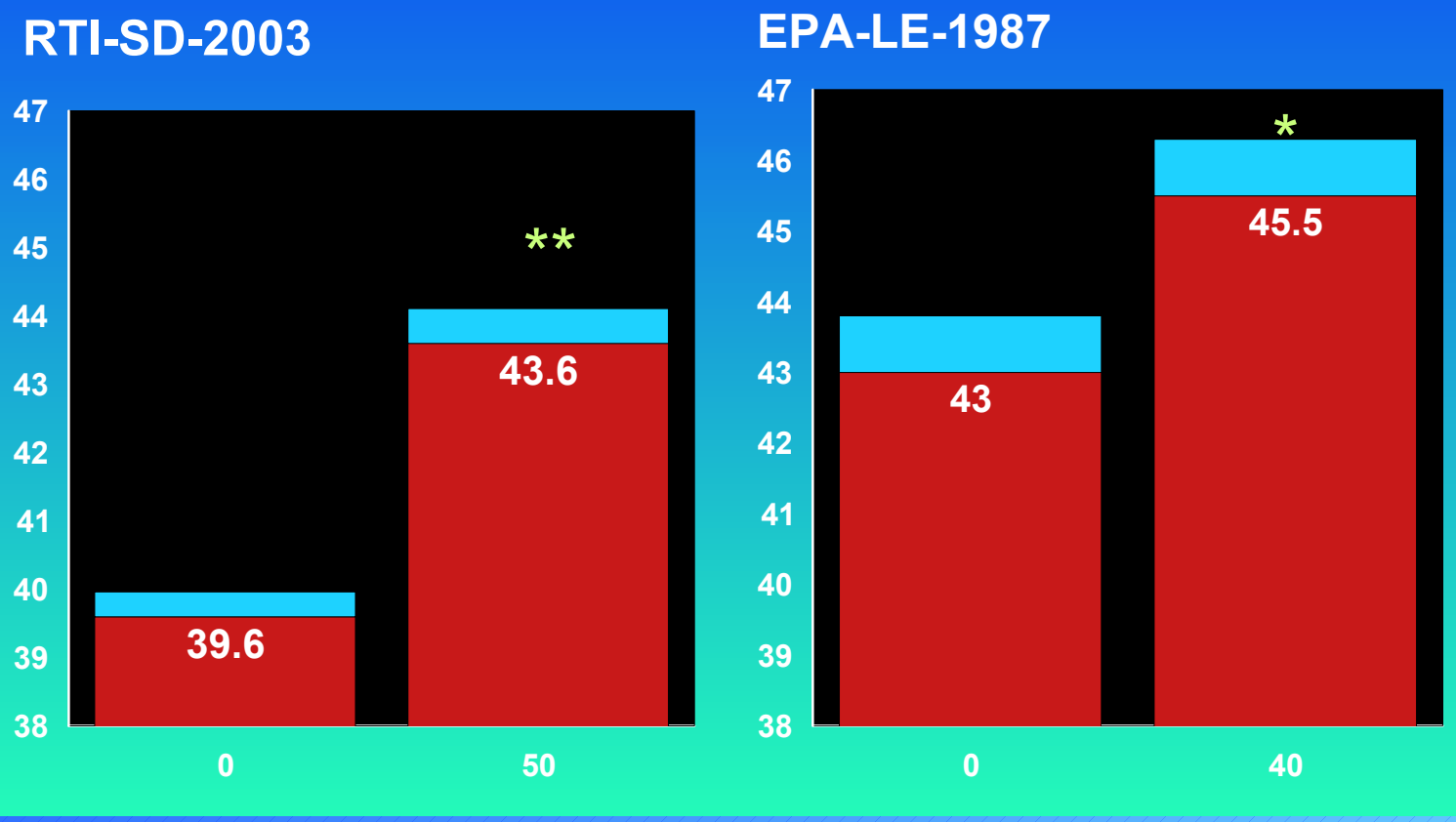
Percent of Male Offspring Malformed



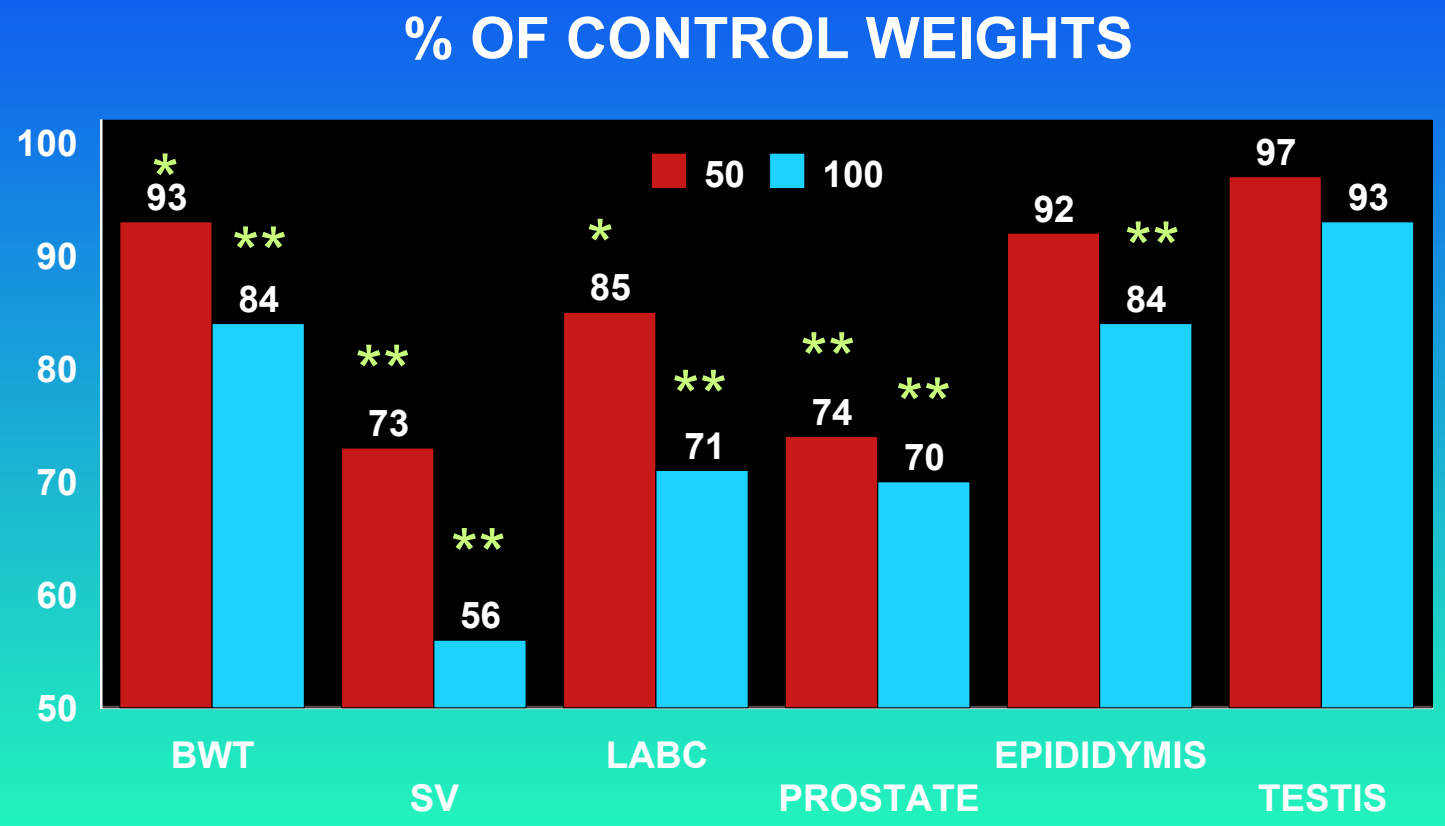
Effects of Linuron in male pubertal rat assay RTI-SD-2003



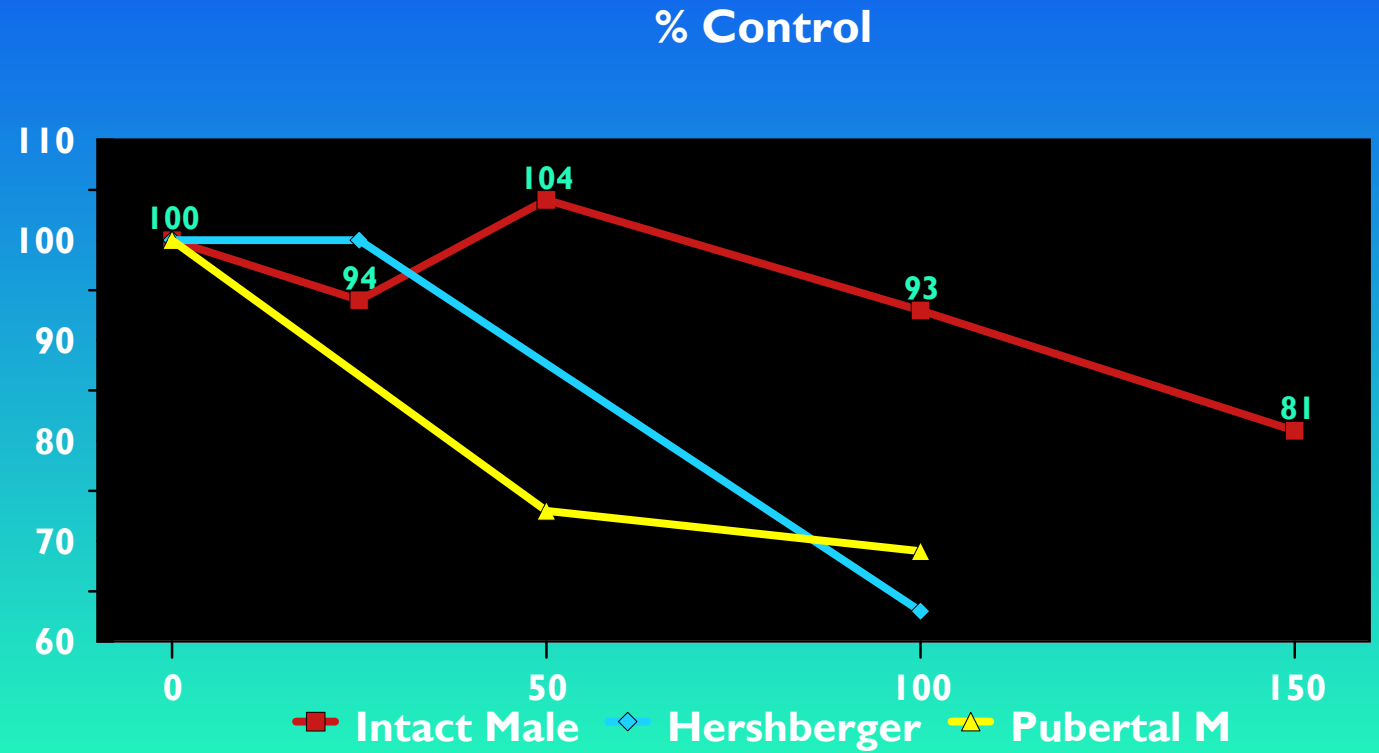
Effects of Linuron on PPS in the EPA-LE-1987 and RTI-SD-2003 male rats



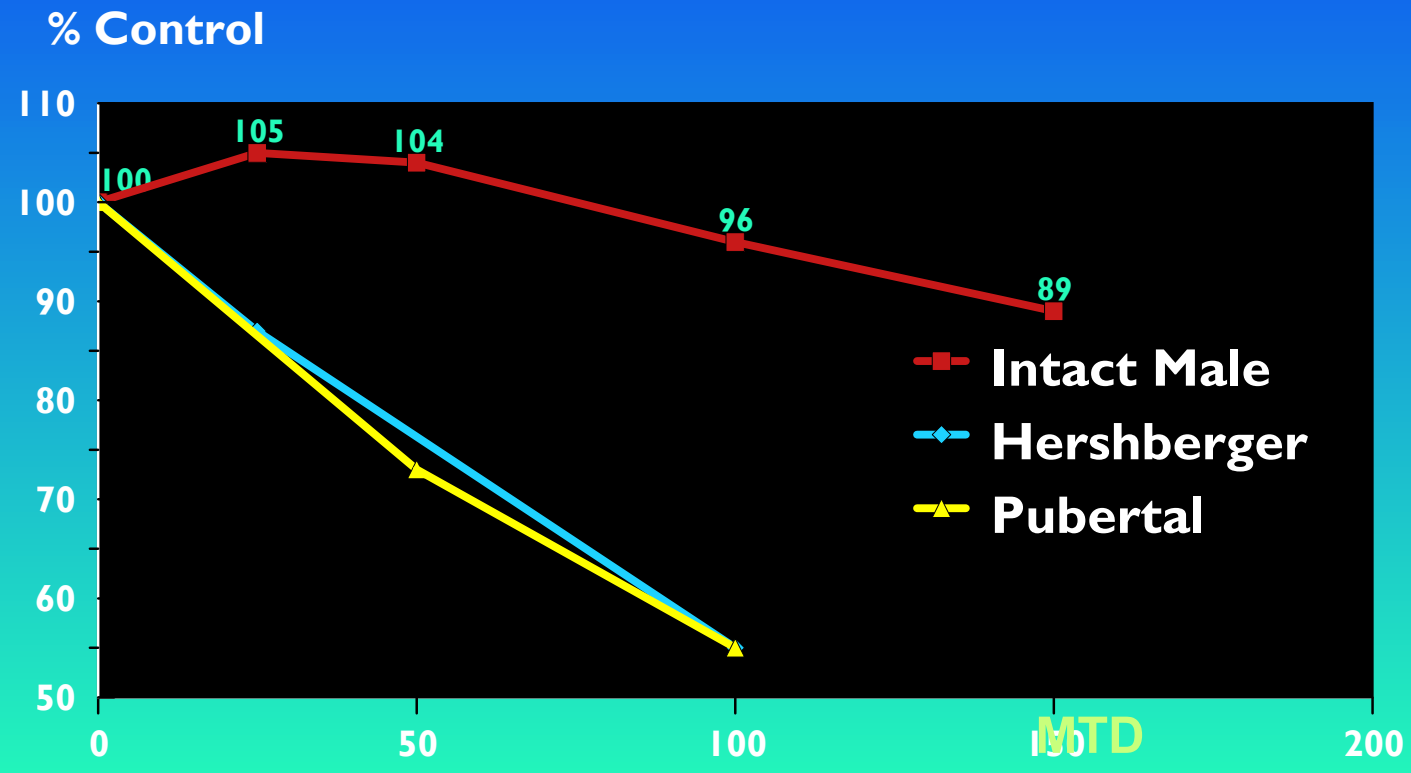
Effects of Linuron in male pubertal rat assay RTI-SD-2003



The Adult intact male assay is much less sensitive to the Effects of Linuron at 100 mg/kg/d on ventral prostate weight. Males treated with TP in the Hershberger Assay.



