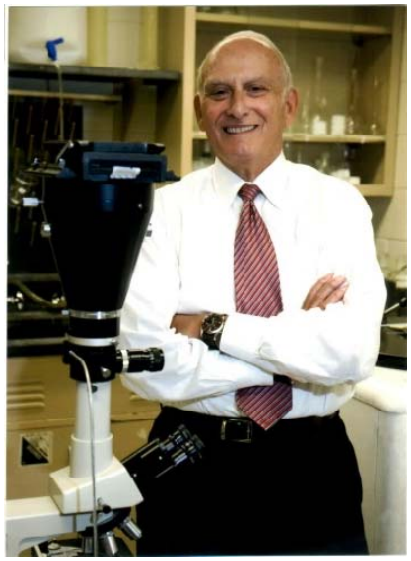
Concepts of Endocrinology: Issues Relevant to Endocrine Disruptor Screening



Raphael J. Witorsch, Ph.D.
Professor Emeritus
Physiology and Biophysics
School of Medicine
Virginia Commonwealth University
Richmond, Virginia

raphaelwitorsch@comcast.net

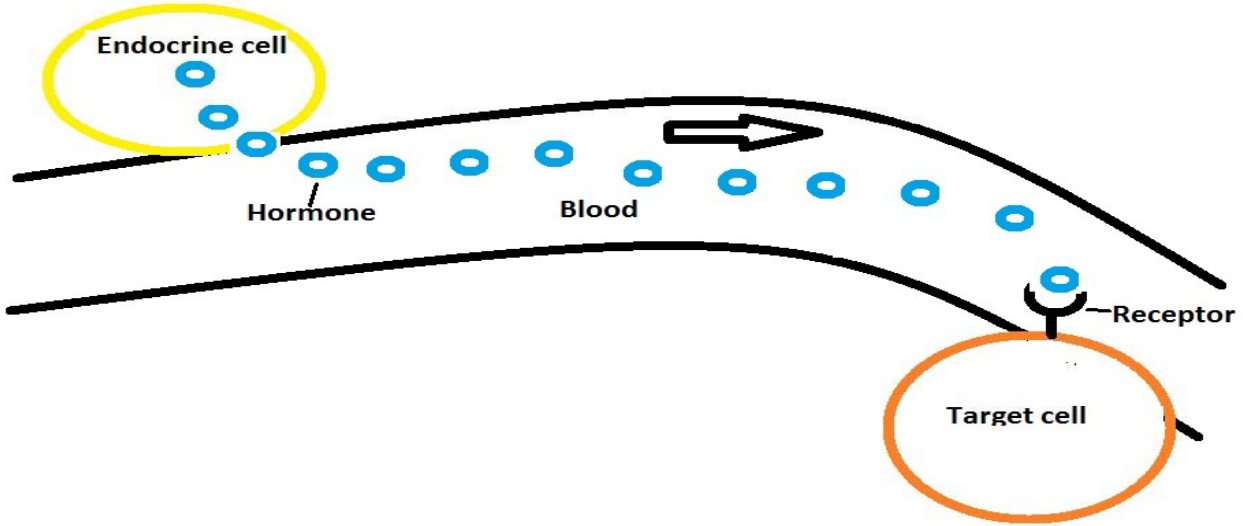
OBJECTIVES OF PRESENTATION

- To provide a basic overview of some of the principles of endocrinology.
- To discuss specific aspects of endocrinology relevant to the assessment of endocrine disruptors (ED) with particular emphasis on issues that may
  - currently be overlooked or underestimated;
  - complicate the results and/or interpretation of ED screening assays or experiments.

Endocrine System: A mode of communication

- Endocrine gland
  - A ductless gland whose secretions are released into the extracellular fluid for action at a remote site.

- Hormone (Gr. “to excite or arouse”)
  - The chemical messenger secreted by an endocrine gland.

