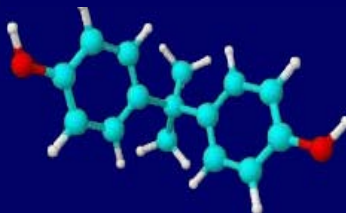


**BISPHENOL A DISRUPTS
REPRODUCTION AND DEVELOPMENT
RESULTING IN DISEASE IN ADULTHOOD**

**Frederick vom Saal
Curators' Professor**

**Division of Biological Sciences
University of Missouri – Columbia**

Bisphenol A



**Support provided by National Institute of
Environmental Health Sciences**

**Conference on Bisphenol A
Sponsored by: NIEHS / EPA
Chapel Hill, NC, November 2006**

**Reproductive Toxicology
(24: 131–138, 2007)**

**Chapel Hill bisphenol A expert panel consensus statement:
Integration of mechanisms, effects in animals and potential
to impact human health at current levels of exposure**

Bisphenol A Research Needs: Determine Sources of Human Exposure

- **Human data suggests continuous exposure:
Food and beverages - can lining, packaging, storage containers.
Water - drinking, bathing?
Dermal exposure: Thermal / carbonless paper?
Inhalation: Associated with dust?
How many other unknown sources?**
- **There is a low rate of metabolism of BPA in early life:
Fetus, infant, child, adolescent, adult.**

**BPA Expert Panel Consensus Statement
Sponsored by: NIEHS / EPA
Chapel Hill, NC, November 2006**

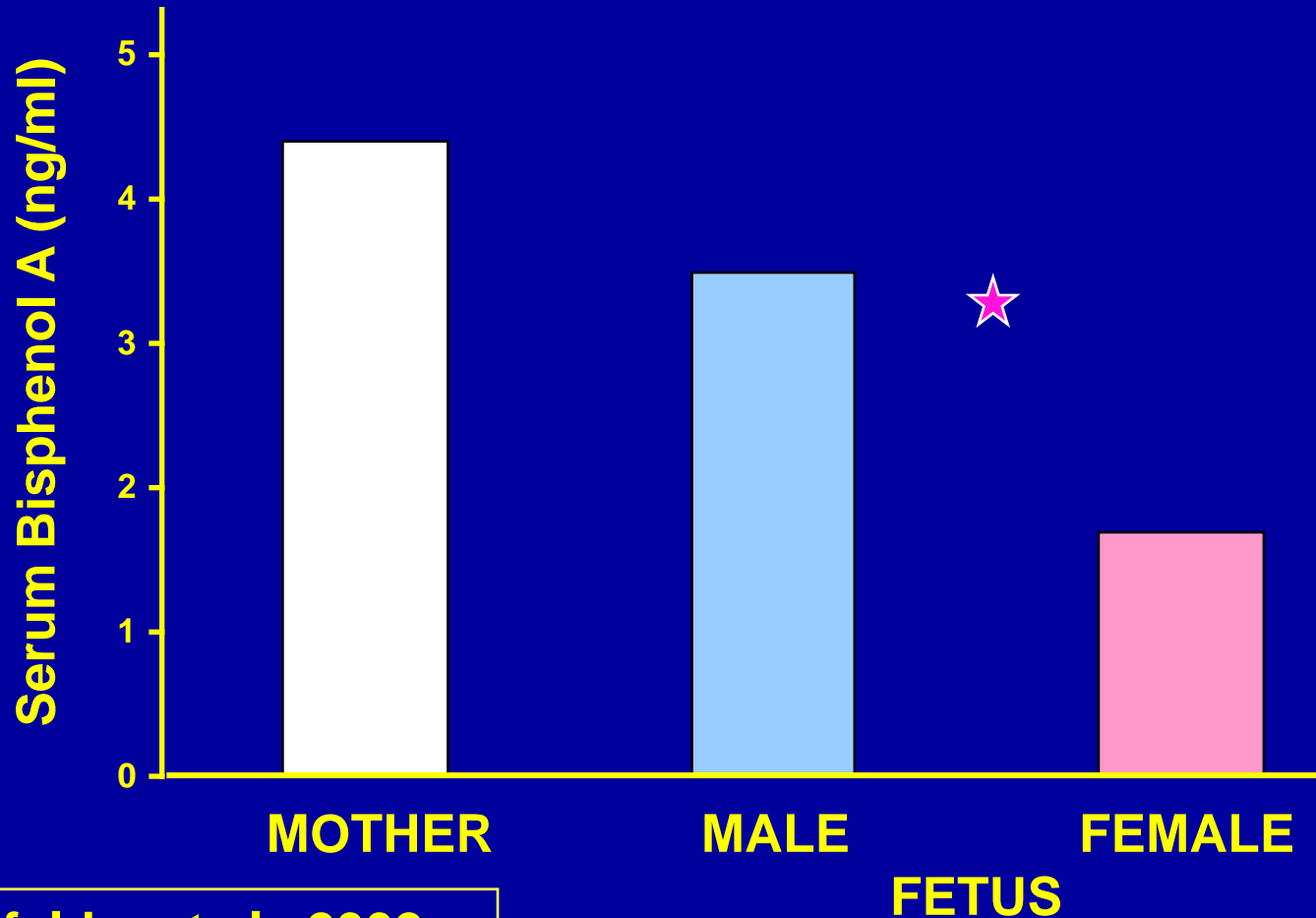
Bisphenol A Data in NHANES Suggest Longer than Expected Half-Life, Substantial Nonfood Exposure, or Both

R.W. Stahlhut, W.V. Welshons and S.H. Swan

Environmental Health Perspectives 117:784–789, 2009.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

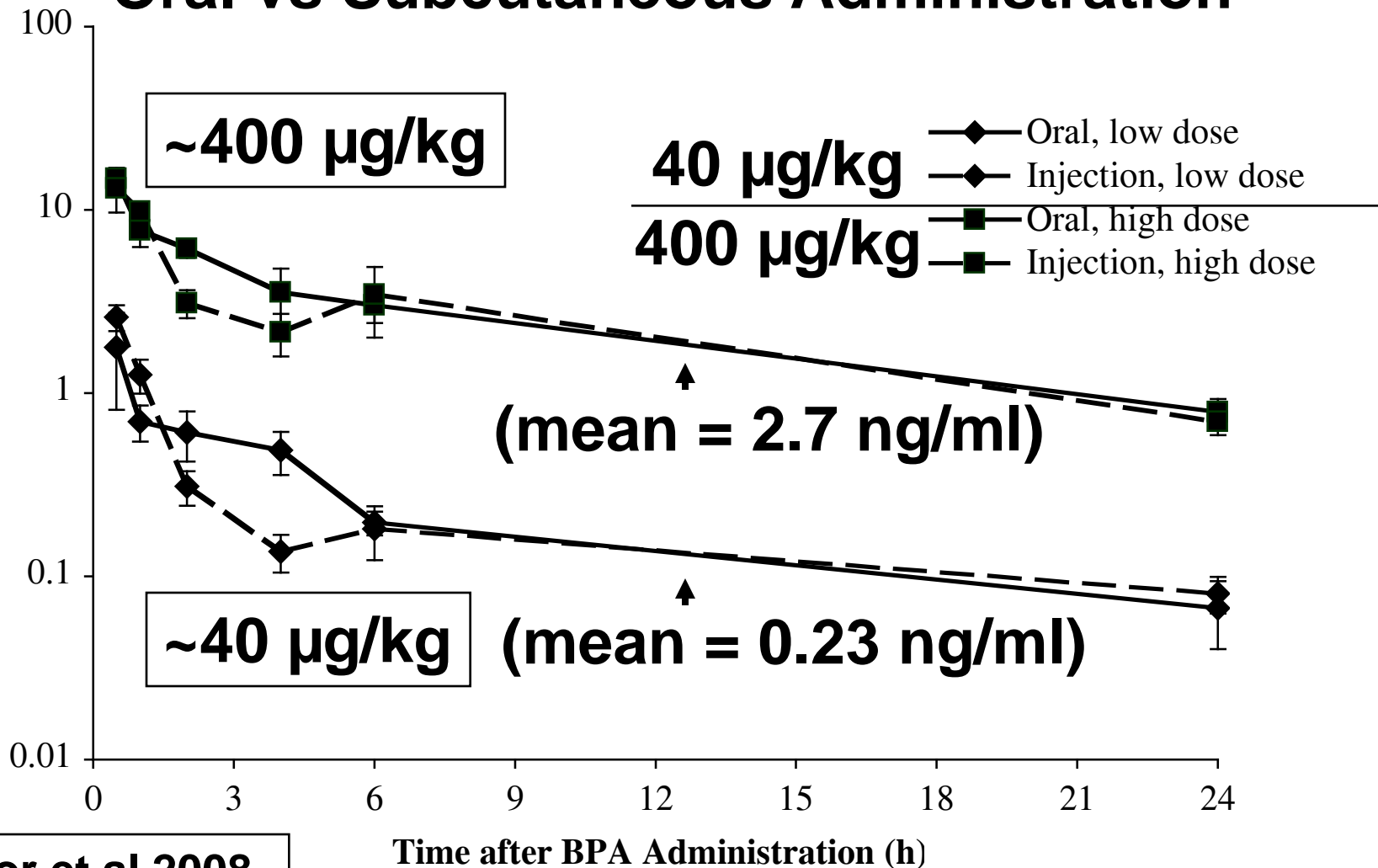
UNCONJUGATED SERUM BISPHENOL A CONCENTRATION IN PREGNANT WOMEN AND FETUSES (Parts Per Billion; ppb)



Schonfelder et al., 2002
Environ. Health Perspect.

PLASMA UNCONJUGATED BISPHENOL A IN NEWBORN MICE (14 / GROUP)

Oral vs Subcutaneous Administration

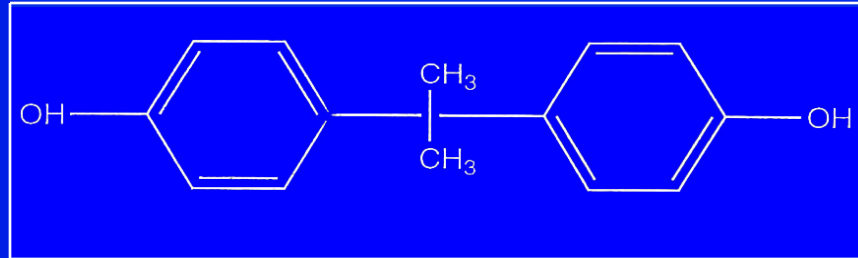


Taylor et al 2008
Reprod. Toxicol..