The Adult intact male assay is much less sensitive to the Effects of Linuron at 100 mg/kg/d on SV versus ASO weight. Males treated with TP in the Hershberger Assay.



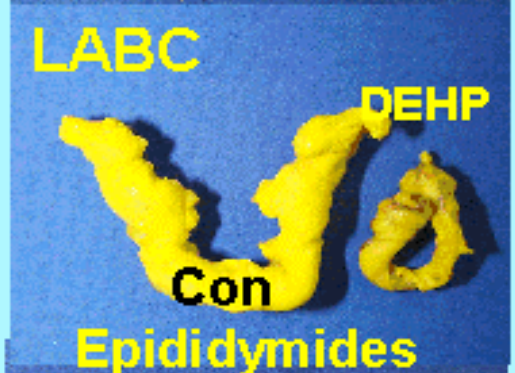
The Phthalate Esters

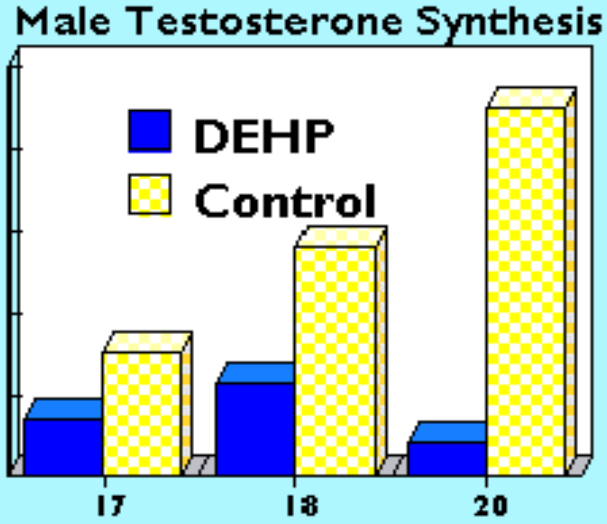
Inhibitors of Fetal Male Rat Testicular Hormone Synthesis. Not an AR antagonist

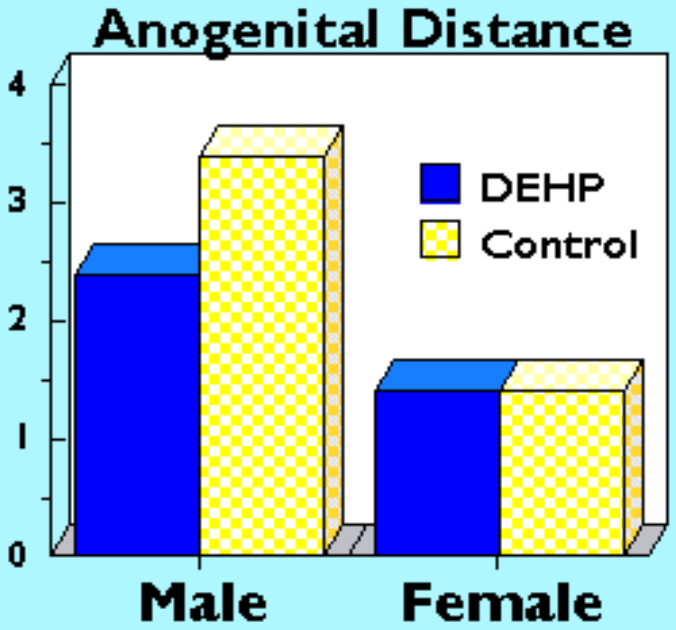
Negative in Developmental Toxicology/Teratology Studies

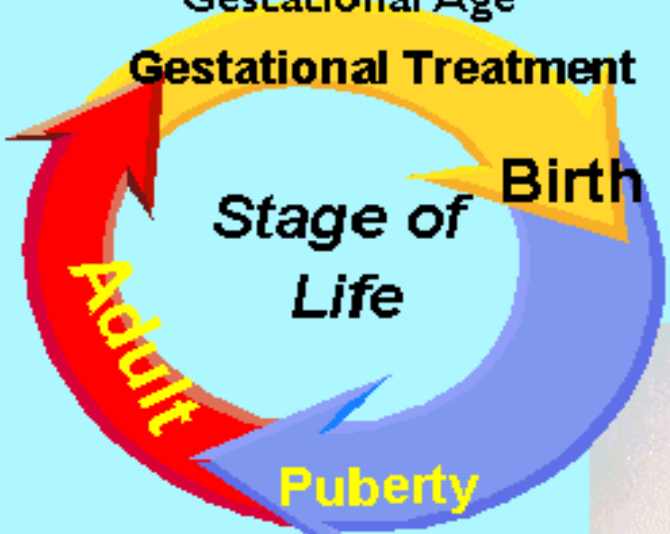
Negative in some Multigenerational Studies

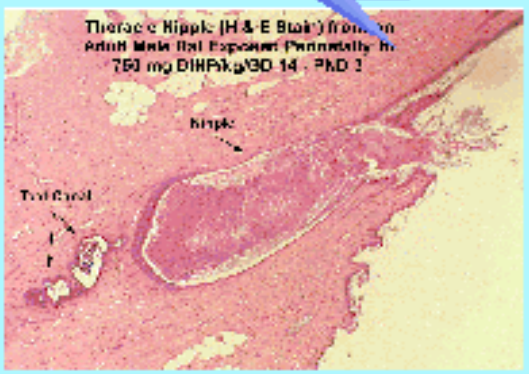
- Results in Transgenerational Studies
  - DEHP ++
  - BBP ++
  - DBP ++
  - DINP +
  - DEP negative
  - DMP negative
  - DOTP negative