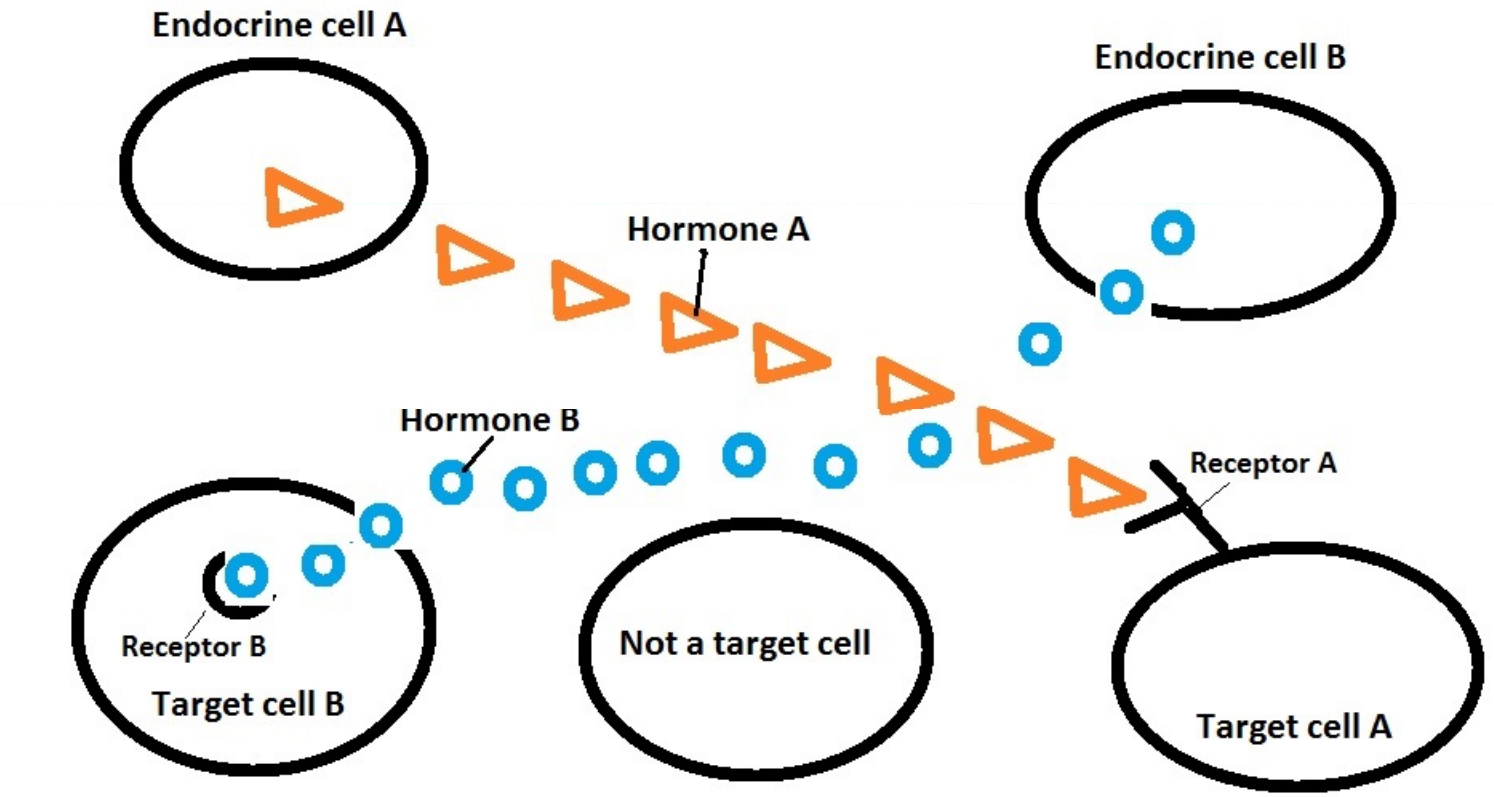


Hormones Synthesized and Secreted by Traditional Endocrine Glands

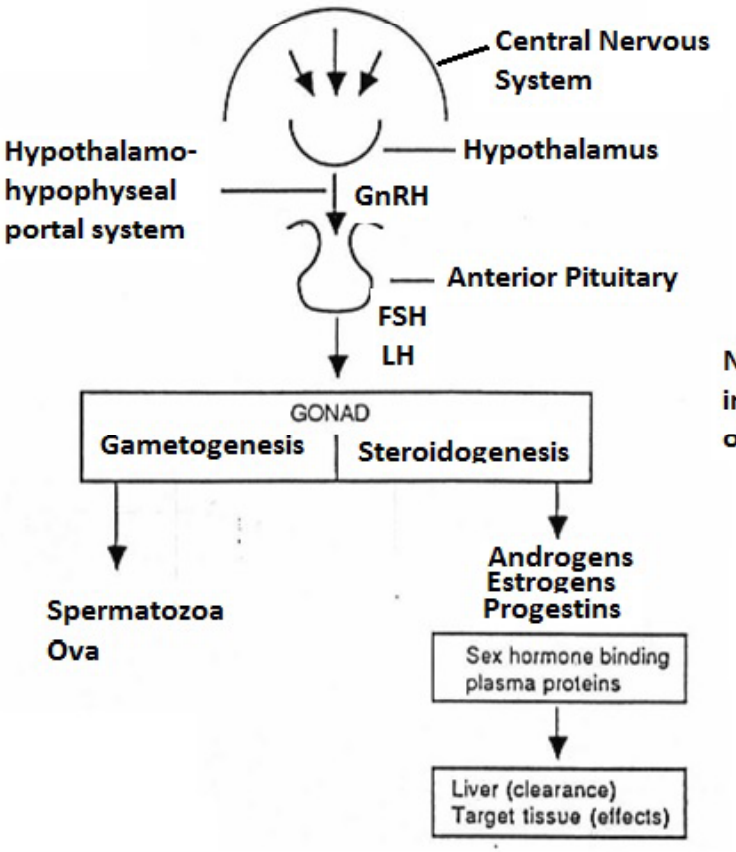
(protein/peptide, modified amino acid, steroid)