NATURAL AND MANMADE ESTROGENS

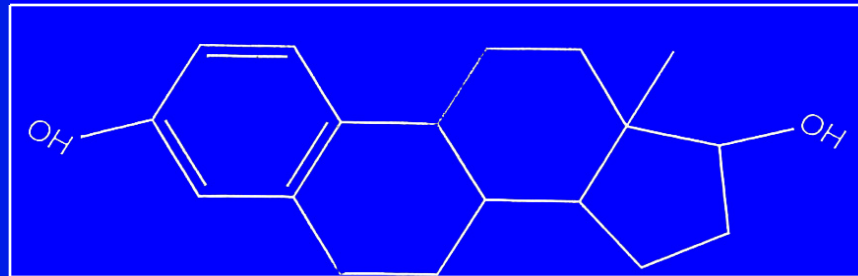
POLYCARBONATE PLASTIC

Bisphenol A



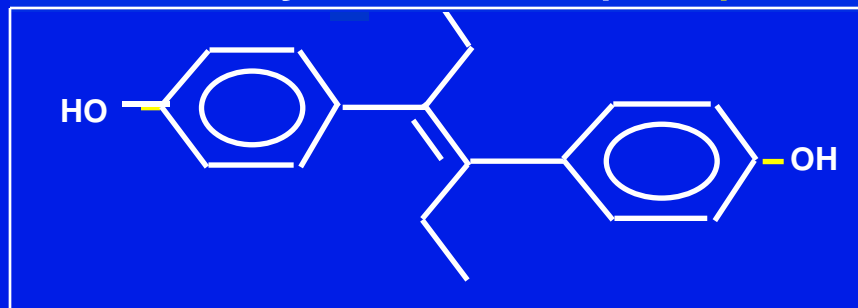
NATURAL HORMONE

Estradiol



ESTROGENIC DRUG

Diethylstilbestrol (DES)



ESTROGENIC POTENCY OF BISPHENOL A

ESTRADIOL AND BISPHENOL A ALTER:

Human Fat Cells

Human Prostate & Breast Cancer Cells

Rat Brain cells

Rat Pituitary Cells

Mouse Pancreatic Cells

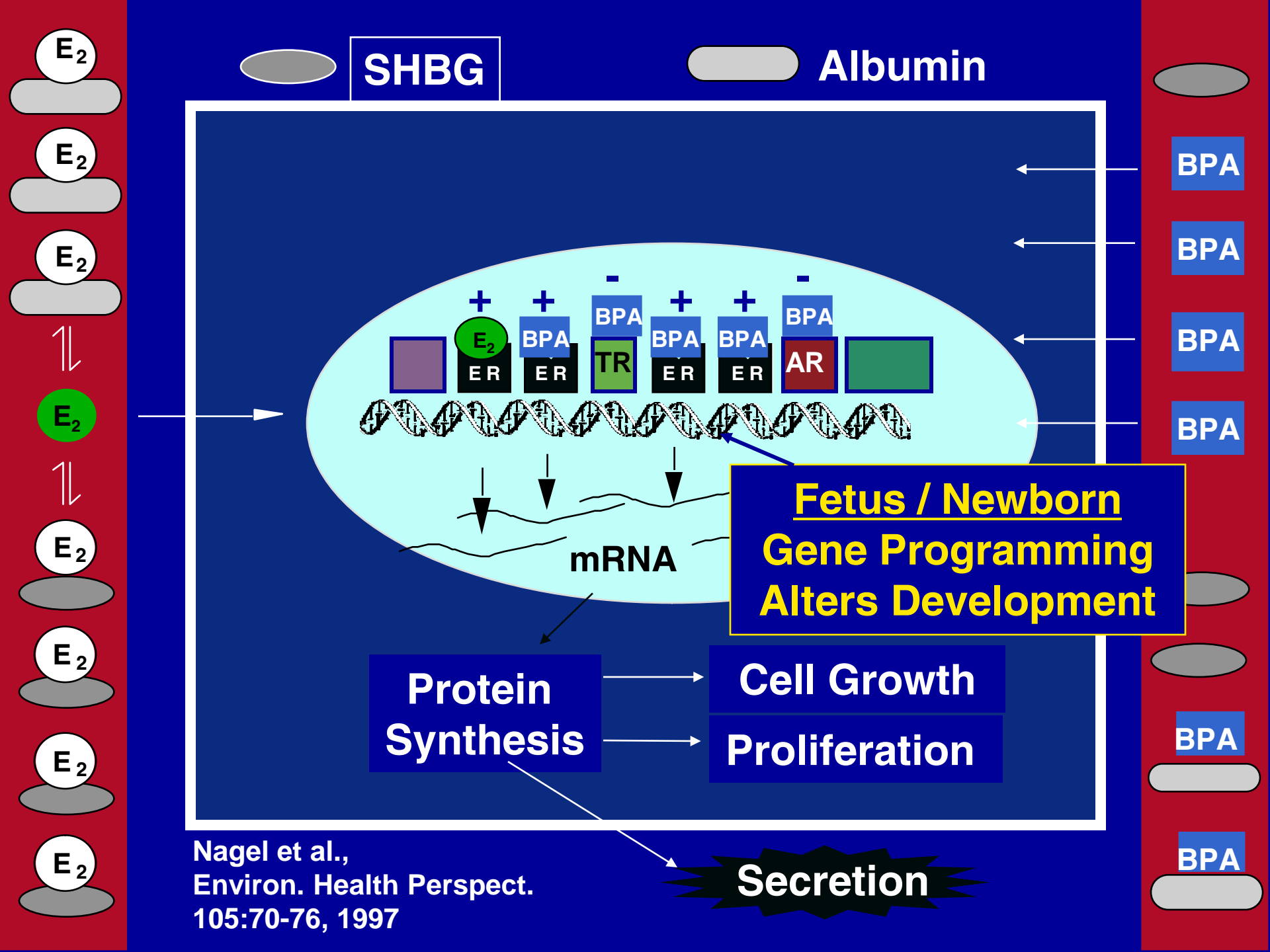
Mouse Prostate Cells

Human Blood Levels

0.01	0.1	1	10	100	1	10	100	1	10	100	1	10	100	1.0
	(pg/ml)				(ng/ml)			(µg/ml)			(mg/ml)			