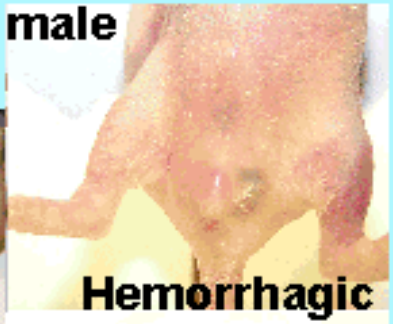


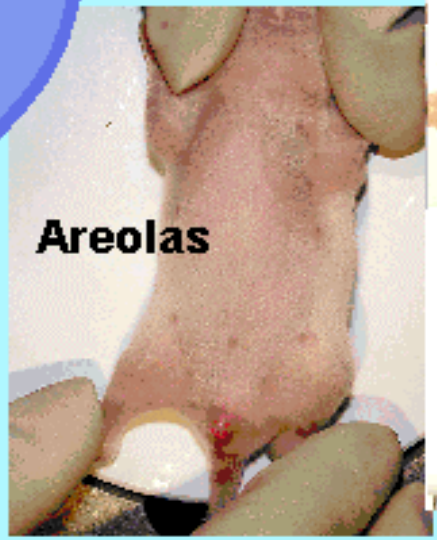




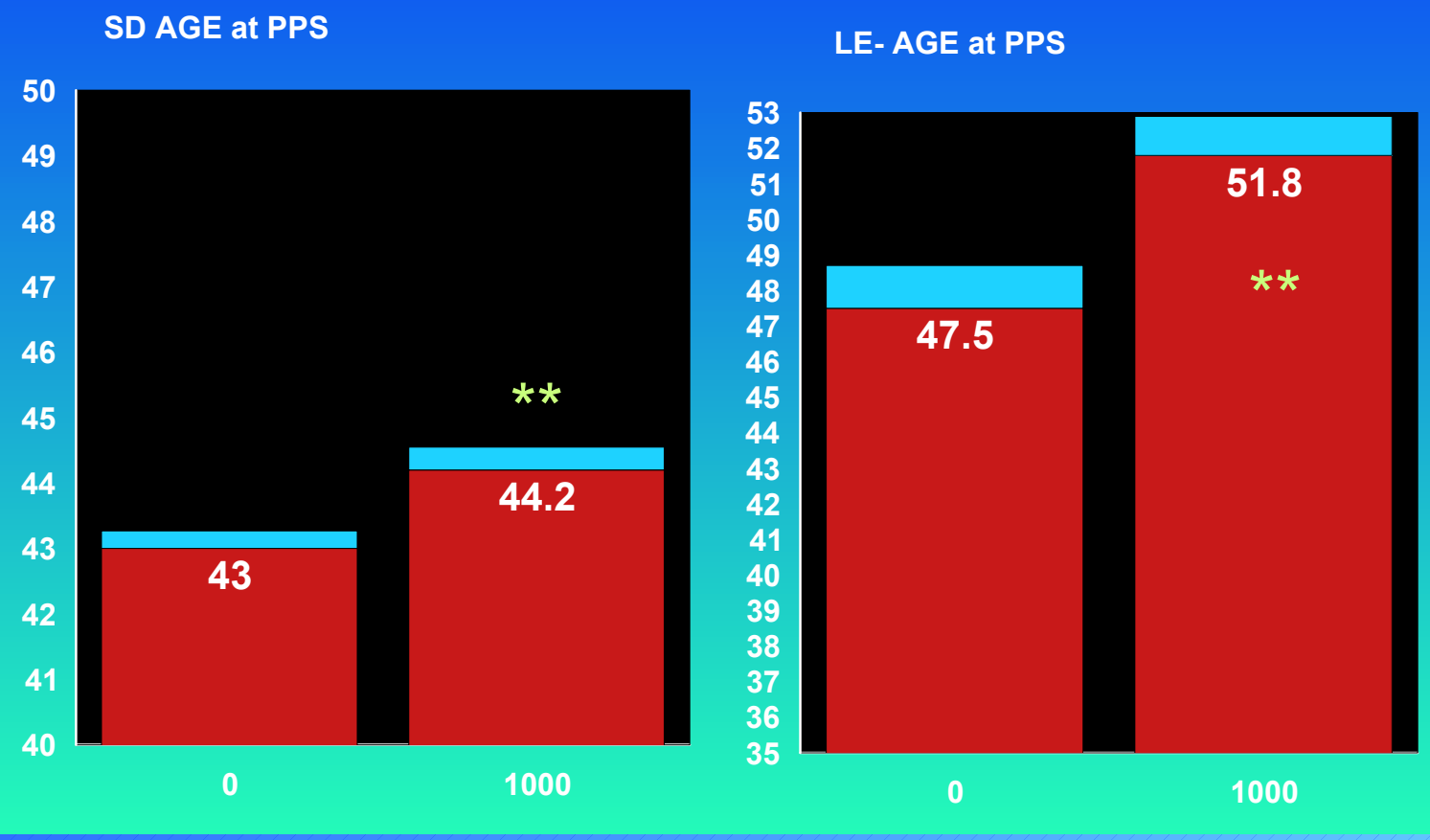


DEHP Infant male

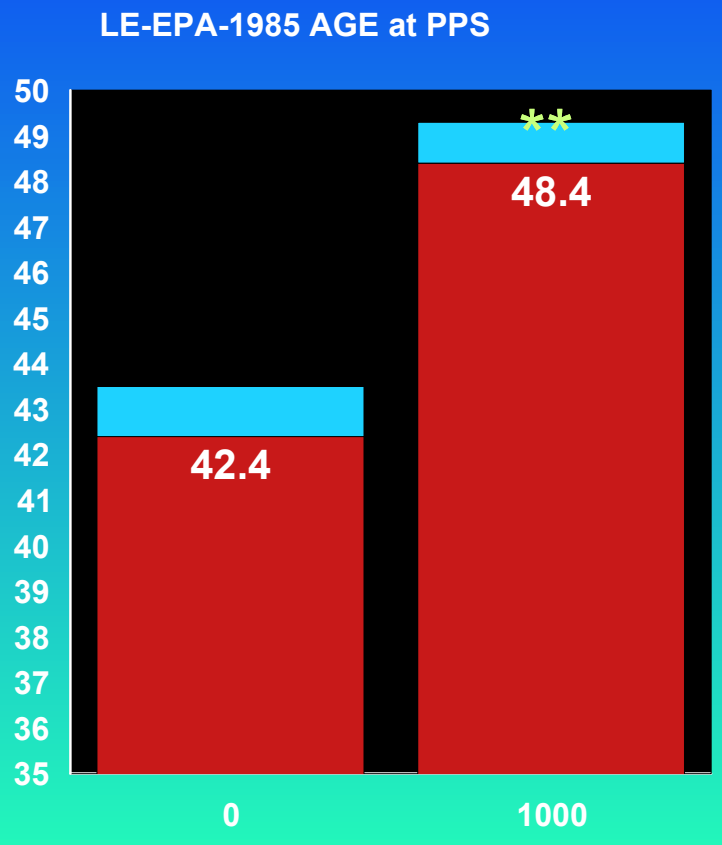


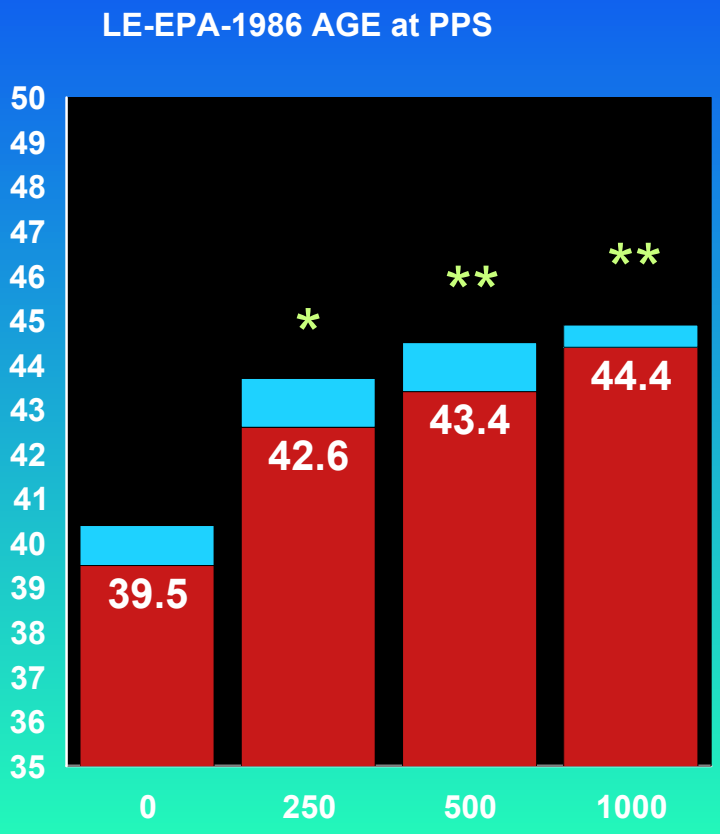


Effects of DBP in male rat TI-2000-SD/LE

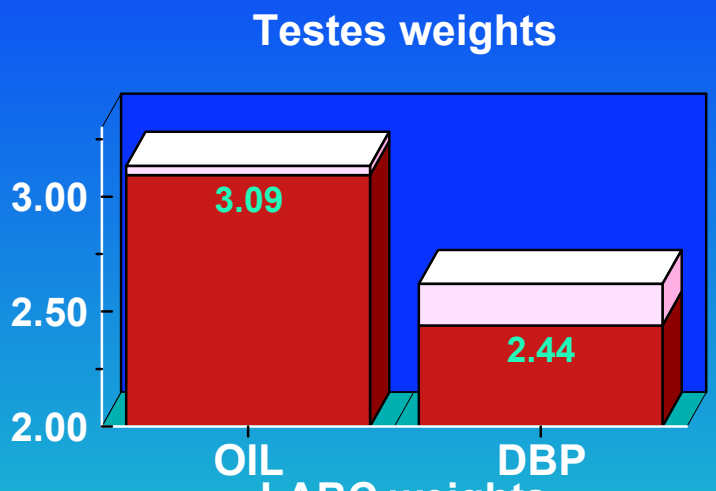


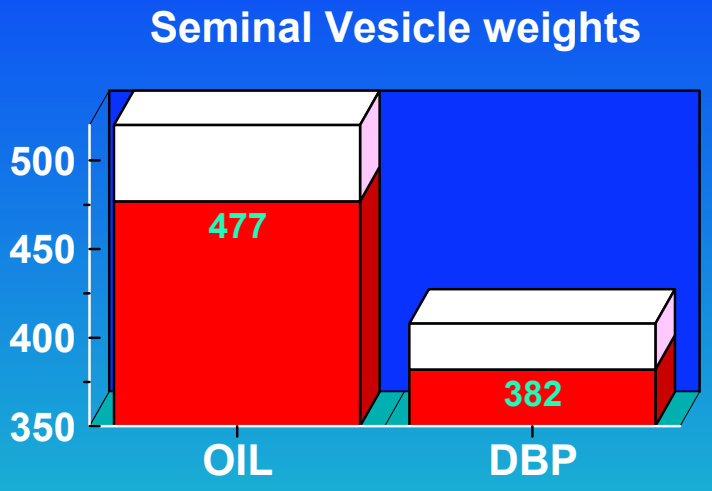
Effects of DBP in male rat EPA-LE-1985, 1986

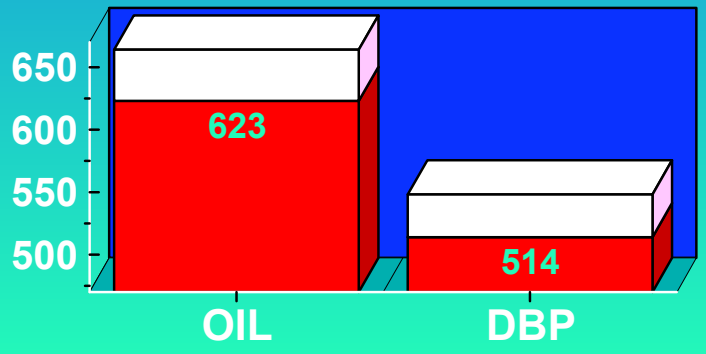


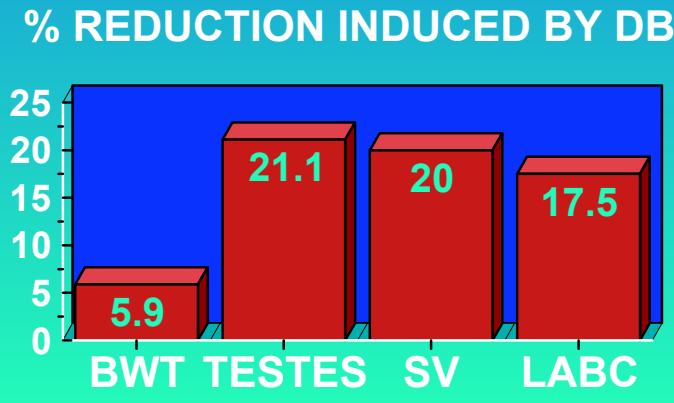


DBP reduces reproductive tissue weights in the pubertal male assay using SD rats, the least affected strain. TI-2000

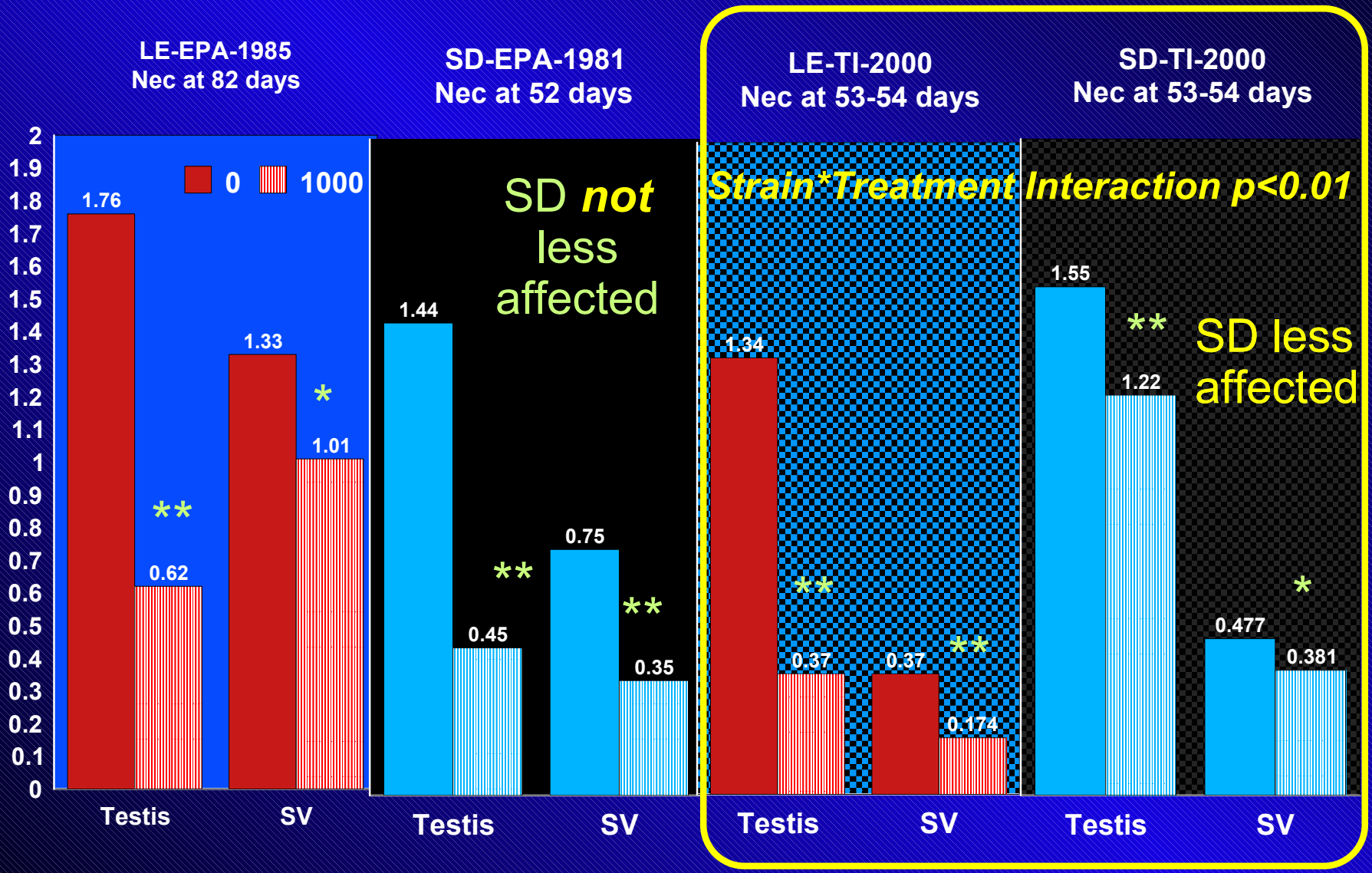




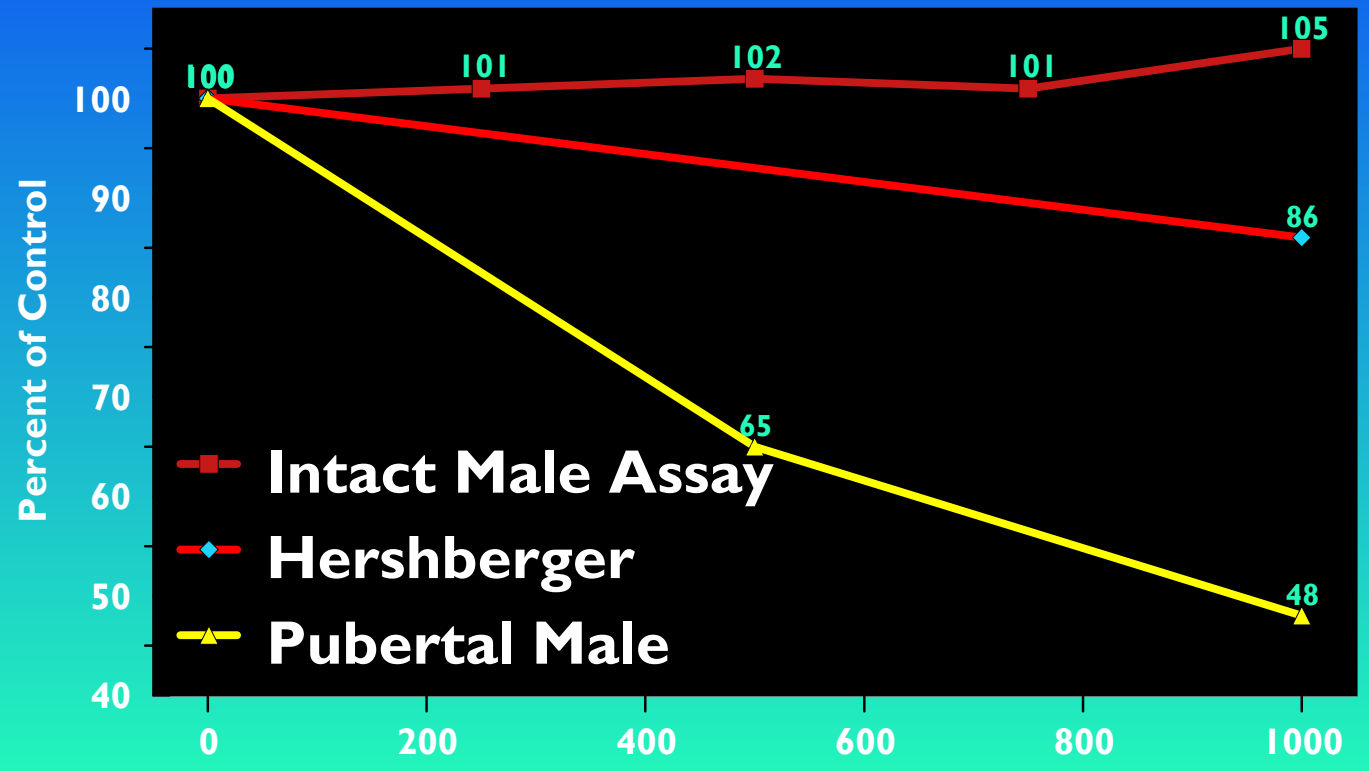




Effects of 1000 mg/kg DBP on testis weight in the LE and SD male rat from studies TI-SD/LE-2002, EPA-LE-1985 and EPA-SD-1981