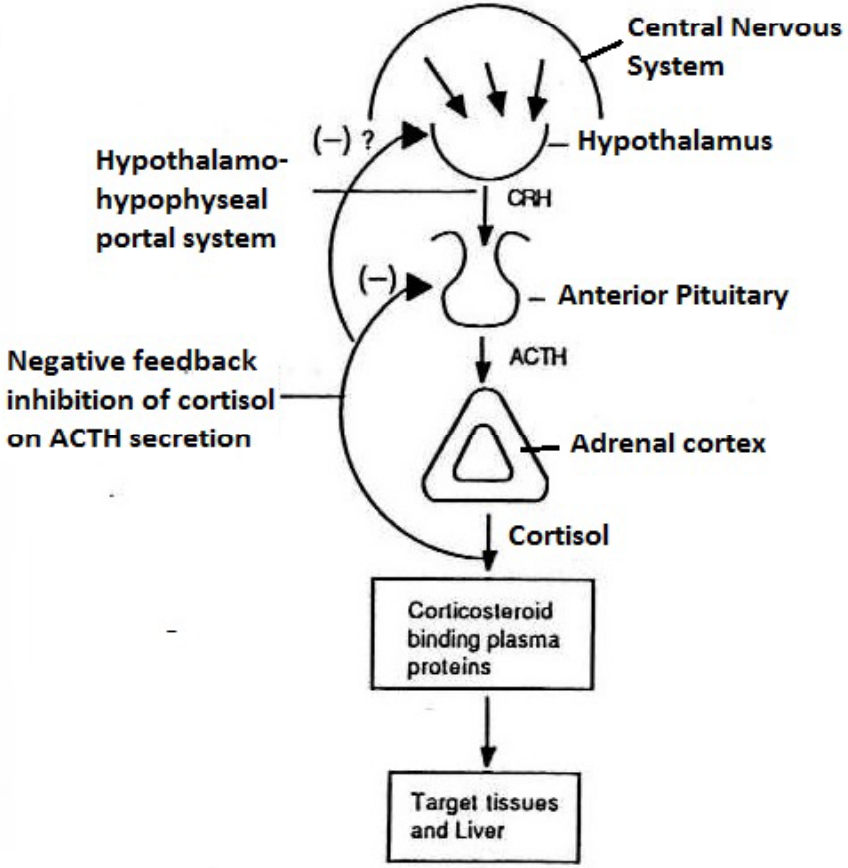
Gland	Hormone
Hypothalamus	releasing and inhibiting hormones: GnRH, TRH, CRH, somatostatin, PIH (dopamine)
Pituitary	anterior - growth hormone, prolactin, ACTH, TSH, FSH, LH posterior - ADH, oxytocin
Thyroid	thyroxine (T4), triiodothyronine (T3), calcitonin
Parathyroid	parathyroid hormone (PTH)
Pancreas	insulin, glucagon, somatostatin
Adrenal	cortex - cortisol, aldosterone, and DHEA medulla - epinephrine, norepinephrine
Ovary	estradiol-17β, progesterone, inhibin
Testes	testosterone, inhibin

Receptors: How a target cell recognizes a hormone



Hypothalamo-Pituitary Target Organ Systems





Endocrine Disruption

Effect of chemicals on endocrine function

Sites Where Endocrine Disruptors Can Act

- Hormone receptor
- Hormone secretion
- Hormone bioavailability, and clearance
- Feedback control