Parts Per trillion | billion | million | thousand

Reviewed in: Welshons et al. Endocrinology 147:S56, 2006



SHBG

Albumin

BPA

BPA

BPA

BPA

**Fetus / Newborn
Gene Programming
Alters Development**

**Protein
Synthesis**

**Cell Growth
Proliferation**

BPA

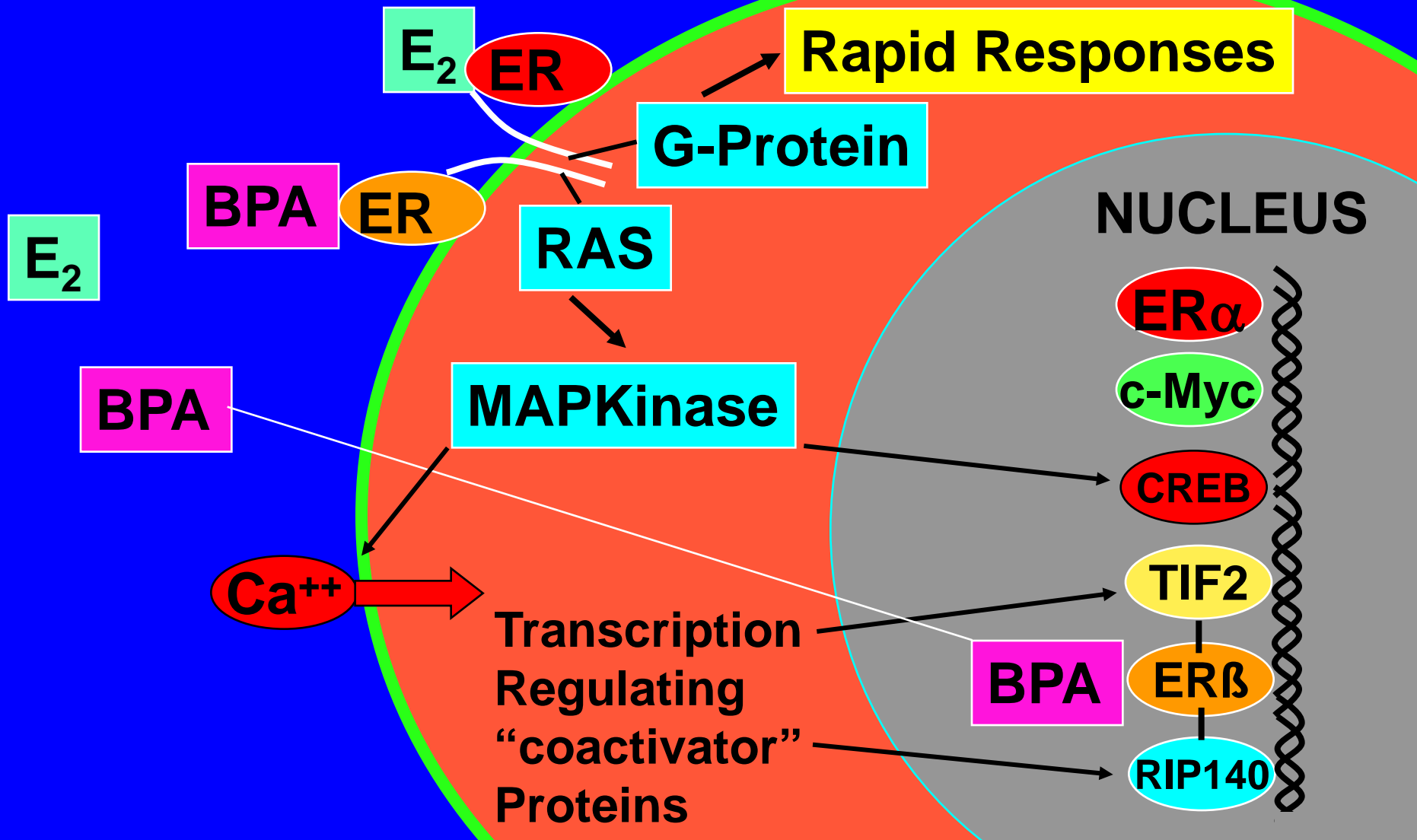
BPA

Secretion

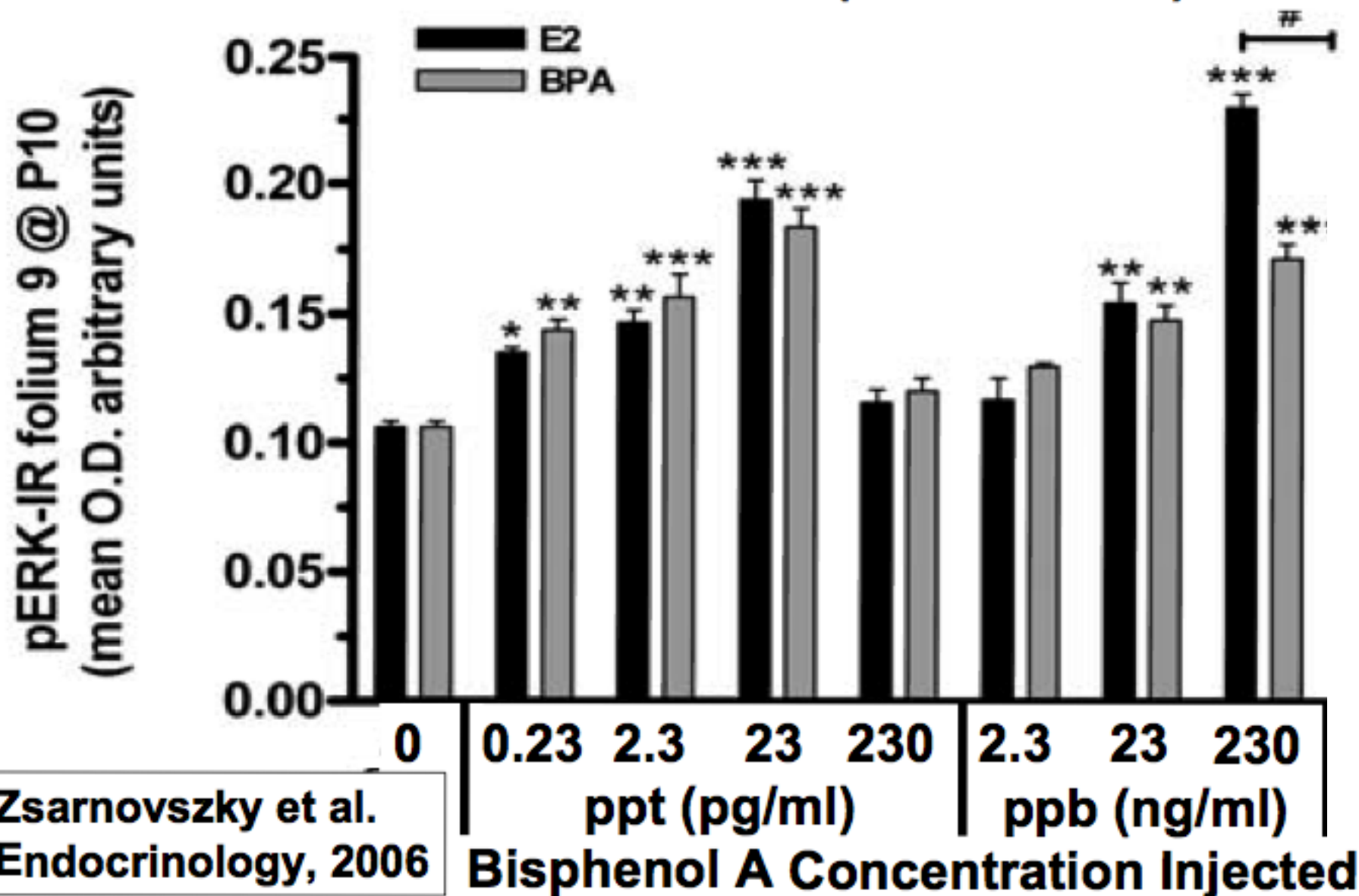
Nagel et al.,
Environ. Health Perspect.
105:70-76, 1997

ESTROGENS ALTER CELL FUNCTION BY:

1. ACTIVATING RAPID RESPONSE ENZYME SYSTEMS
2. BINDING TO NUCLEAR RECEPTORS
RECRUITING COACTIVATORS



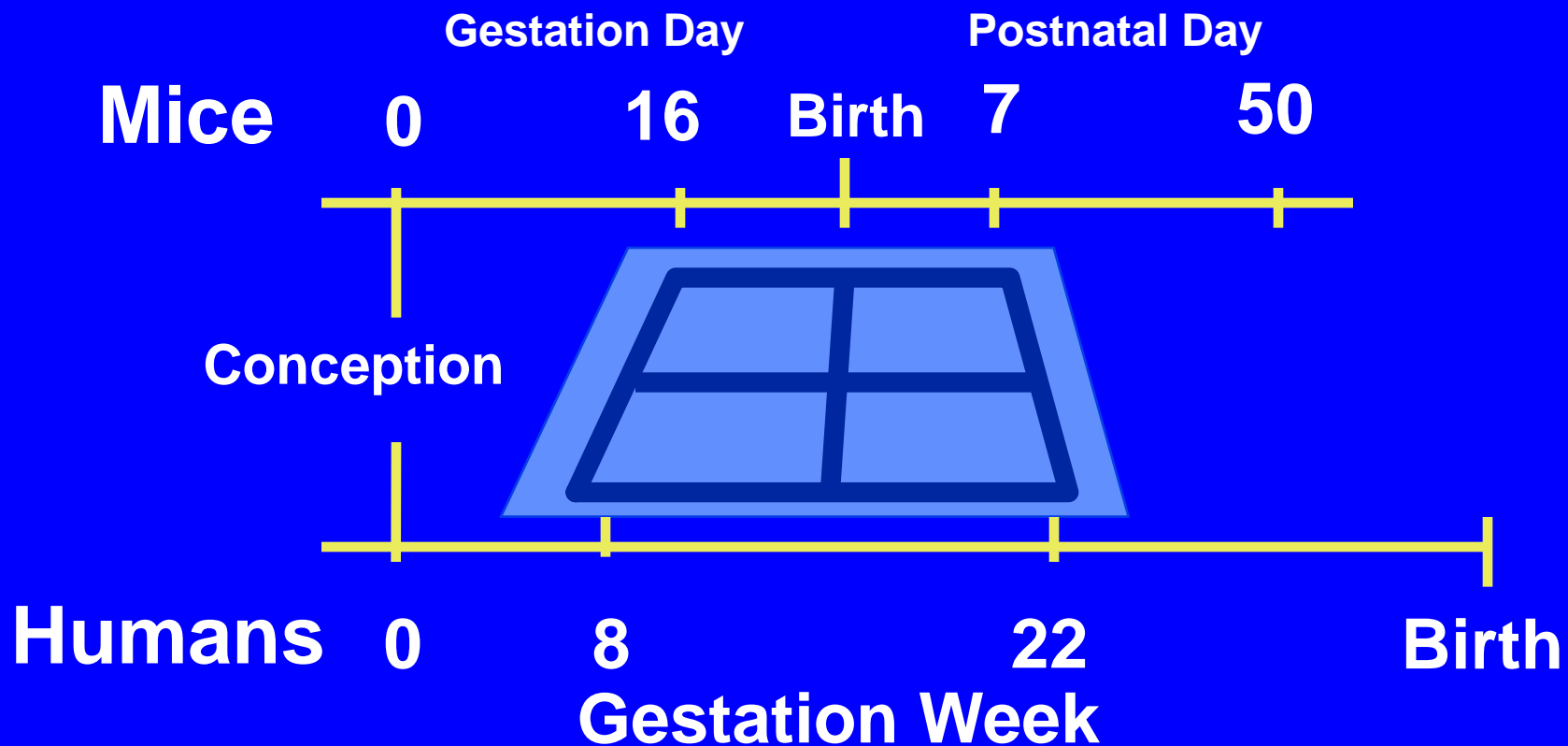
BISPHENOL A (AND ESTRADIOL) INJECTED INTO 10-DAY-OLD RAT CEREBELLUM INCREASES CELLS SHOWING PHOSPHORYLATION (ACTIVATION) OF ERK