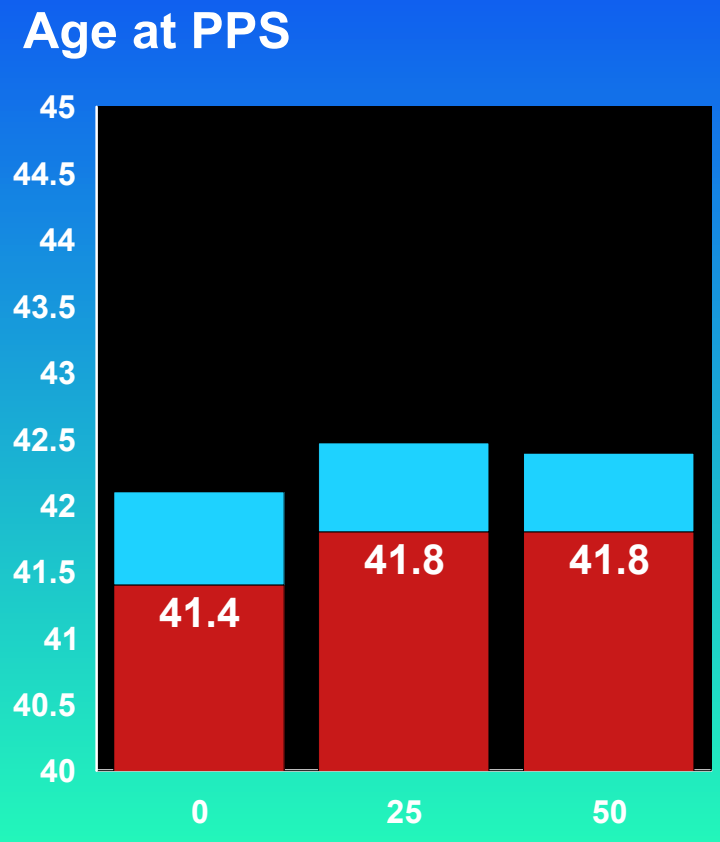
The intact male assay is less sensitive to the effects of the phthalate ester DBP at the limit dose of 1 g/kg/d on ASO weight than is seminal vesicle weights in our Hershberger or pubertal male assays.

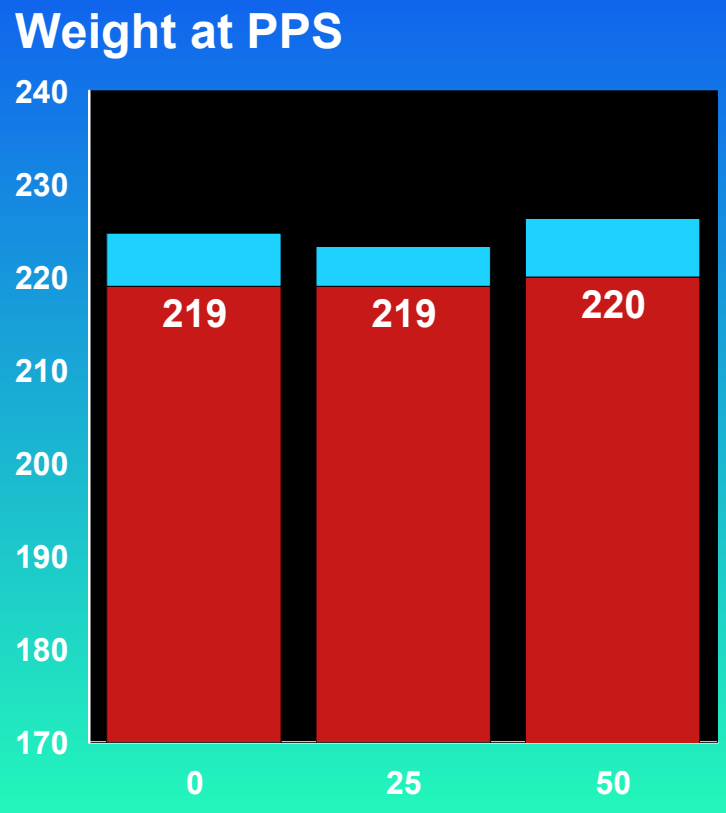


EFFECTS OF AN ESTROGENIC AND POTENTIALLY ANTIANDROGENIC PESTICIDE

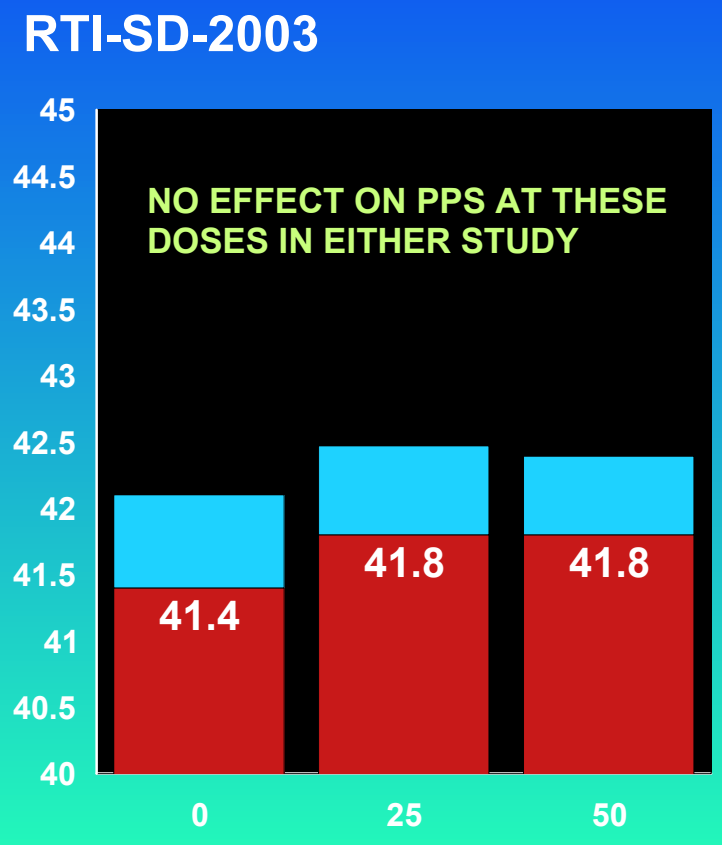
- NO EFFECT ON PPS, AS EXPECTED FROM EARLIER WORK
- REDUCED ANDROGEN-DEPENDENT TISSUE WEIGHTS
- POSITIVE IN THE PUBERTAL MALE

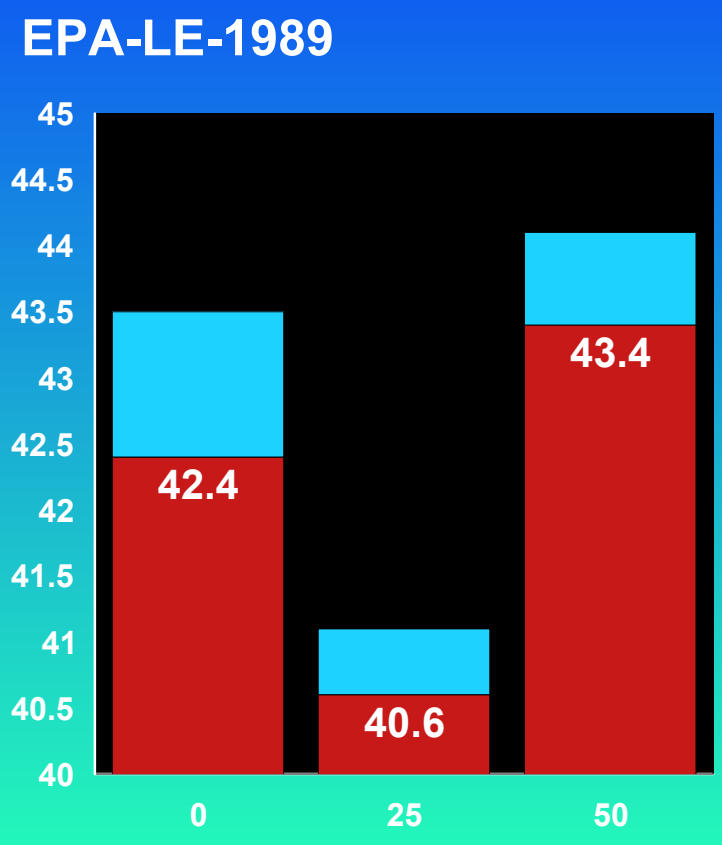
Effects of Methoxychlor in male pubertal rat assay RTI-SD-2003



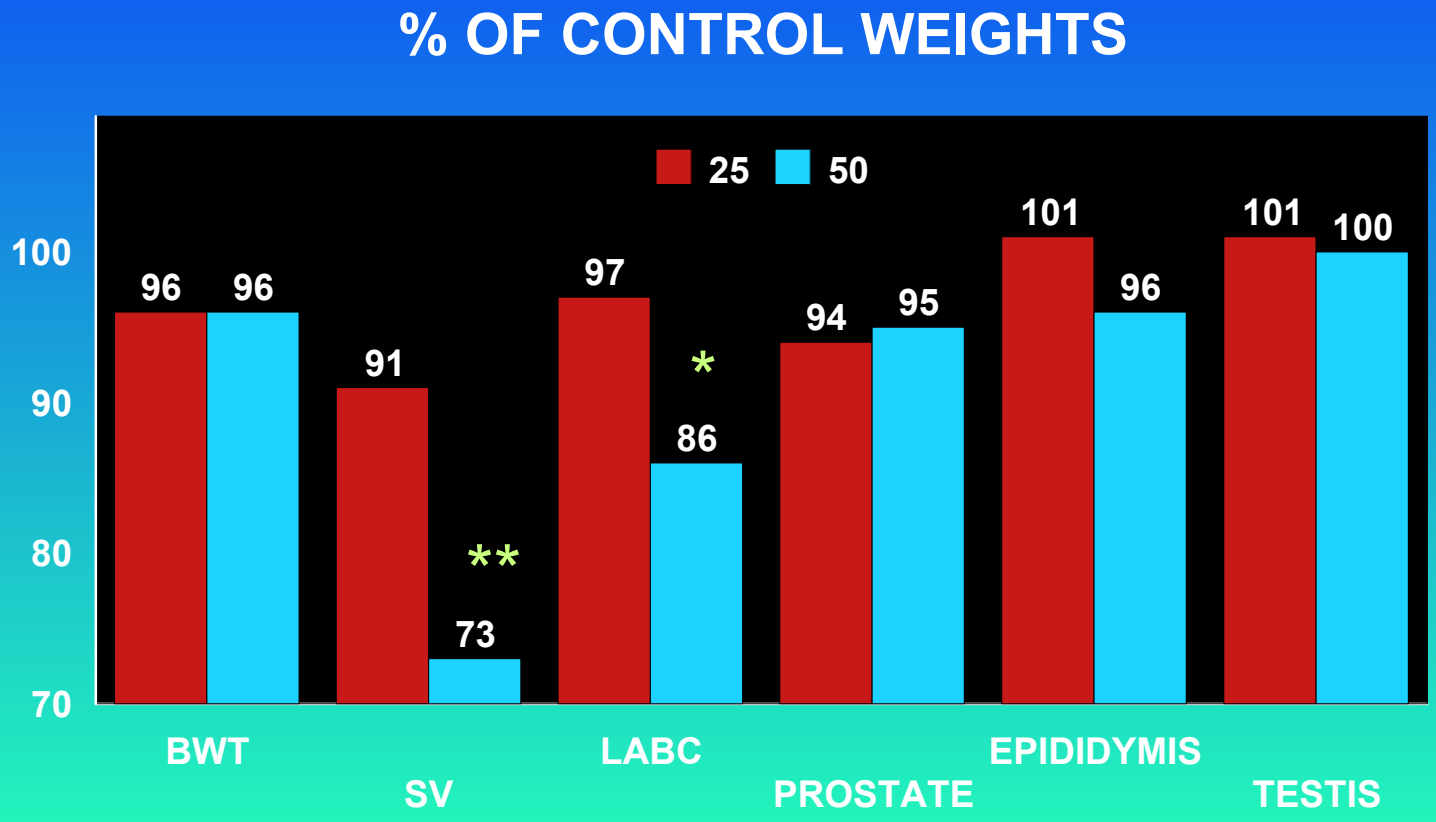


Effects of Methoxychlor on PPS in the male rat from EPA-LE-1989 study verus RTI-SD-2003