EDSP Tier 1 Battery of Screening Assays

In vitro

- ER binding (rat uterine cytosol)
- hERα transcriptional activation (ERTA) (HeLa-9903 cells)
- AR binding (rat prostate cytosol)
- Steroidogenesis H295R (human adrenocortical tumor)
- Aromatase (human recombinant microsomes)

In vivo

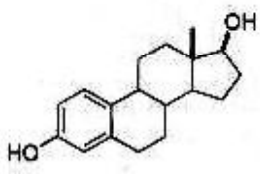
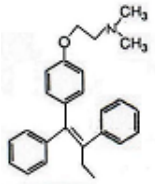
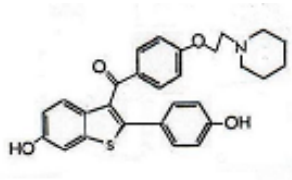
- Uterotrophic (rat, sc, ovex, uterine wt.)
- Hershberger (rat, oral, orchidex, peripubertal, sex organ wts)
- Pubertal female (rat, oral, time of vaginal opening, TSH, T4, etc.)
- Pubertal male (rat, oral, preputial separation, TSH, T4, etc.)
- Amphibian metamorphosis (tadpole to frog, thyroid histology)
- Fish short-term reproduction (male, female fathead minnows, morphological and biochemical endpoints)

Other Assays Relevant to Endocrine Disruptor Assessment

- Multigeneration studies
- In utero effects on offspring

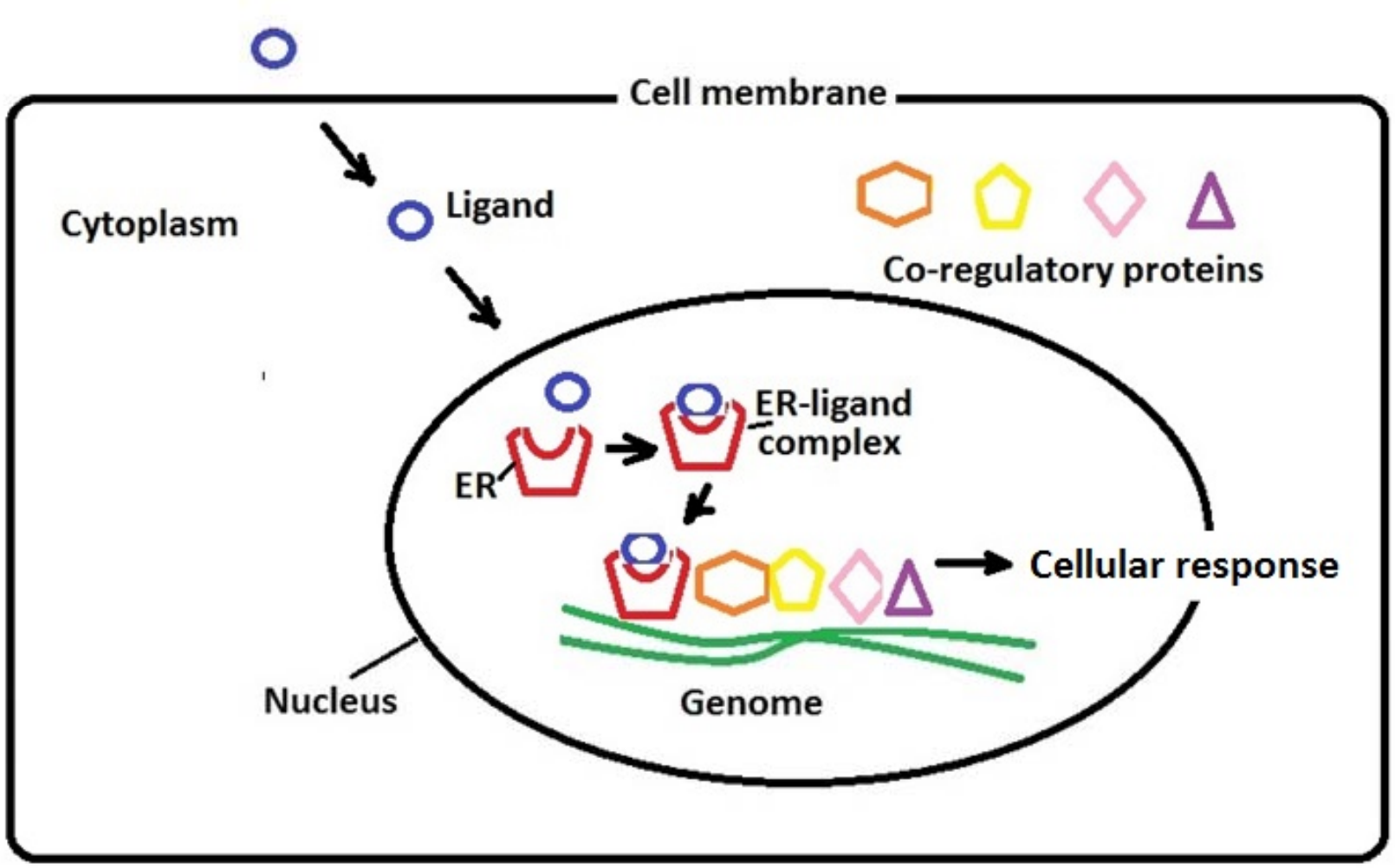
Receptor Binding Does Not Predict The Nature of a Biological Effect