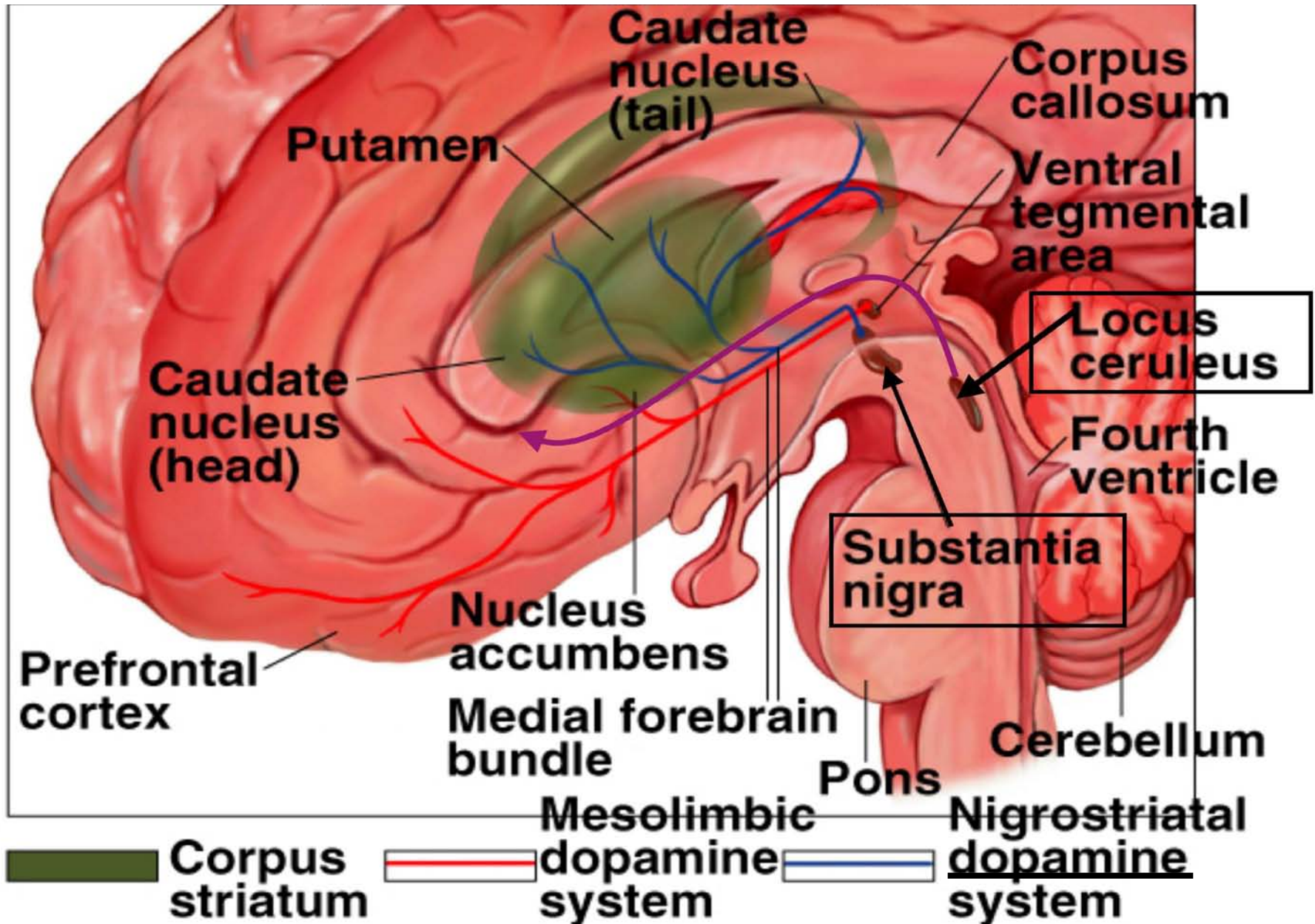
NEURO-BEHAVIORAL EFFECTS

**DEVELOPMENTAL EXPOSURE TO
LOW DOSES OF BISPHENOL A**

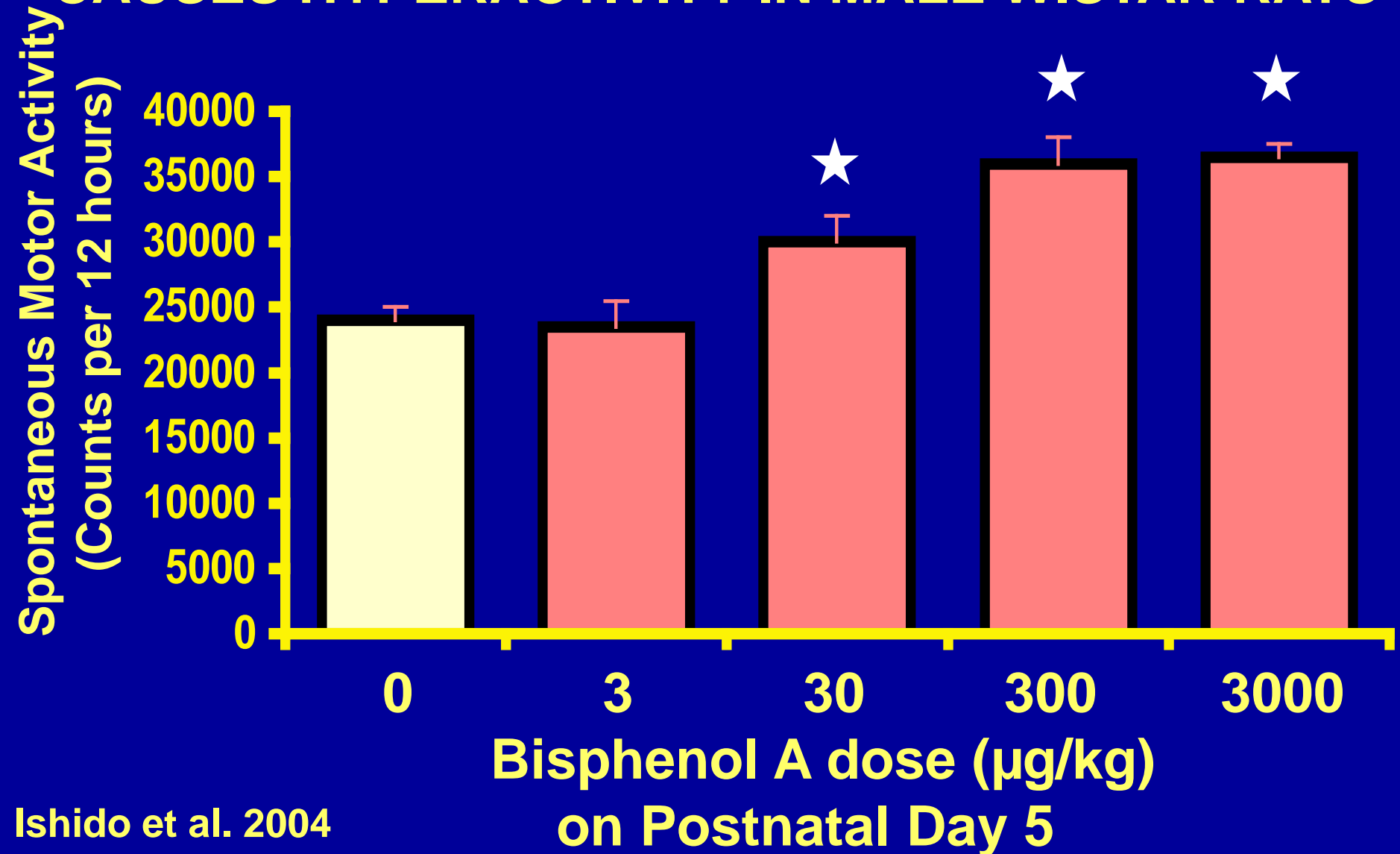
Developmental Window of Vulnerability of Reproductive Organs to Estrogenic Chemicals in Humans and Mice



BRAIN AREAS INVOLVED IN PARKINSON'S DISEASE

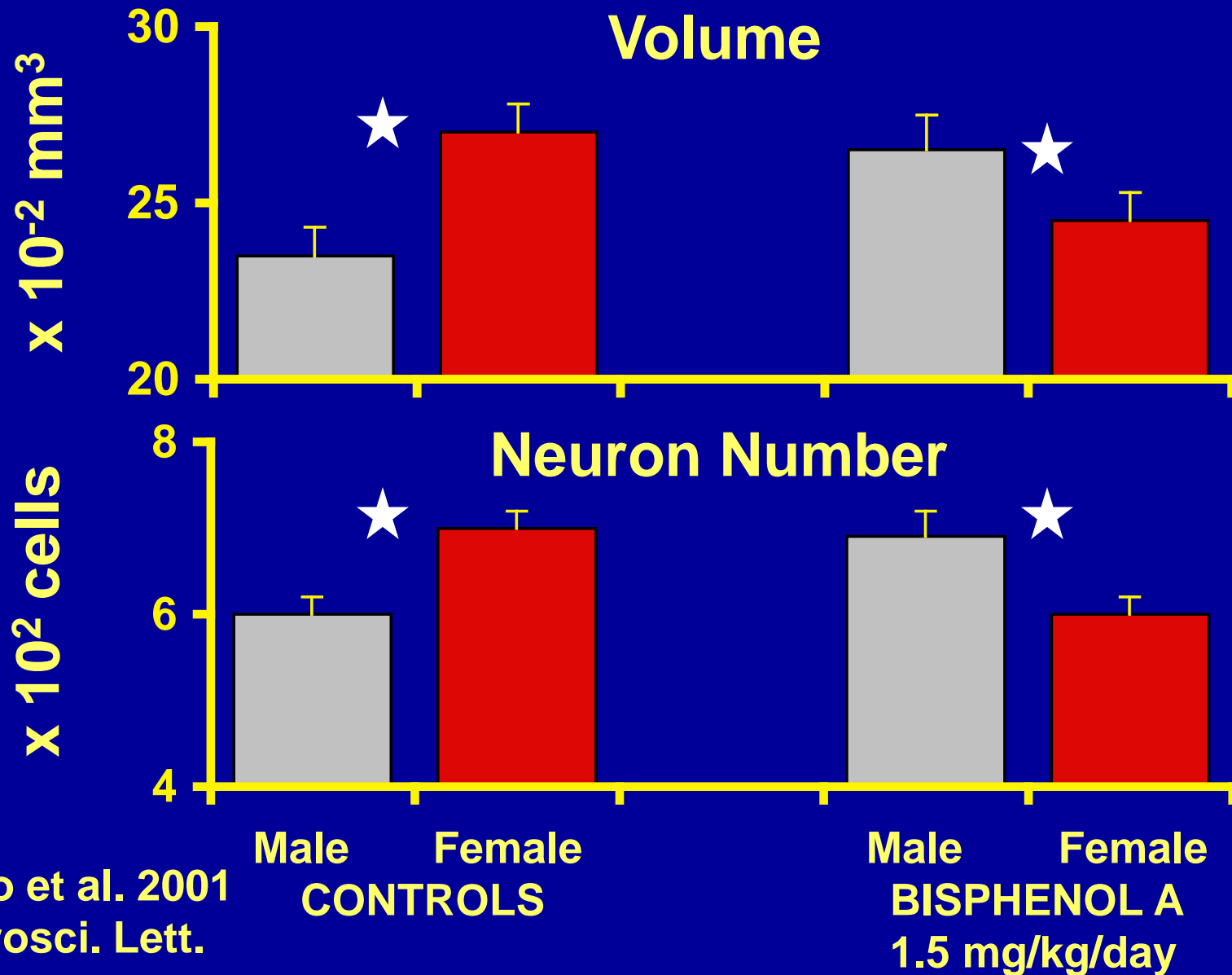


INTRACEREBRAL BISPHENOL A INJECTION: CAUSES HYPERACTIVITY IN MALE WISTAR RATS



Ishido et al. 2004
J. Neurosci. Res.

FETAL AND NEONATAL BISPHENOL A EXPOSURE ALTERS THE LOCUS COERULEUS IN WISTAR RATS



Kubo et al. 2001
Neurosci. Lett.

Number of Tyroxine Hydroxylase Positive Neurons in the Anterior Periventricular Preoptic Area (AVPV)

Number of Neurone

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

F M

Cont

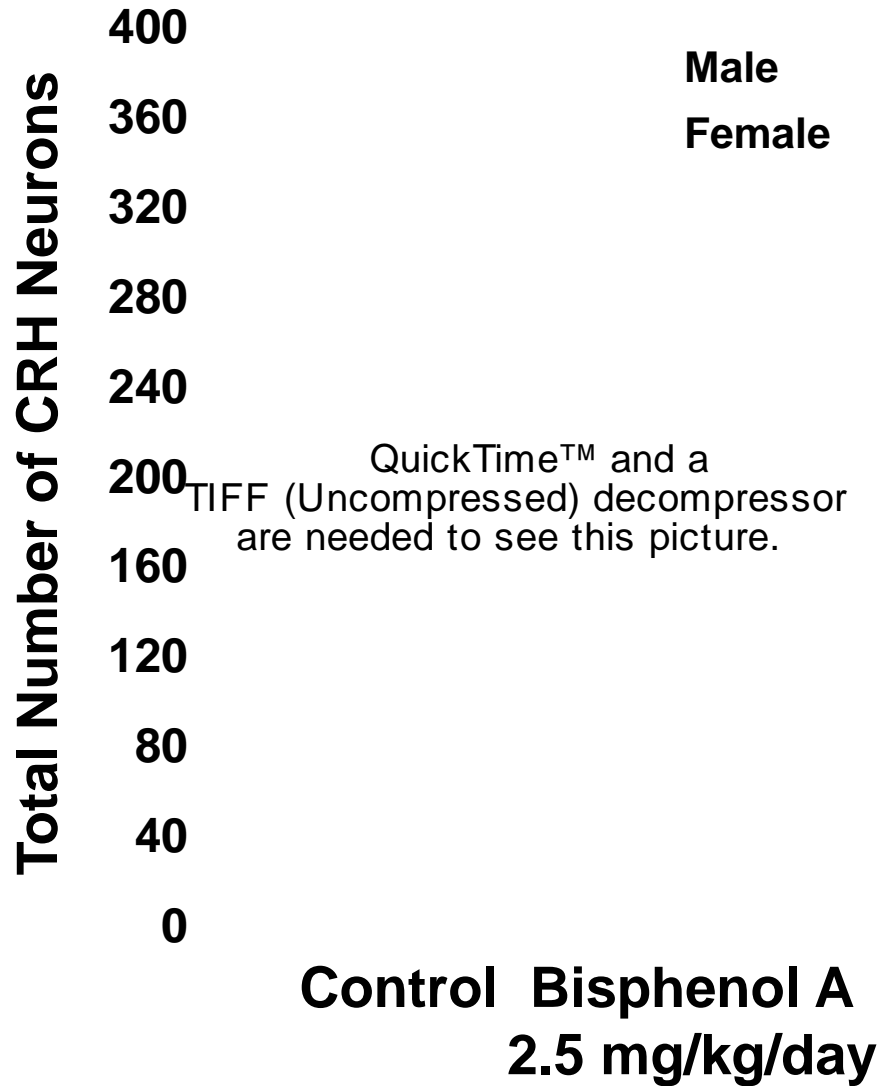
F M

25 ng/kg

F M

250 ng/kg

MATERNAL BISPHENOL A ELIMINATES SEX DIFFERENCES IN CORTICOTROPIN RELEASING HORMONE (CRH) NEURON NUMBER IN THE ANTERIOR BED NUCLEUS OF THE STRIA TERMINALIS IN RAT OFFSPRING



Cynomolgus Monkeys

Outward Looking

By Neonate By Mother

Frequency
(/120 5-sec Samples)
120
60
0

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

M and a
decompressor
this picture.