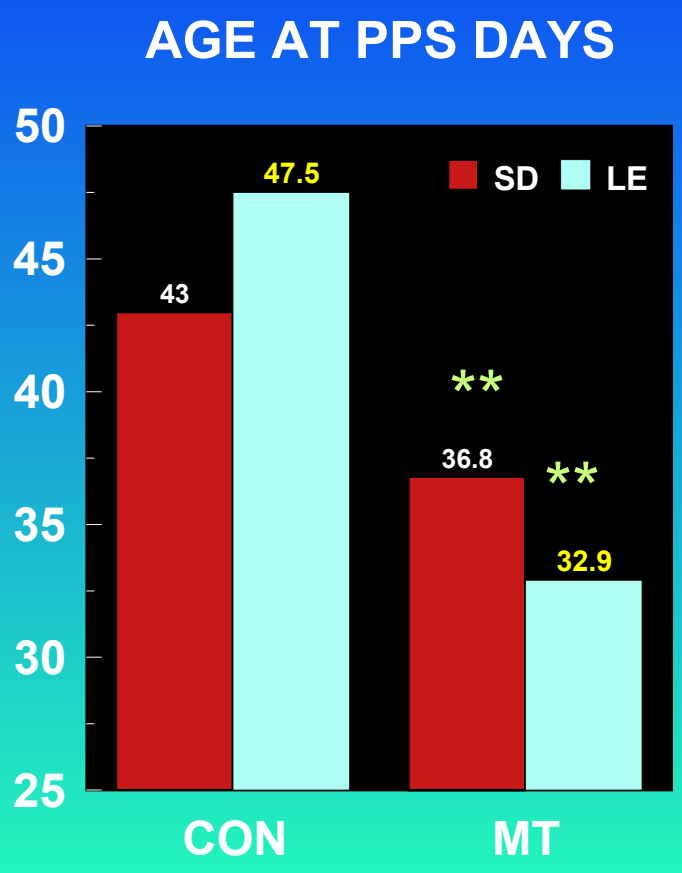
Effects of Methoxychlor in male pubertal rat assay

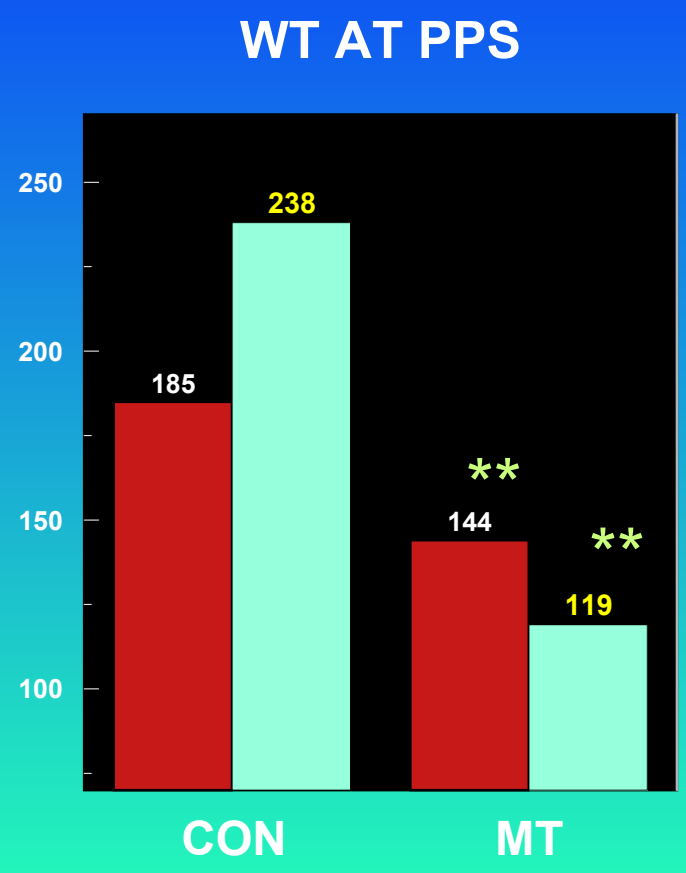


ANDROGENIC EFFECTS IN THE PUBERTAL MALE ASSAY

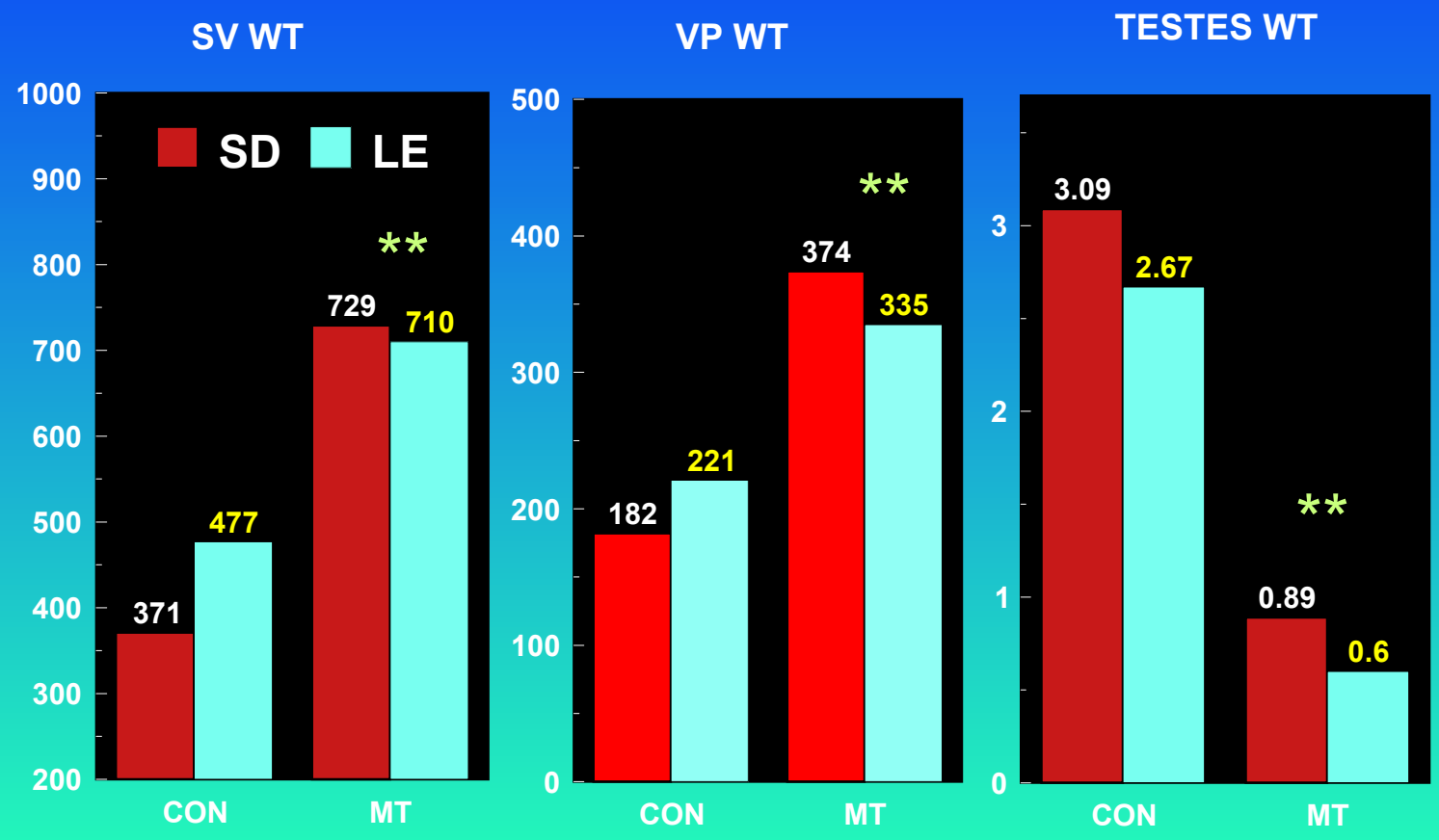
- VERY POSITIVE
- RESPONSES EXACTLY AS EXPECTED ON PPS, TESTIS AND SEX ACCESSORY TISSUES

Effects of METHYLTESTOSTERONE at 80 mg/kg on puberty in the male rat TI-SD/LE-2000





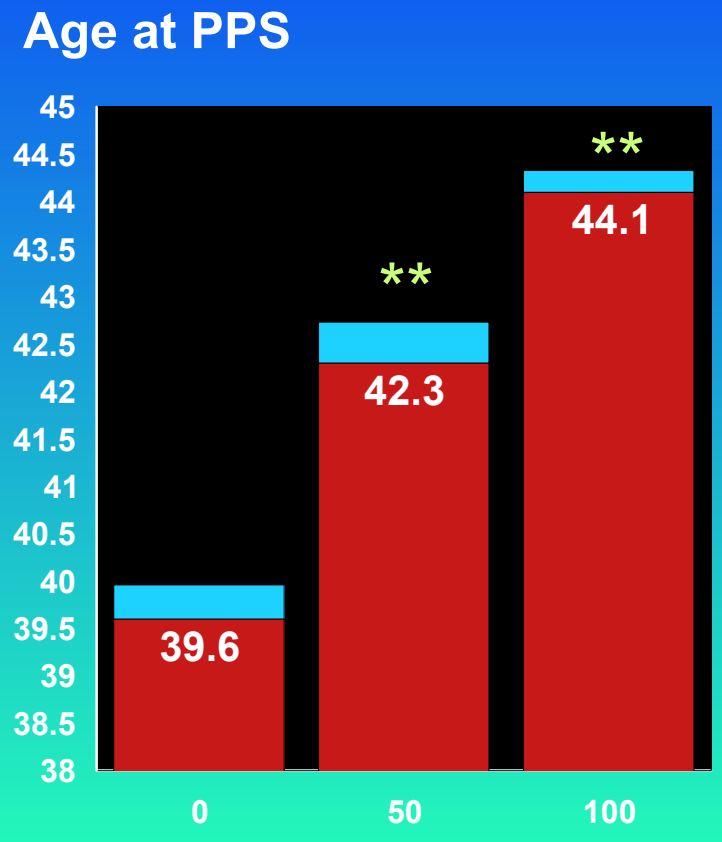
Effects of METHYLTESTOSTERONE at 80 mg/kg In the male rat TI-SD/LE-2000