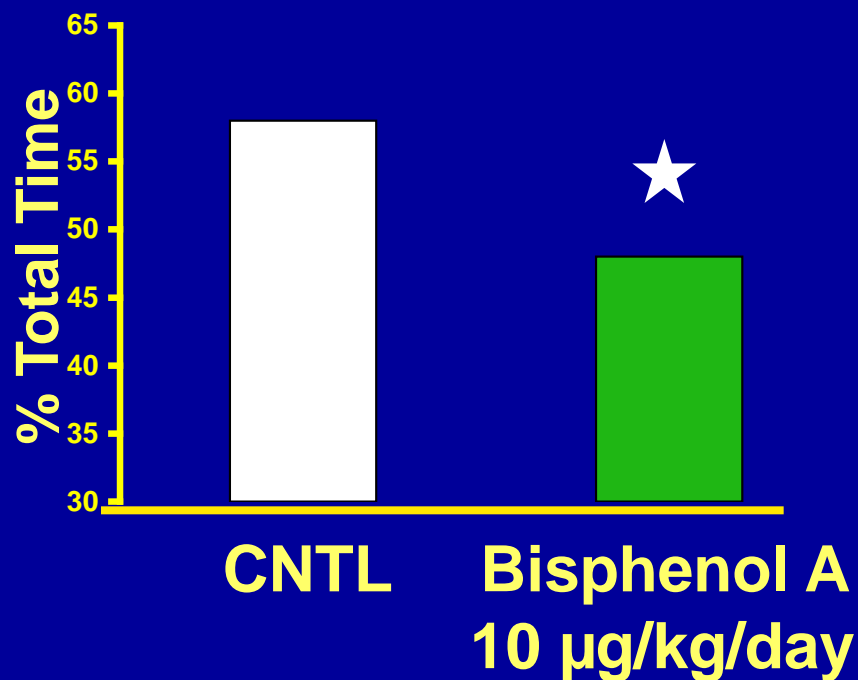
C BPA C BPA C BPA C BPA
Male Female Male Female

(Prenatal BPA Dose - 10 µg/kg/day)

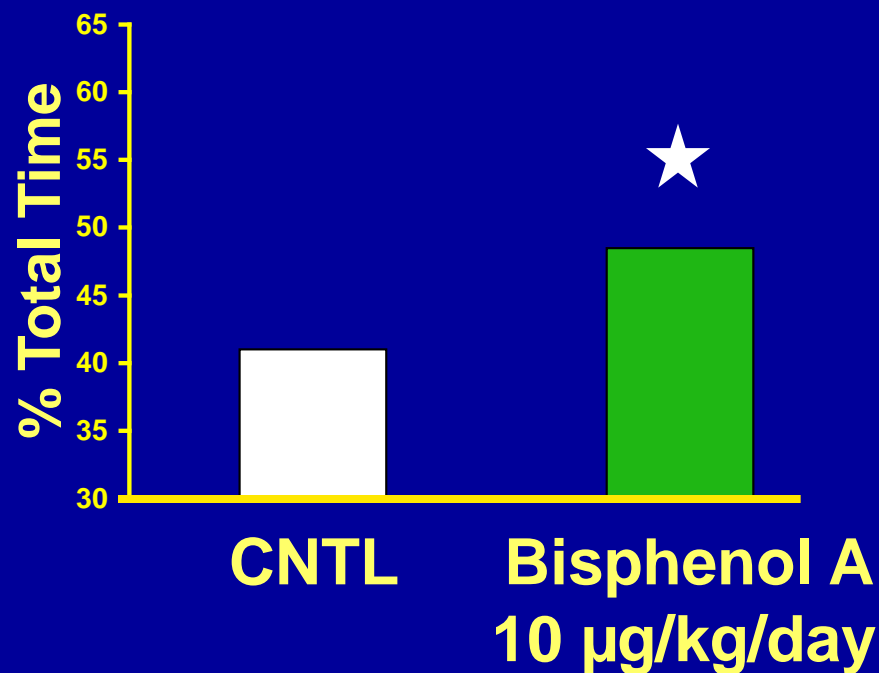
Nakagami et al. 2009
Psychoneuroendocrinol

ORAL EXPOSURE TO BISPHENOL A DURING PREGNANCY DECREASES NURSING BEHAVIOR AFTER PARTURITION IN CD-1 FEMALE MICE

PERCENT OF TIME NURSING

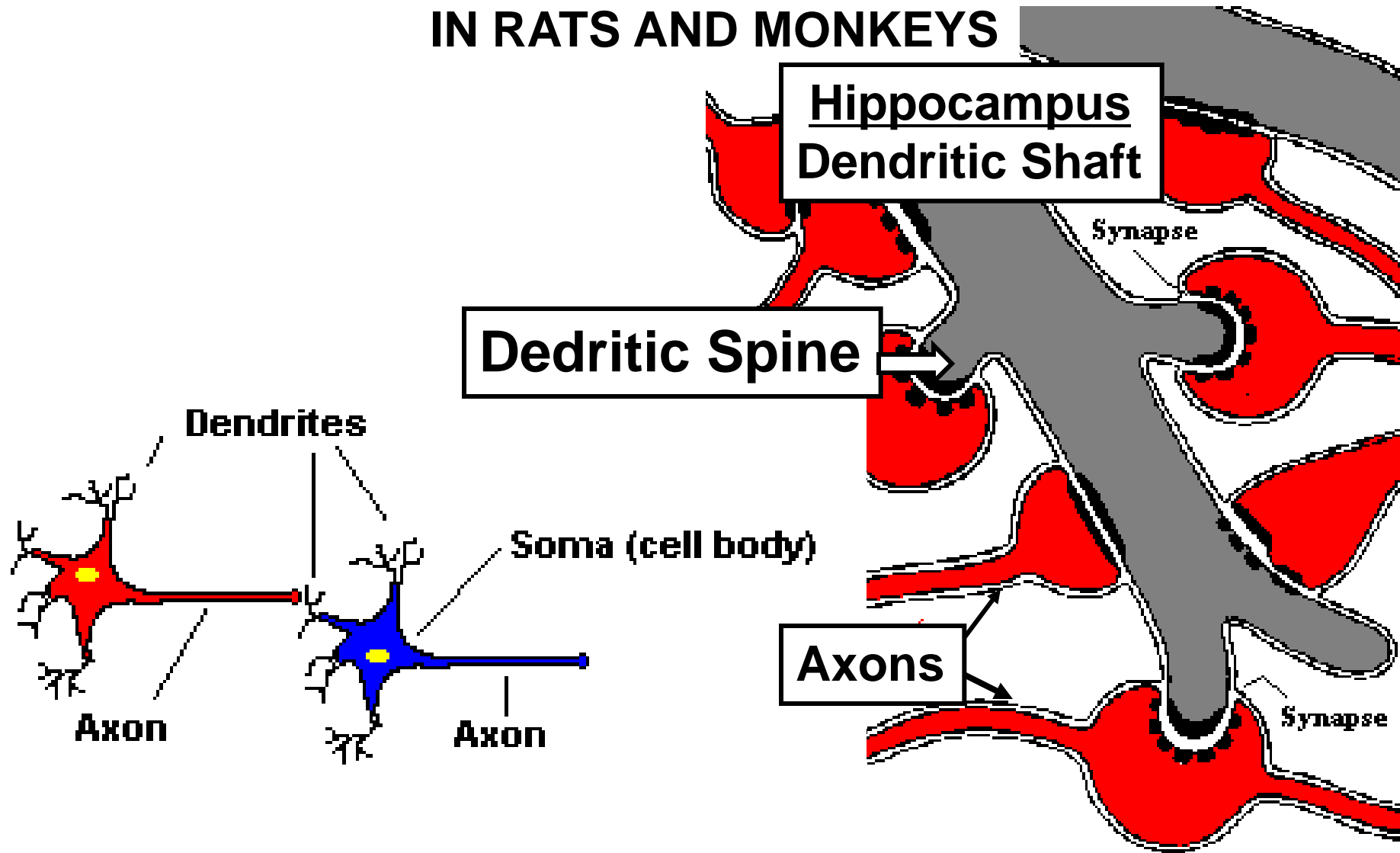


PERCENT OF TIME OUT OF THE NEST



Palanza al., 2002
Environ. Health Perspect.

BISPHENOL A REDUCES HIPPOCAMPAL DENDRITIC SPINES IN RATS AND MONKEYS



MacLusky et al. 2005
Env. Health Perspect.

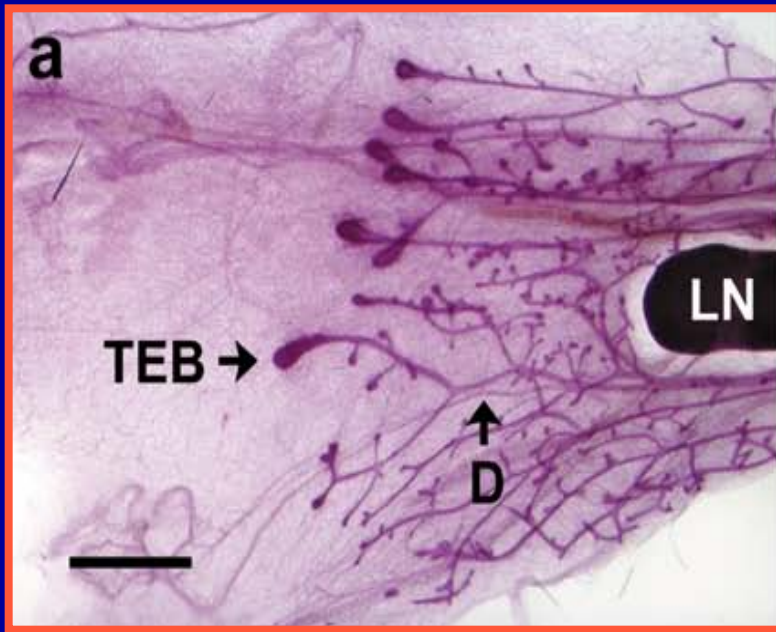
Leraith et al.
Endocrinol. 2007

Leraith et al.
PNAS 2008

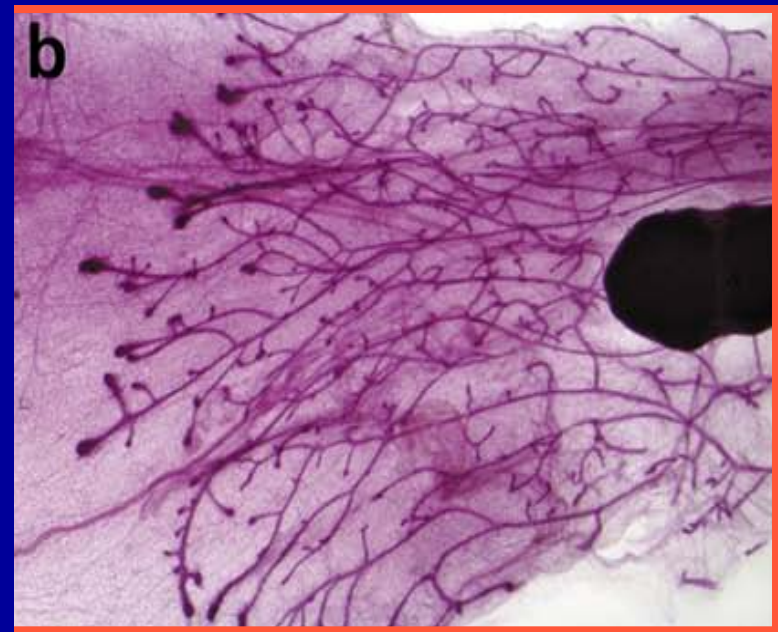
FEMALE REPRODUCTIVE SYSTEM EFFECTS

**DEVELOPMENTAL EXPOSURE TO
LOW DOSES OF BISPHENOL A**

Prenatal bisphenol A increases mammary gland duct size and number of terminal end buds in CD-1 mice 200,000-times below the current No Effect Dose



Control



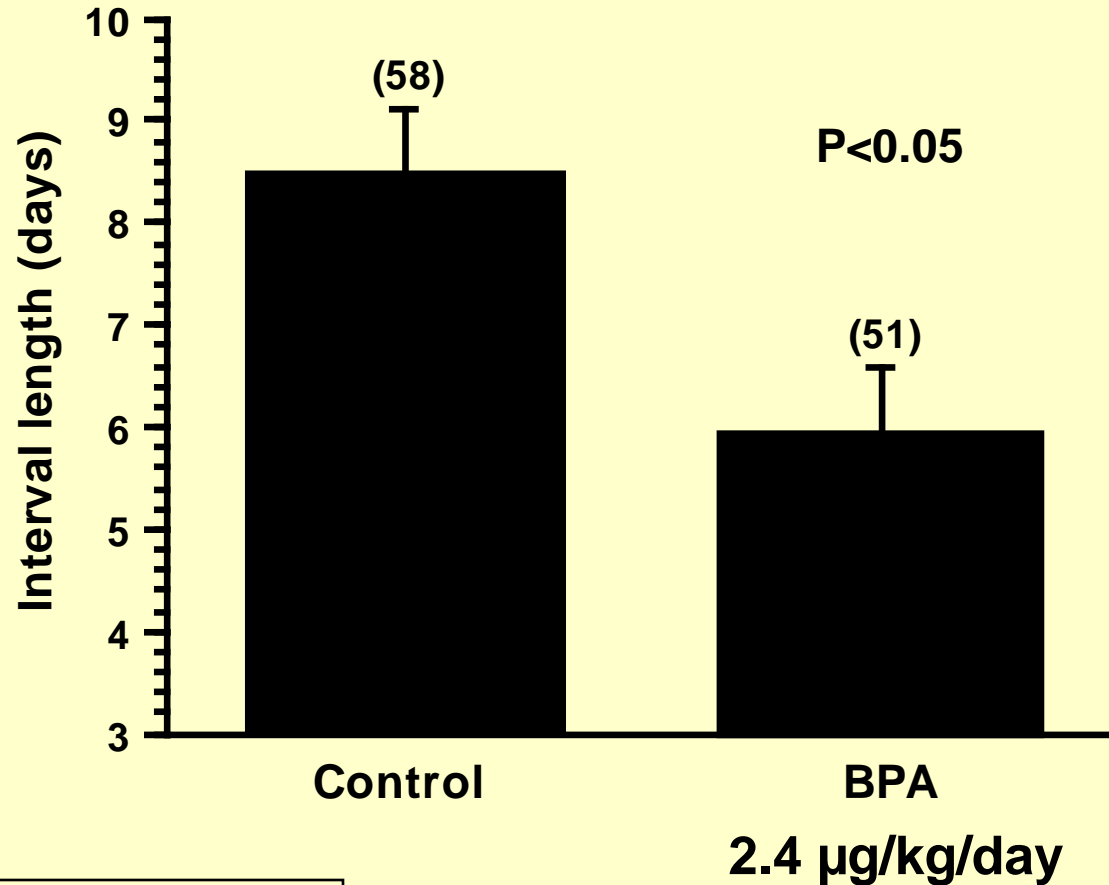
**0.025 µg/kg/day
BISPHENOL A**

**Markey et al., 2001
Biol. Reprod.**

DMBA INDUCED MAMMARY TUMORS IN OFFSPRING OF RATS EXPOSED ORALLY TO BPA DURING LACTATION

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

PRENATAL ORAL MATERNAL BPA SHORTENS THE INTERVAL BETWEEN VAGINAL OPENING (PUBERTY ONSET) AND FIRST VAGINAL ESTRUS (END OF PUBERTY - OVULATION) IN MICE



Howdeshell et al.
Nature 401: 763-764, 1999

Neonatal Bisphenol A Advances the Onset of Puberty in Female Rats

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Adewale et al. 2009
Biology of Reproduction

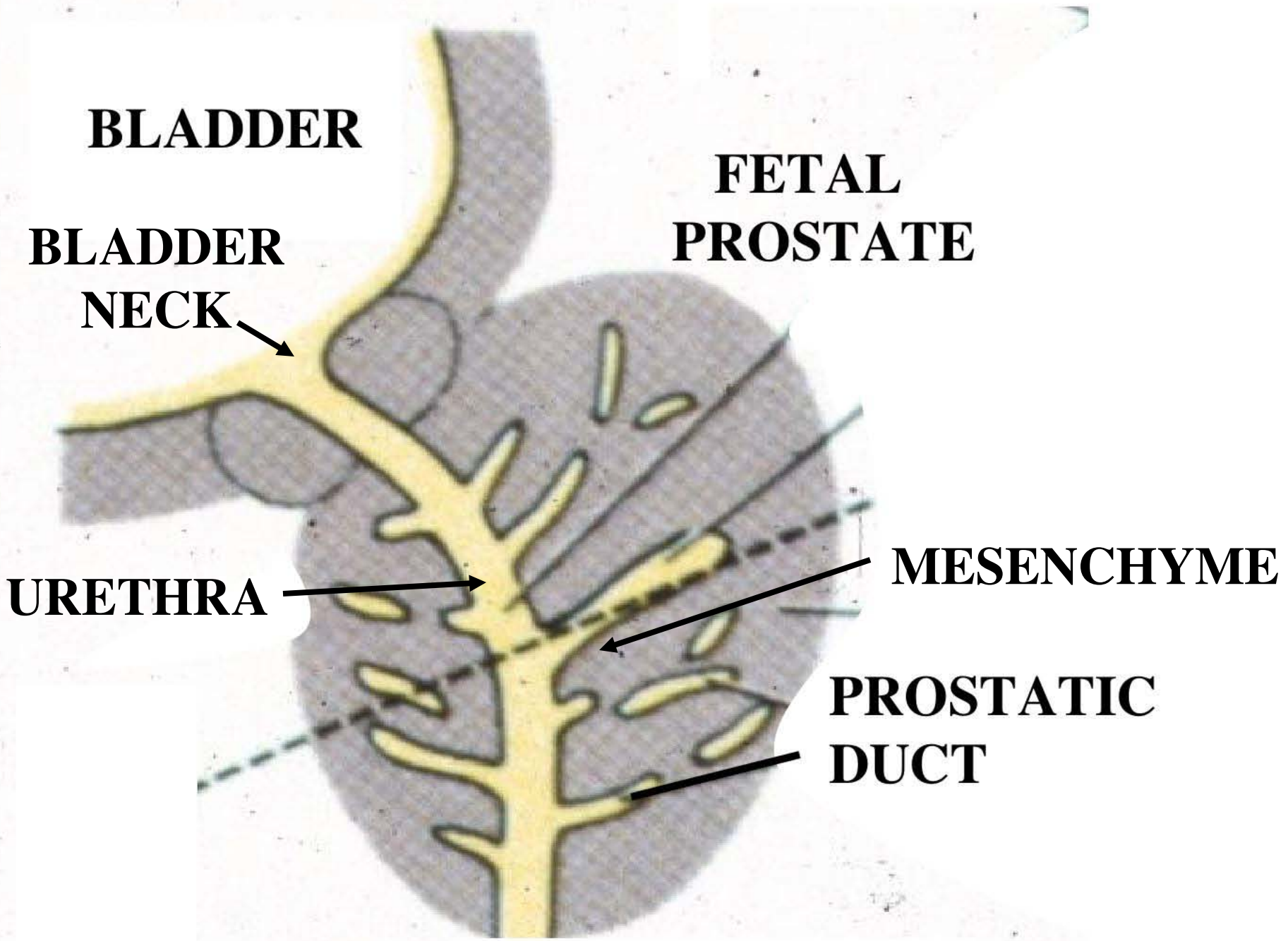
Neonatal Bisphenol A Causes Early Reproductive Senescence in Female Rats

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Adewale et al. 2009
Biology of Reproduction

MALE REPRODUCTIVE SYSTEM EFFECTS

**DEVELOPMENTAL EXPOSURE TO
LOW DOSES OF BISPHENOL A**



BLADDER

**BLADDER
NECK**

**FETAL
PROSTATE**

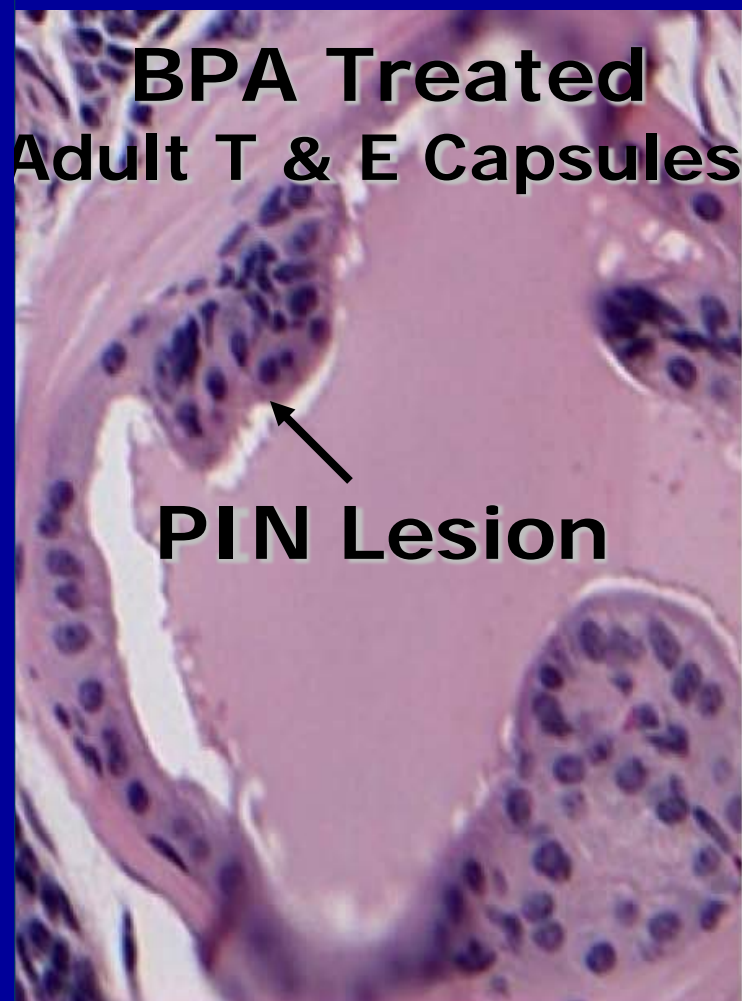
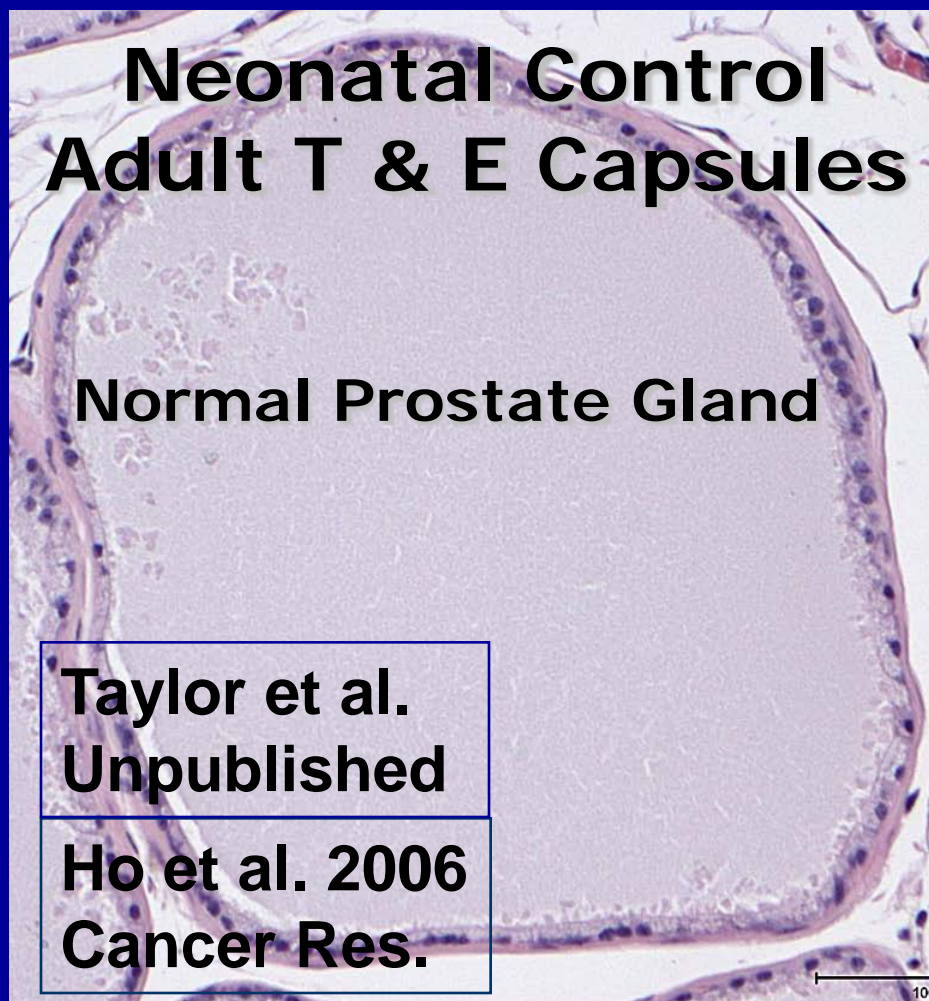
URETHRA