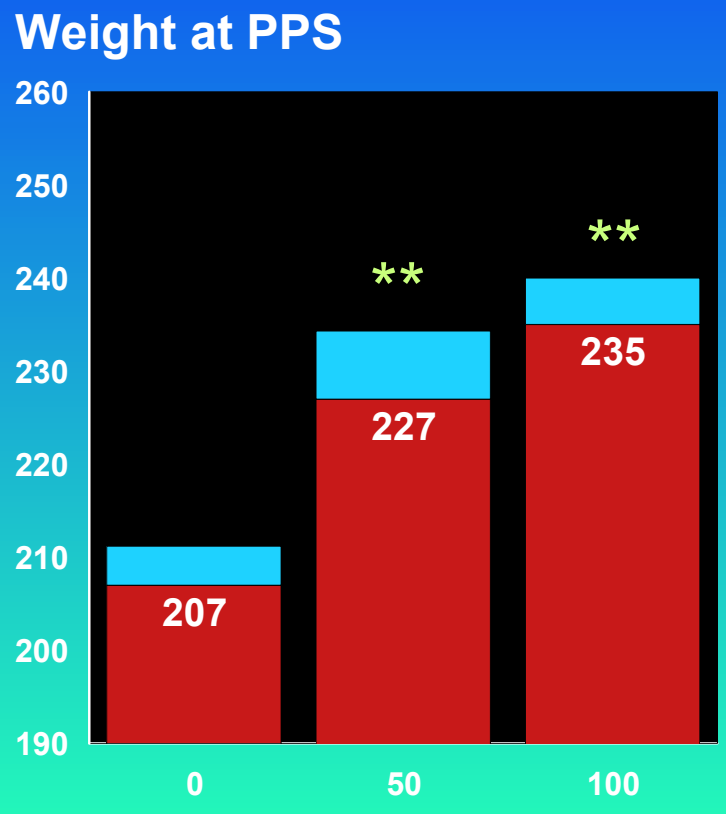


AN INHIBITOR OF P450 ENZYMES, INCLUDING STEROIDOGENESIS

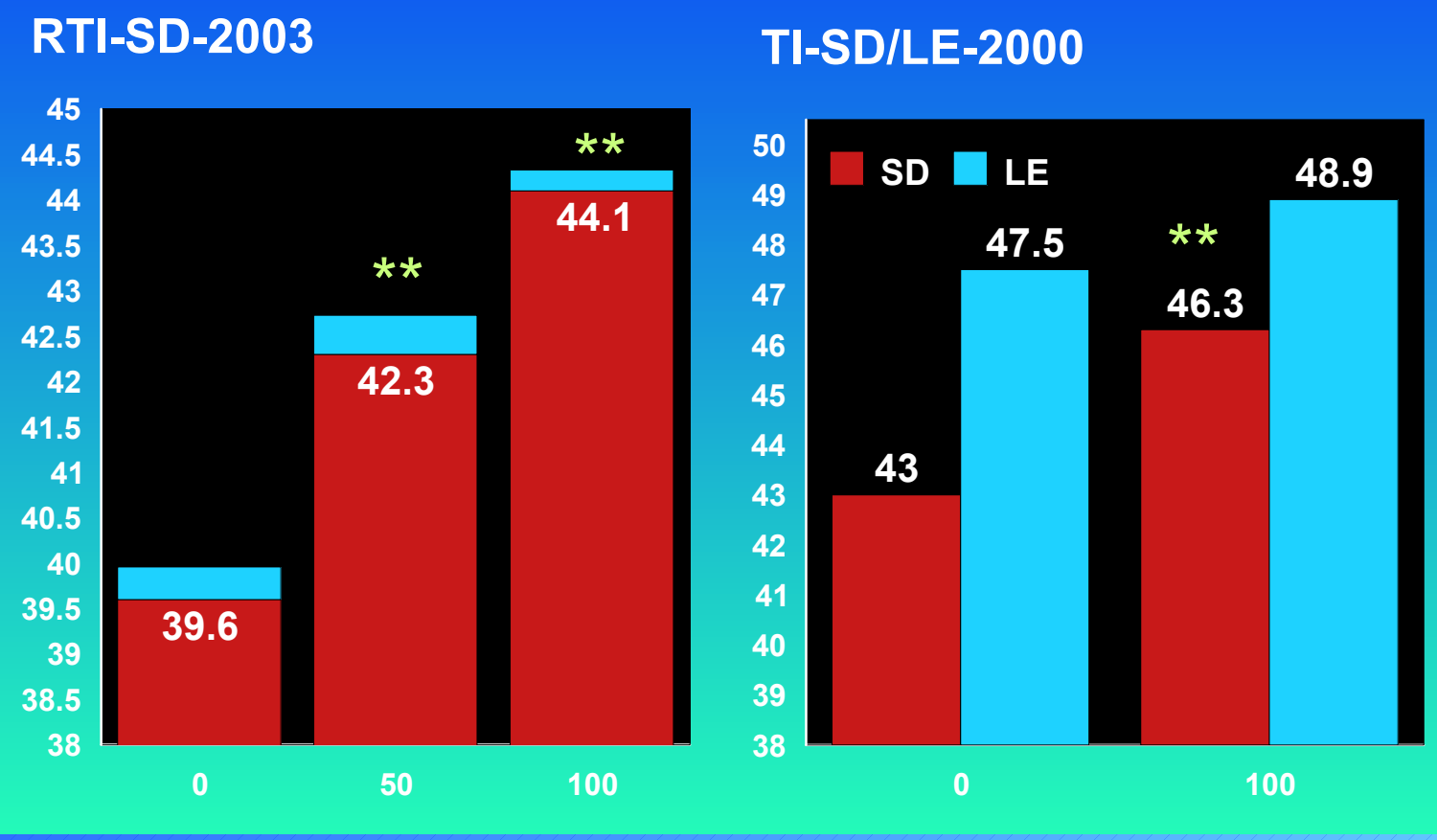
- VERY POSITIVE
- REPRODUCIBLE REPRODUCTIVE AND OTHER ENDOCRINE (ADRENAL) AND ORGAN (LIVER) EFFECTS

Effects of Ketoconazole in male pubertal rat assay RTI-SD-2003

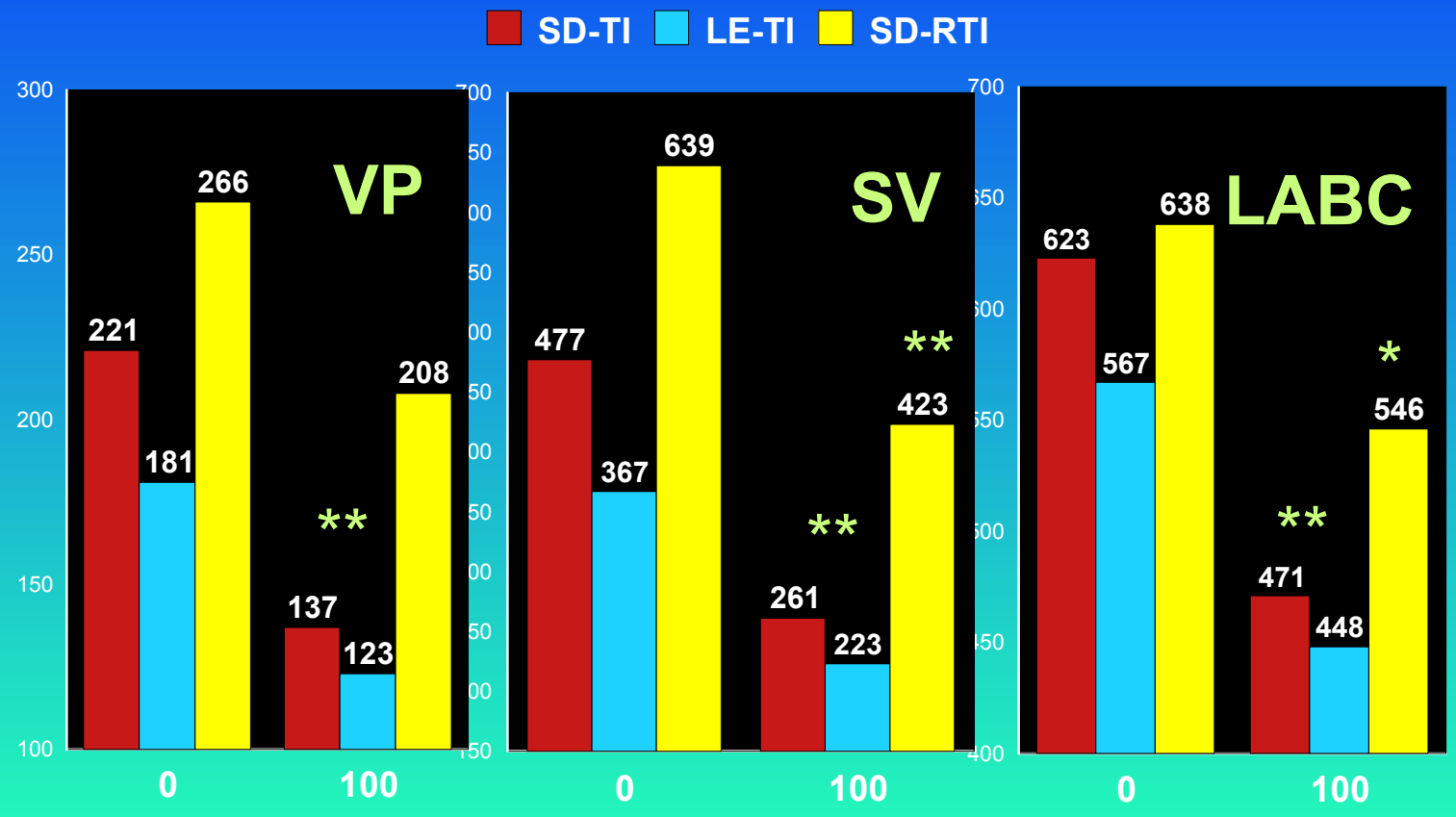




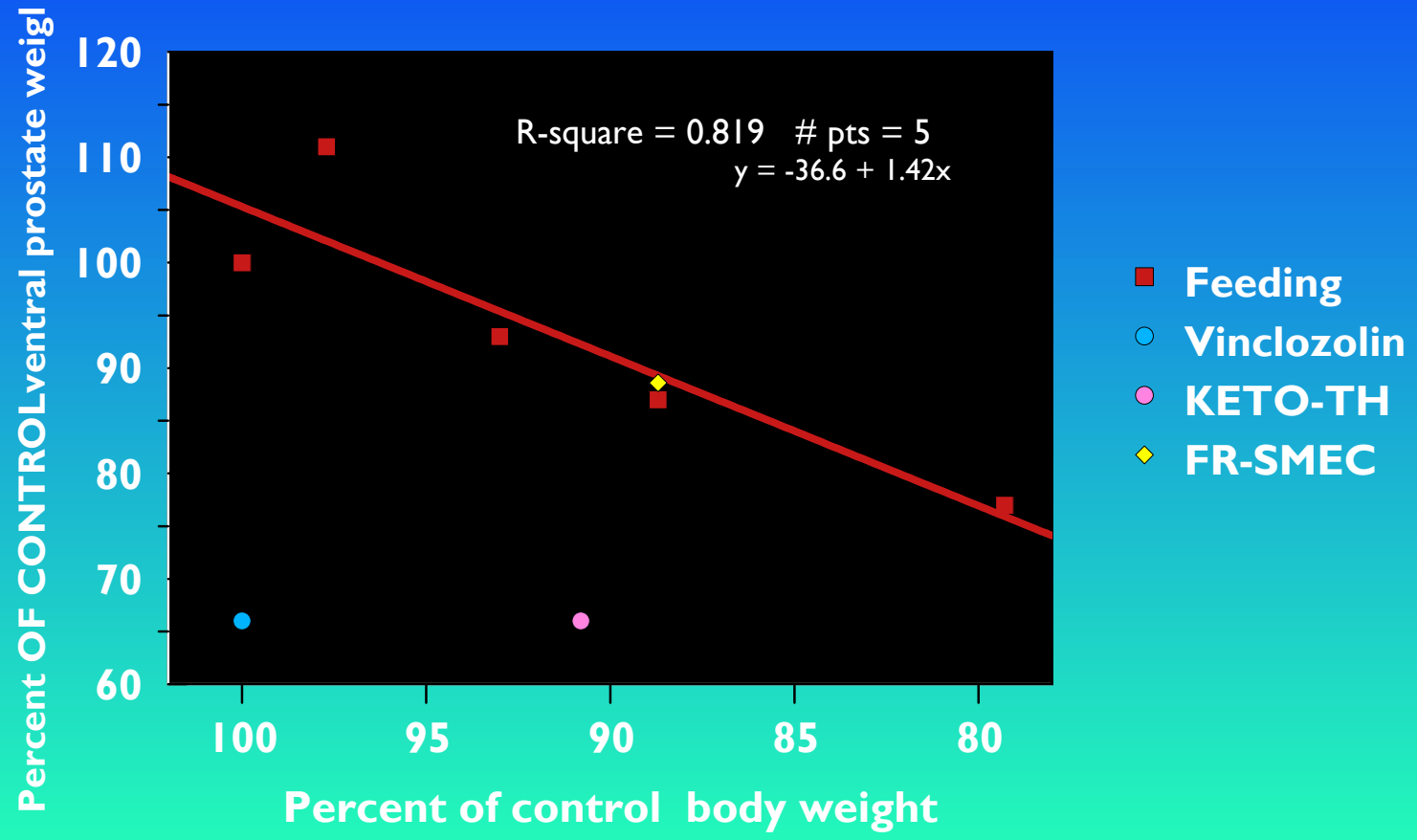
Effects of Ketoconazole ON THE AGE AT PPS



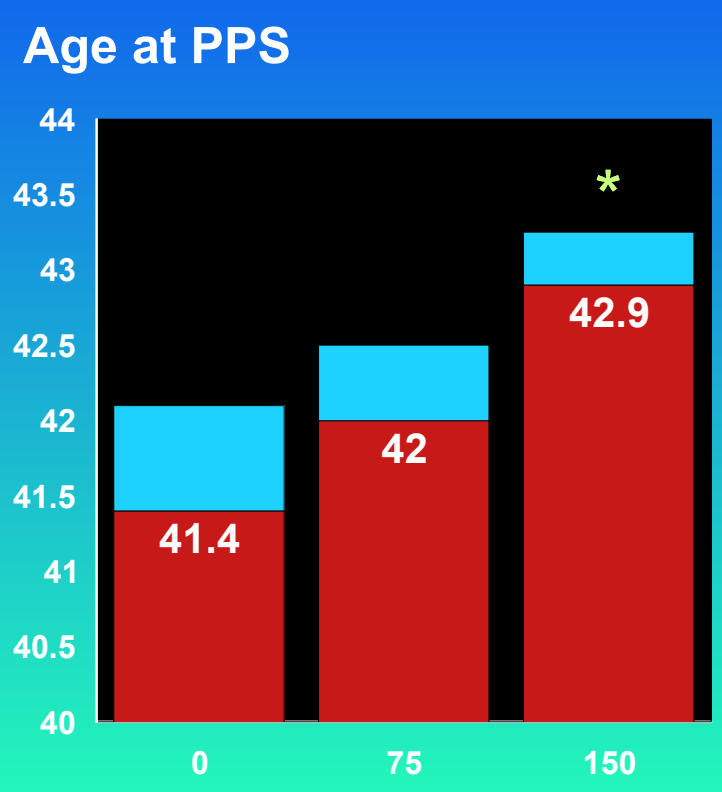
Effects of Ketoconazole on reproductive organs in TI-SD/LE-2000 and RTI-SD-2003. Also, consistent increases in adrenal and liver weights at both doses.