MESENCHYME

**PROSTATIC
DUCT**

QuickTime™ and a
TIFF (PackBits) decompressor
are needed to see this picture.

Neonatal Exposure to 10 $\mu\text{g}/\text{kg}/\text{day}$ BPA Prostatic Intraepithelial Neoplasia (PIN) (Early Stage Cancer) in Adult Mice and Rats



REPLICATED FINDINGS FROM PROSTATE STUDIES CONDUCTED IN INDEPENDENT LABORATORIES

- ◆ **Increase in Prostate Gland Number & Size**
- ◆ **Increase in Prostate Androgen Receptors**
- ◆ **Basal (Stem) Cell Hyperplasia / Metaplasia**
- ◆ **Prostatic Intraepithelial Neoplasia (Cancer)**
- ◆ **Changes in Epigenetic Programming
Associated with Cancer**

FINDINGS REJECTED BY THE NTP CERHR PANEL: “FAILED” EXPERIMENTS DUE TO NO EFFECT OF POSITIVE CONTROL

◆ Ashby et al. (1999) - 2, 20 µg/kg/day BPA

Prenatal Low dose BPA and DES (0.2 µg/kg/day):

No significant change in prostate weight.

Utility (Adequacy) for CERHR Evaluation Process: This paper is inadequate for the evaluation process due to absence of response of the positive control group and small sample sizes.

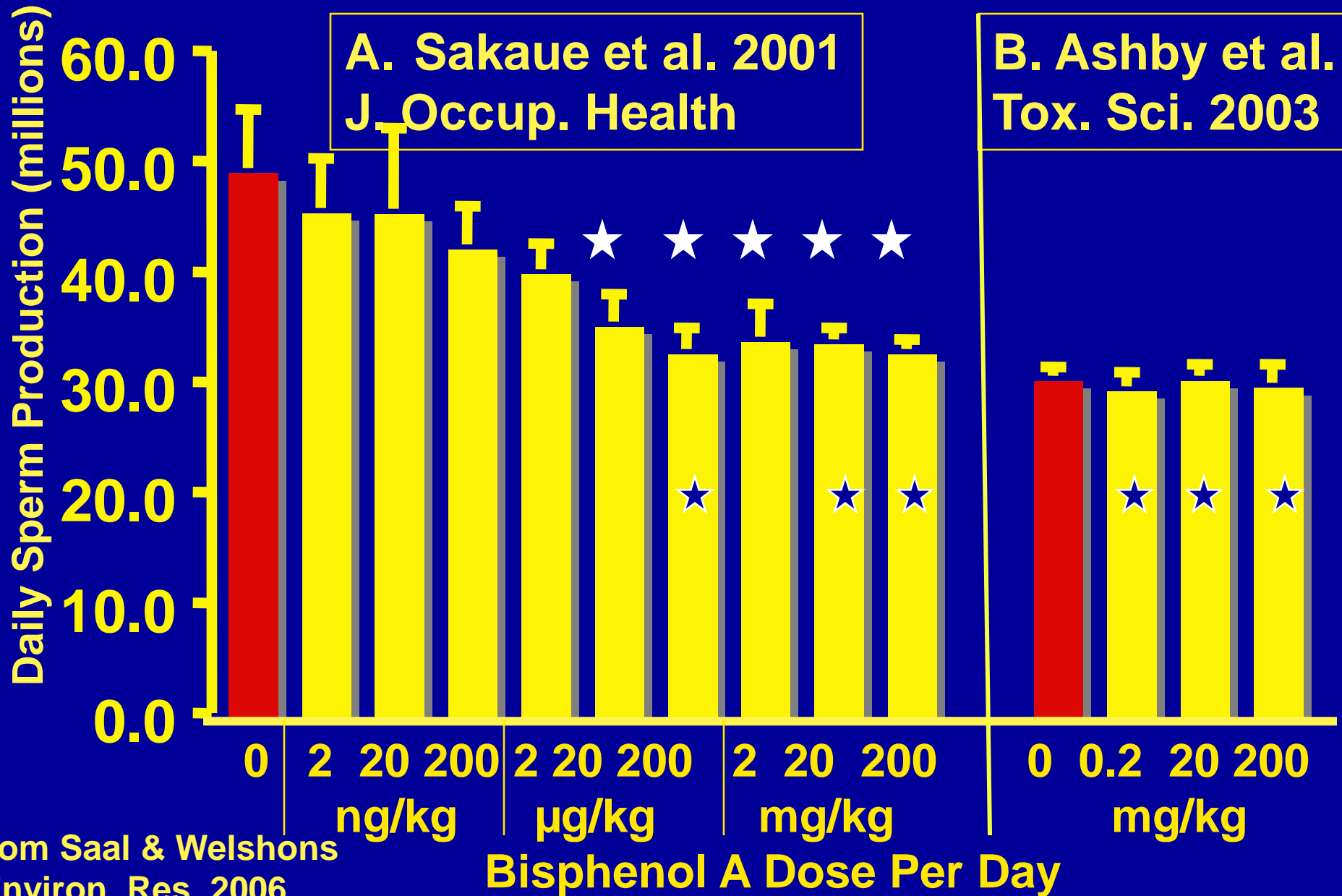
◆ Cagen et al. (1999) - 0.2, 2, 20, 200 µg/kg bw/day BPA

Prenatal Low dose BPA and DES (0.2 µg/kg/day):

No significant change in prostate weight.

Utility (Adequacy) for CERHR Evaluation Process: This paper is inadequate for the evaluation process due to absence of response of the positive control group.

COMPARISON OF RESULTS FROM: A. JAPANESE NIH AND B. CHEMICAL INDUSTRY STUDIES OF BPA



IN VIVO BISPHENOL A STUDIES THAT USED THE CHARLES RIVER SPRAGUE-DAWLEY (CD-SD) RAT

REPORTED STUDY OUTCOME

HARM	NO HARM	TOTAL
1	14	15

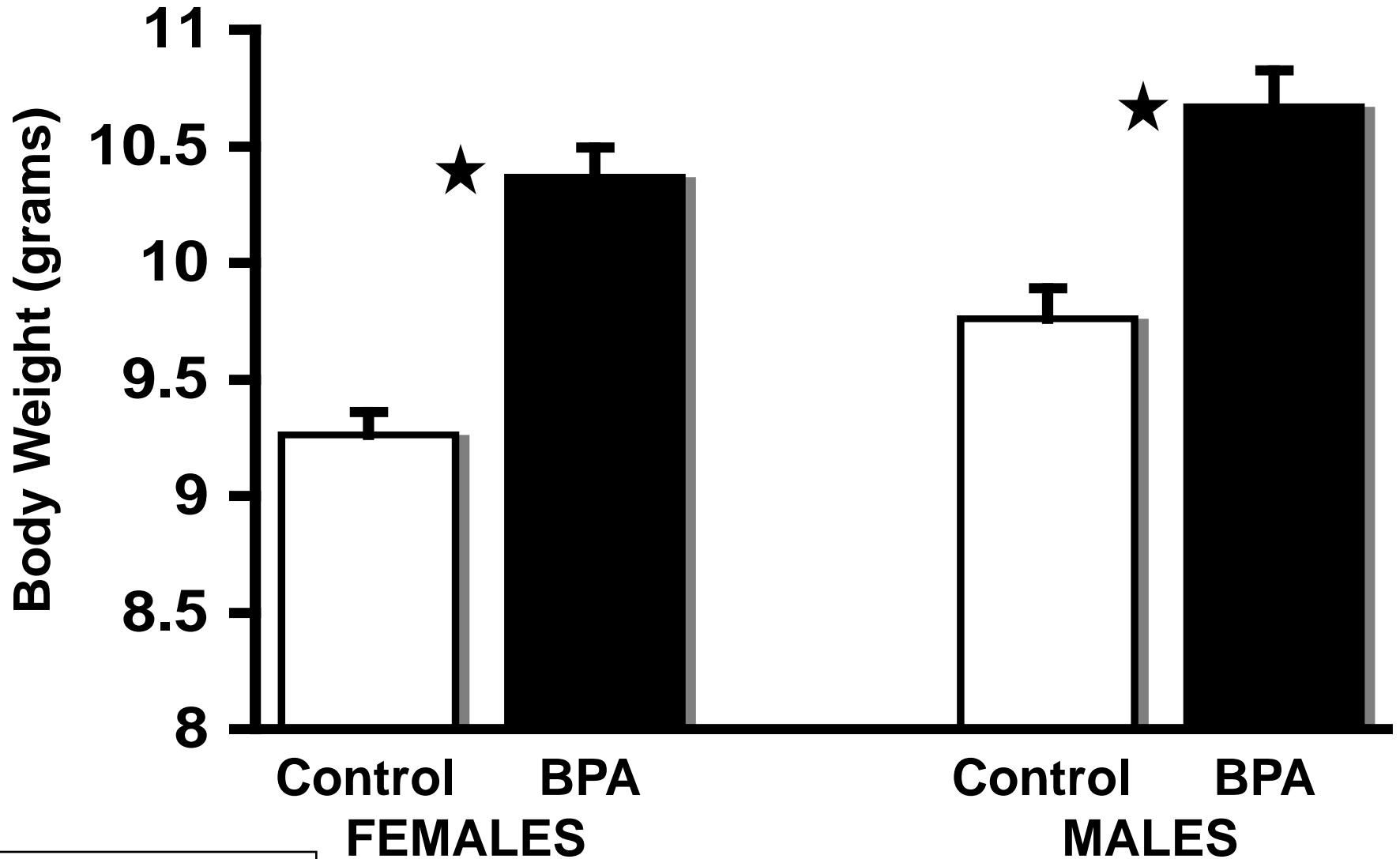
These rats do not respond to doses of the estrogenic drug ethinylestradiol (EE) in birth control pills (0.3 µg/kg/day) They require 5 - 200 µg/kg/day EE to show any gross effects.

**vom Saal and Hughes, 2005
Environ. Health Perspect.**

METABOLIC EFFECTS

**DEVELOPMENTAL EXPOSURE TO
LOW DOSES OF BISPHENOL A**

PRENATAL EXPOSURE TO BISPHENOL A INCREASES POSTNATAL BODY WEIGHT IN MICE



Howdeshell et al.
Nature, 1999

Maternal BPA dose = 2.4 $\mu\text{g}/\text{kg}/\text{day}$

PRENATAL AND NEONATAL MATERNAL ORAL BISPHENOL A (~70 $\mu\text{g}/\text{kg}/\text{day}$) INCREASES ABDOMINAL BODY FAT AND UPREGULATES ACTIVITY OF REGULATORY GENES IN FAT

BPA 
CONTROL 

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

BPA Inhibited Gene Methylation

Normal Gene Methylation

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

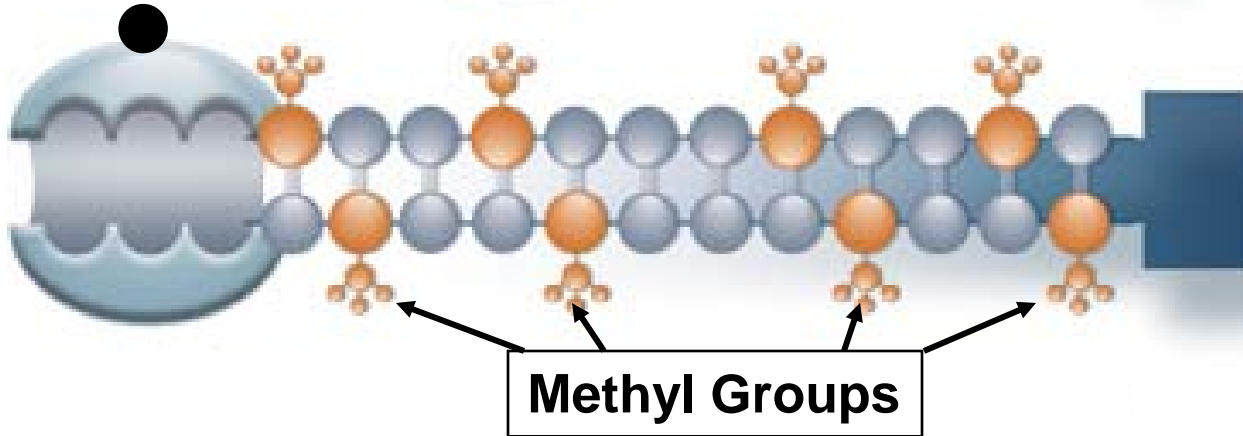
Dolinoy, Huang and Jirtle
PNAS 2007

PROMOTER REGION OF ESTROGEN-RESPONSIVE GENE

ESTROGENIC CHEMICAL

ESTROGEN RECEPTOR

Activates Gene Transcription

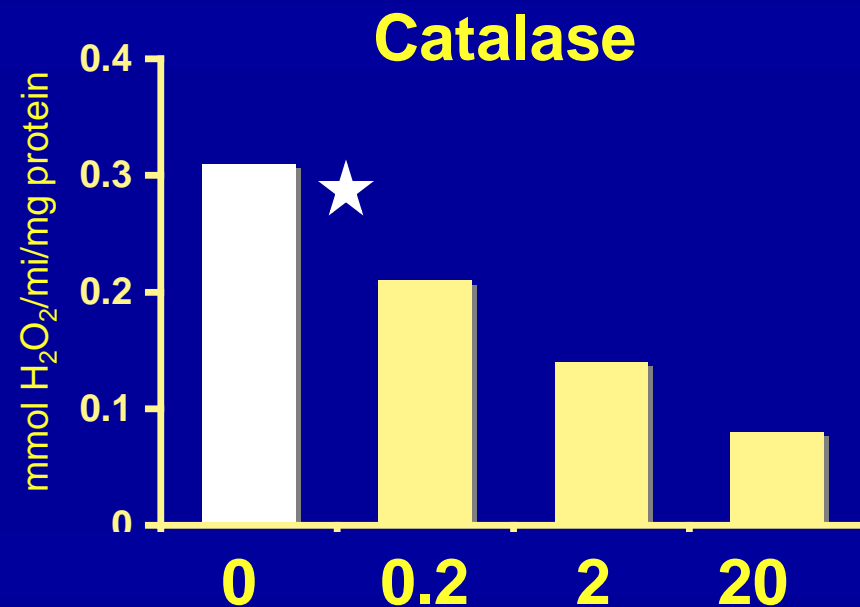
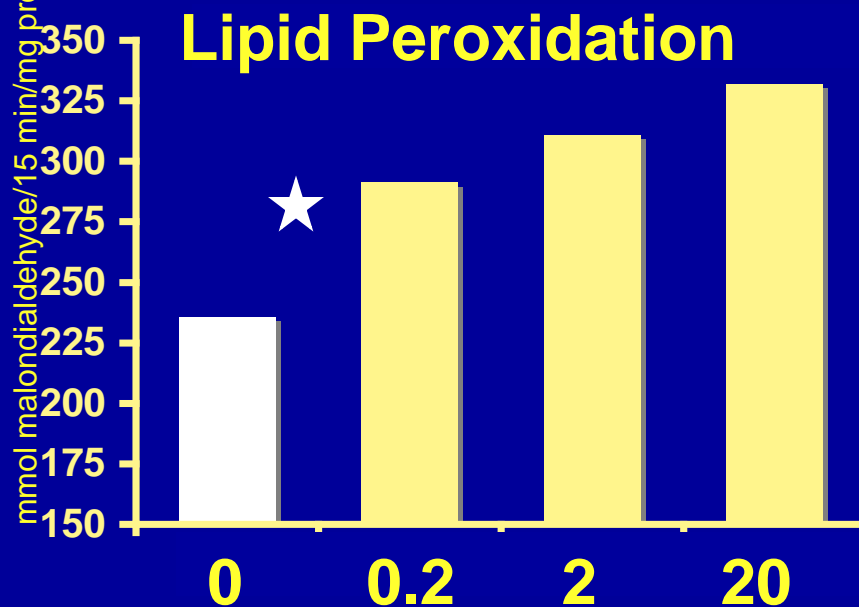
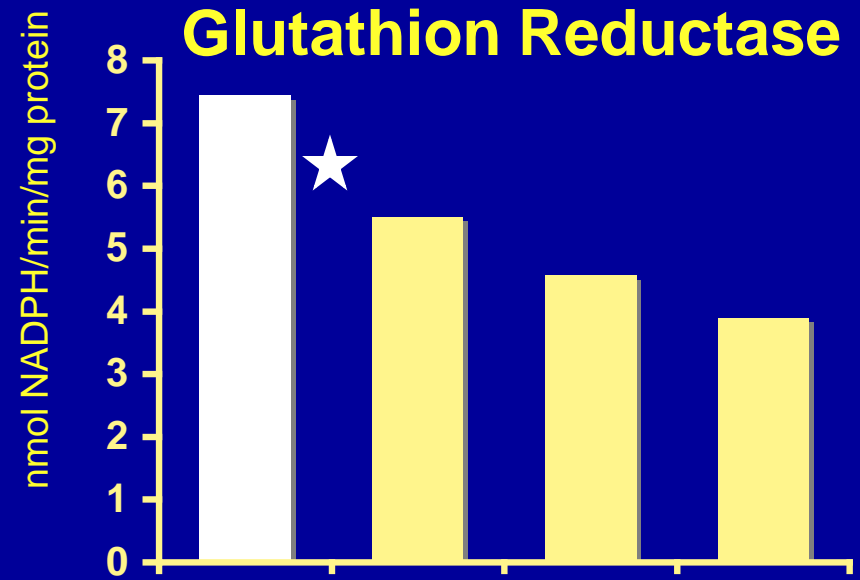
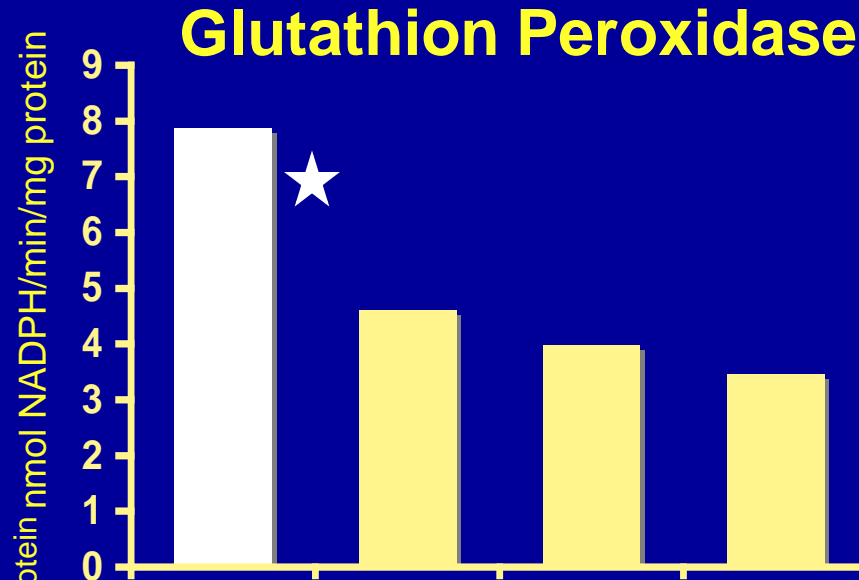


Gene Transcription Blocked

Methyl Groups

METHYLATION OF CYTOSINE BASES RESULTS IN "GENE SILENCING"

Prenatal Bisphenol A Alters Liver Enzymes in Rats



Bisphenol A ($\mu\text{g/kg/day}$)

Linear Regression Coefficients Associated with One Standard Deviation Increase in Bisphenol A Concentration

(Adjusted for age, sex and urinary creatinine)

β



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



Lang et al.
JAMA 2008

Odds Ratios of Disease Associated with One Standard Deviation Increase in Bisphenol A Concentration

(Adjusted for age, sex and urinary creatinine)



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



BPA EFFECTS IN MICE & RATS

□ (Developmental Exposure)

CANCER

Prostate hyperplasia & cancer

Mammary hyperplasia & cancer

HUMAN HEALTH TRENDS

Prostate cancer increase

Breast cancer increase

MALE AND FEMALE REPRODUCTIVE SYSTEM

Abnormal urethra / Obstruction

Sperm count decrease

Early puberty in females

Ovarian cysts / Uterine fibroids

Abnormal oocyte chromosomes

Early reproductive senescence

Hypospadias

Sperm count decrease

Early sexual maturation

PCOS / Uterine fibroids

Miscarriage

METABOLIC DISEASE

Body weight increase

Insulin resistance

Obesity increase

Type 2 diabetes

BRAIN AND BEHAVIOR

Hyperactivity / Impaired learning ADHD

Abnormal socio-sexual behavior