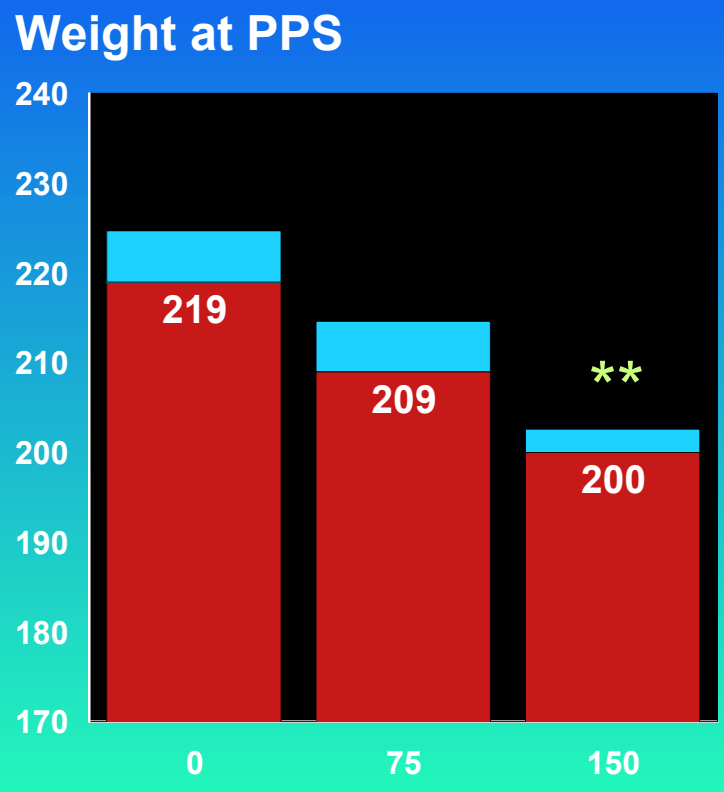


Relationship between reduction in body weight due to restricted feeding versus ventral prostate weight



Effects of Atrazine in male pubertal rat assay

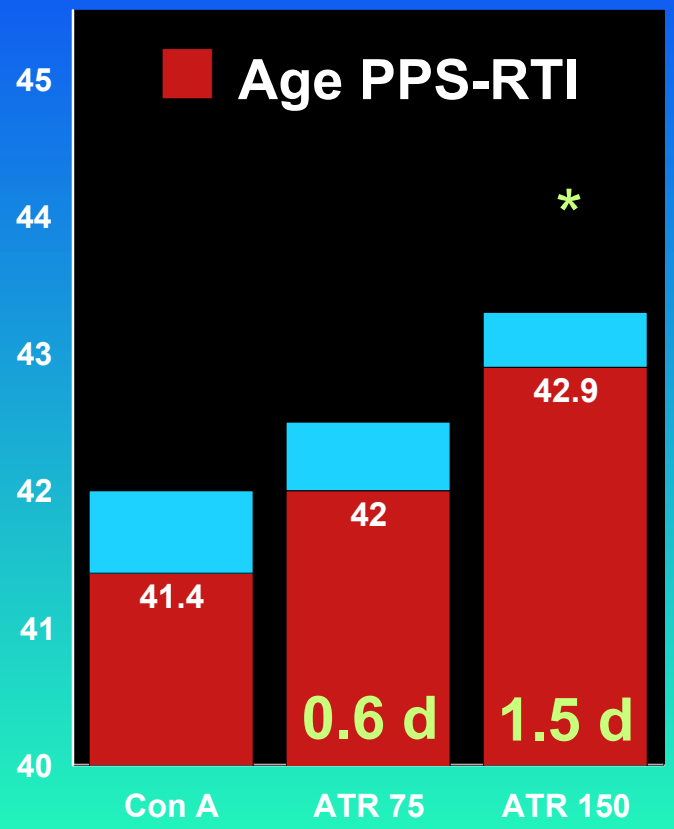


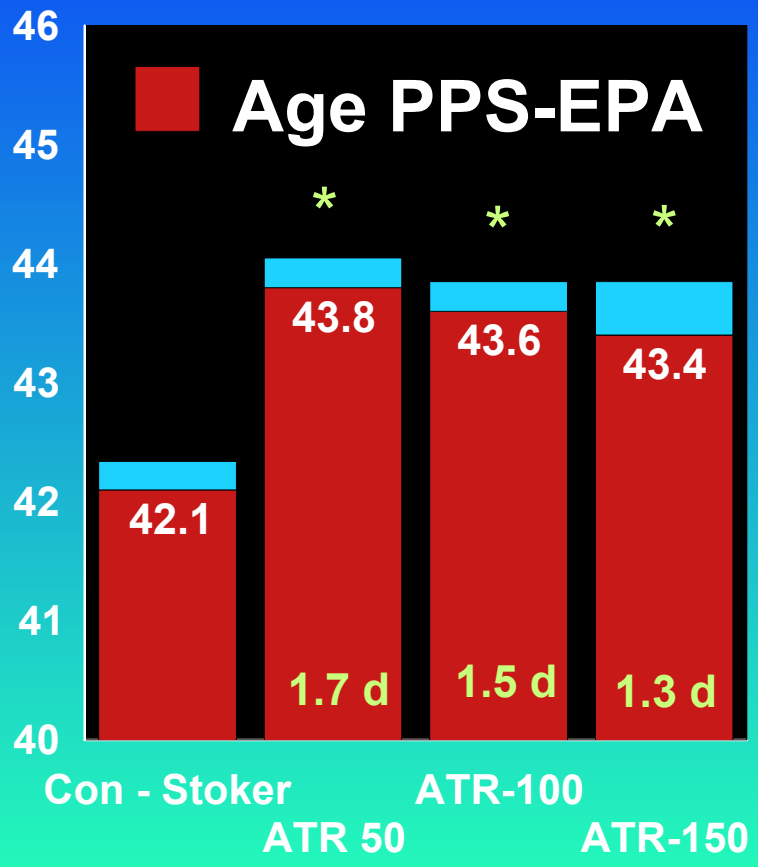


ATRAZINE A CNS ACTIVE CHEMICAL

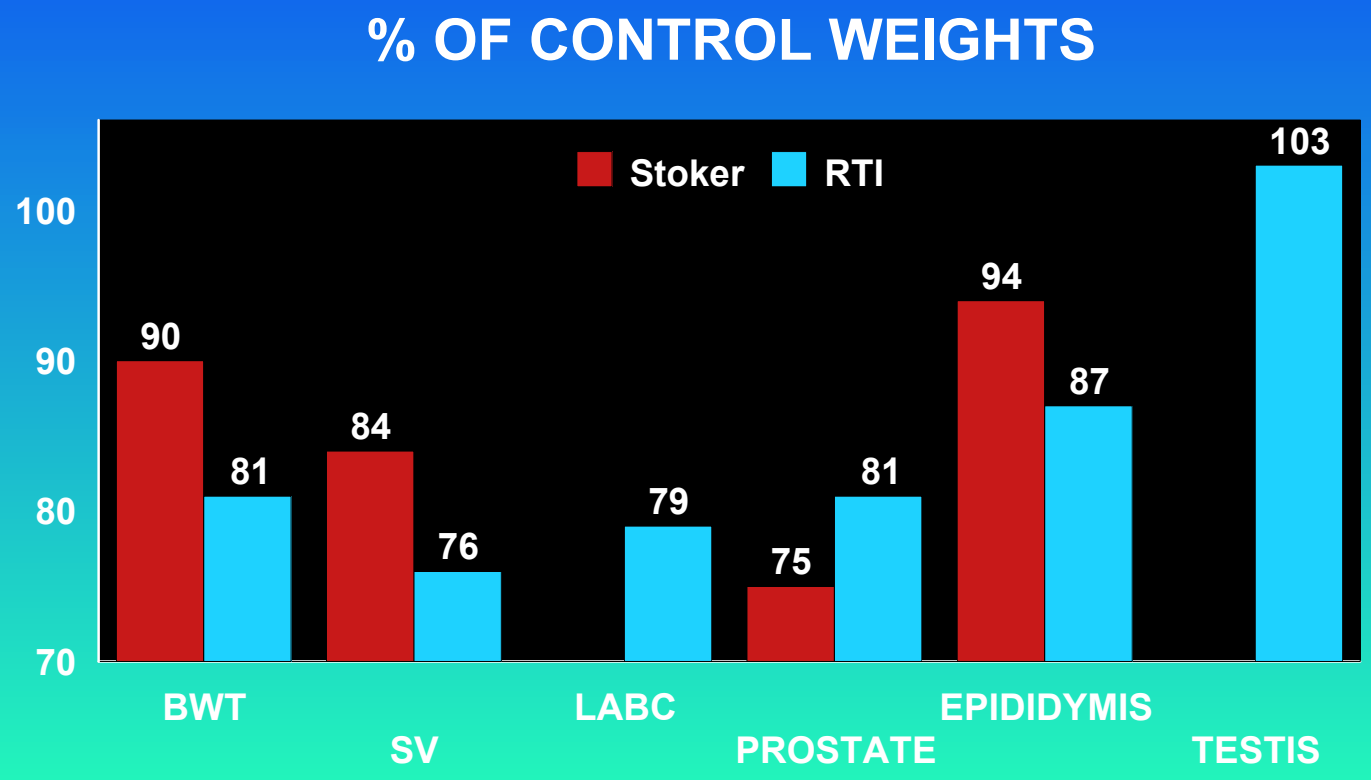
- DELAYS PPS IN ALL STUDIES
- VARIABLE EFFECTS ON BODY WEIGHT

Effects of ATRAZINE in the Pubertal Male rat assay RTI versus Stoker al.





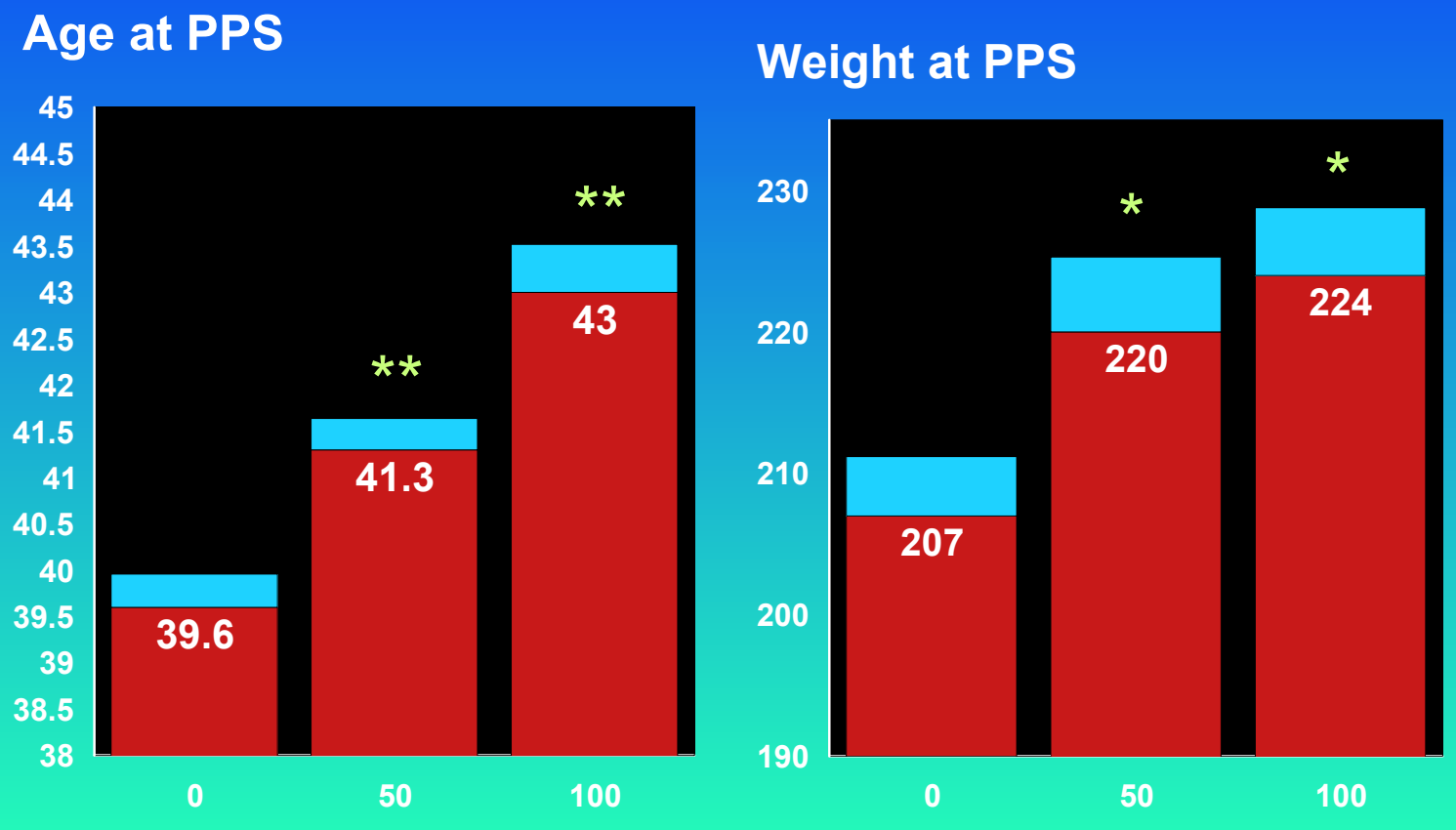
Effects of Atrazine in male pubertal rat assay. RTI versus Stoker data at 150 mg/kg/d



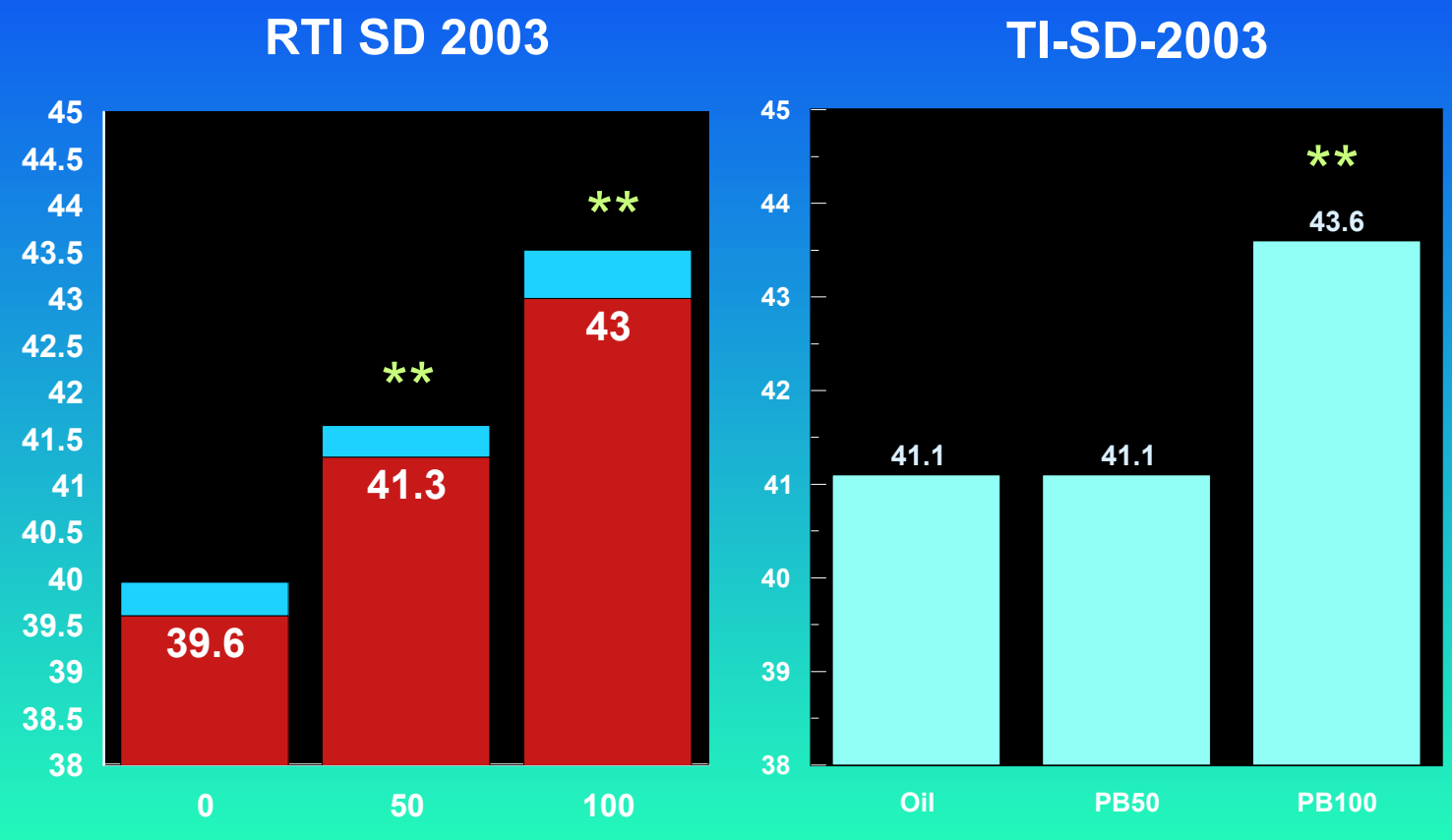
PHENOBARBITAL AFFECTS CNS, LIVER METABOLISM AND ENDOCRINE ORGANS DIRECTLY

- ROBUST, REPRODUCIBLE RESPONSES ON MALE PPS AND REPRODUCTIVE ORGAN WEIGHTS

Effects of Phenobarbital in male pubertal rat assay

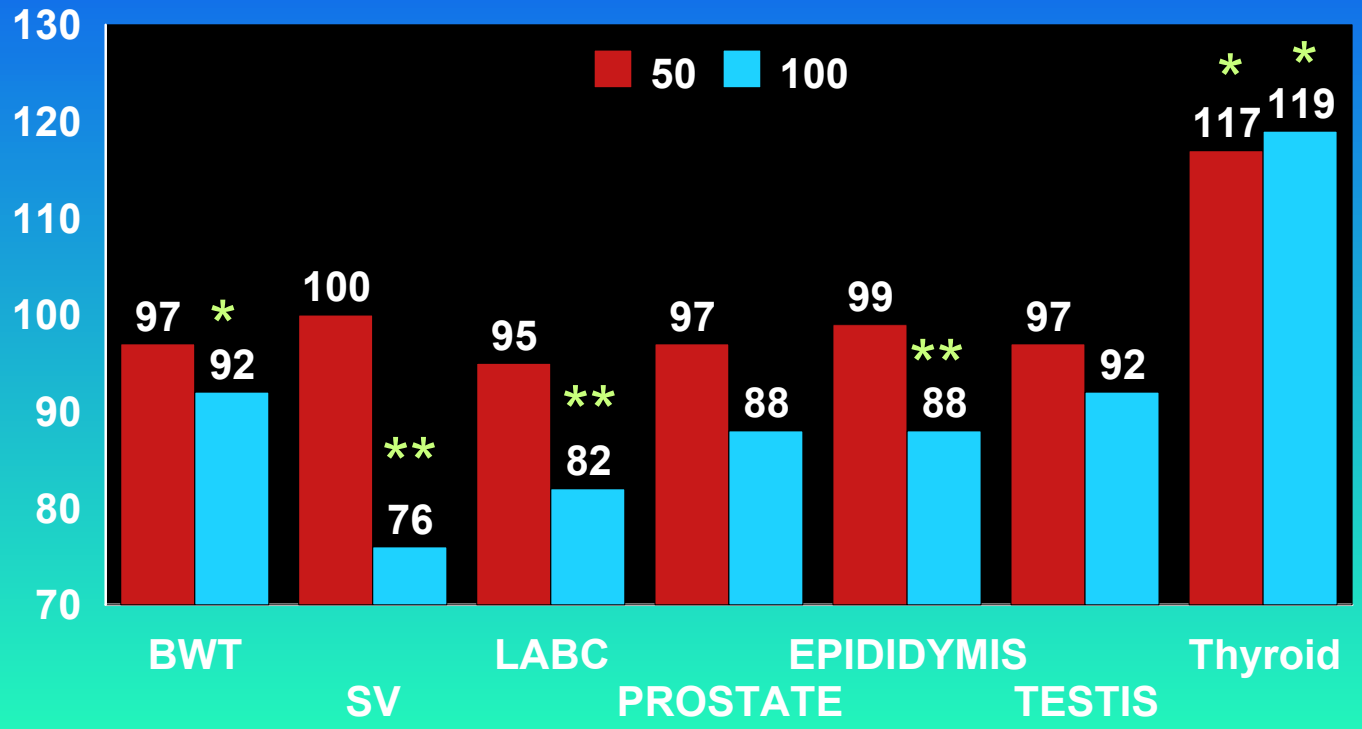


Effects of Phenobarbital in male pubertal rat assay



Effects of Phenobarbital in male pubertal rat assay

% OF CONTROL WEIGHTS



OBSERVATIONS ON THE PUBERTAL MALE ASSAY

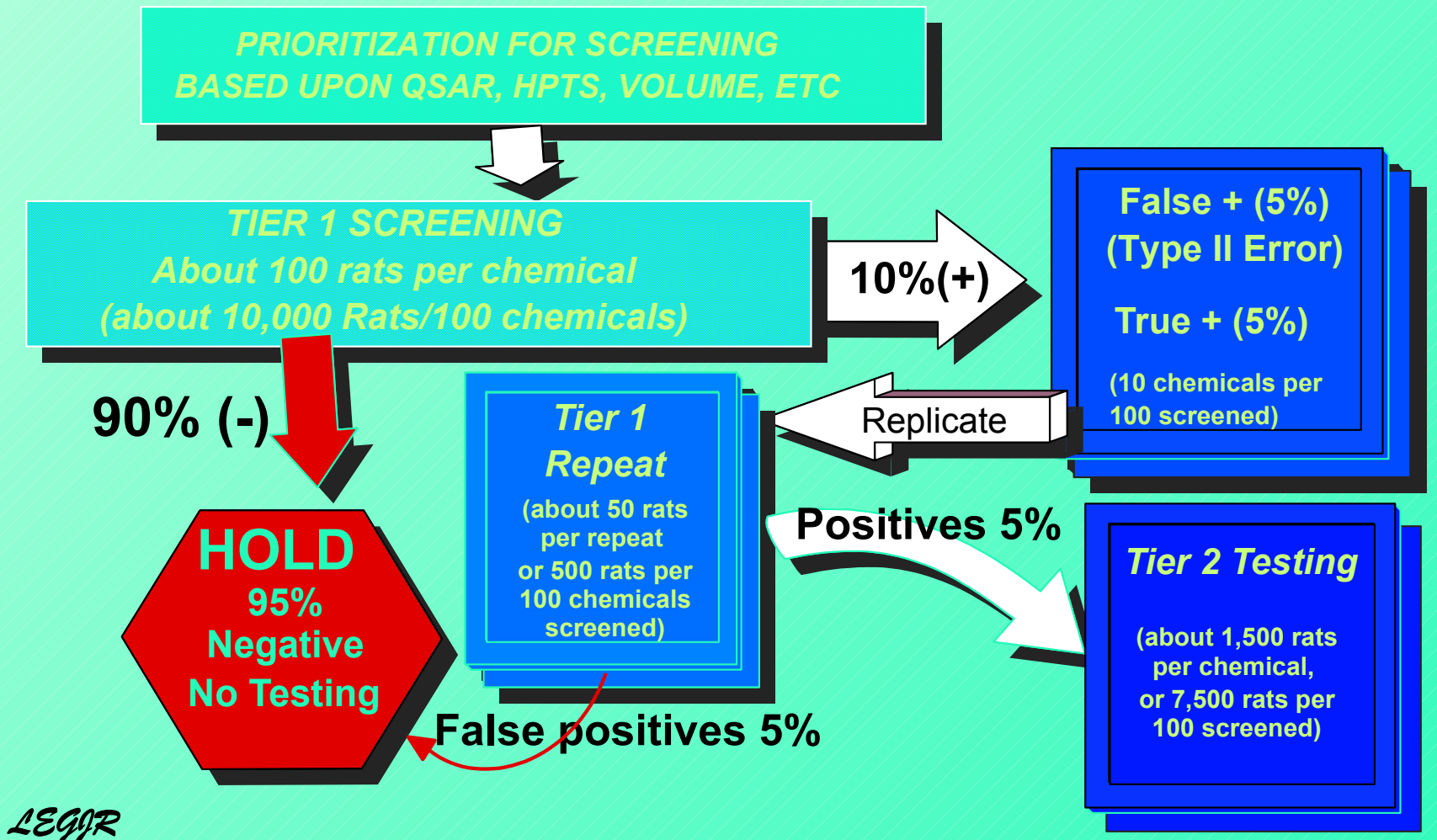
- Produced expected results for all chemicals
- Both SD and LE strains responded significantly
- Appears to detect all the activities as expected
- More sensitive than the Adult Intact Male Assay
- More sensitive than the Hershberger to DBP but not to the AR antagonists

Estimate of Animal Use in EDSP

(Assuming 5% of Chemicals are endocrine active)

18,000 Rats per Hundred Chemicals Evaluated

Gray et al., in press. Toxicology Letters. ICT IX



LEGJR