Diversity of Biological Effects of ER Ligands

ER ligand	Mammary gland	Uterus	Bone
 17β-estradiol	mitogenic	mitogenic	anti-osteoporotic
 tamoxifen	anti-mitogenic	mitogenic	anti-osteoporotic
 raloxifene	anti-mitogenic	anti-mitogenic	anti-osteoporotic

Adapted from:
Witorsch, Toxic Substance Mechanisms 19: 53-78, 2000.

ER Mechanism of Action



Factors Contributing to Diversity of Response of Estrogen Receptor Ligands

- Heterogeneity of ER subtypes (ERα, ERβ)
- Ligand specific alterations in ER conformation
- Heterogeneity of co-regulatory proteins
- Heterogeneity of ER binding sites within the genome (estrogen response elements)
- Alternate (non-genomic) pathways
- ER signaling may “cross-talk” with other pathways (e.g., aryl hydrocarbon)

Species Differences May Be Important

Endocrinology of Pregnancy: Murine vs. Human

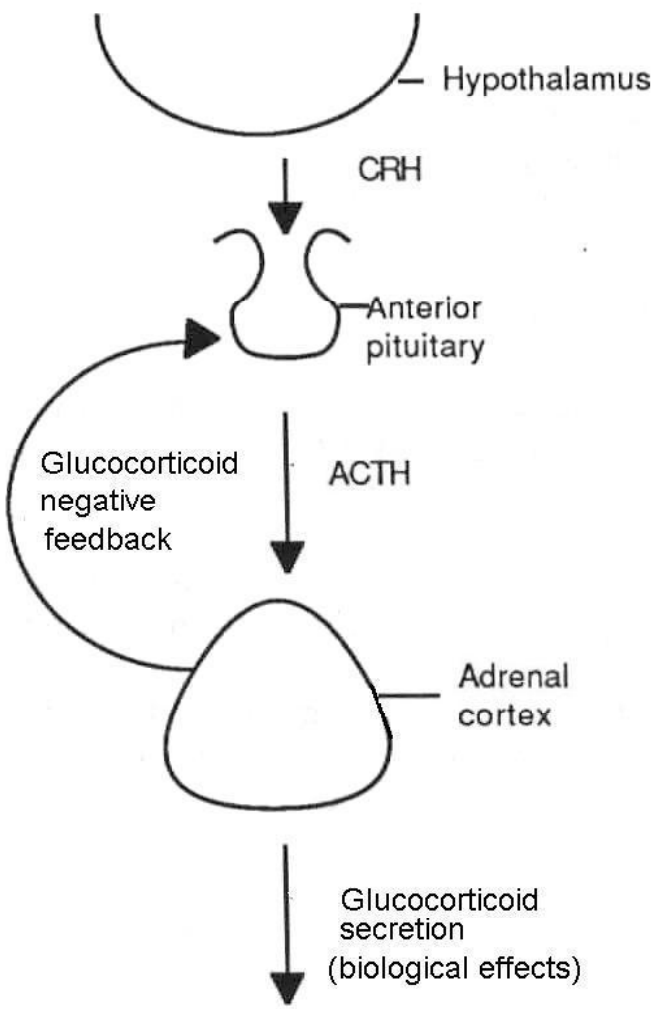
(*Witorsch,RJ, Food and Chemical Toxicology 40: 905-912, 2002)*

Endpoint	Murine	Human
Dominance of corpus luteum	Throughout gestation	First trimester *(luteal-placental shift)*
Adrenal DHEA as estrogen precursor	No	Yes
Estrogens produced	Estradiol, estrone	Estradiol, estrone, *estriol*
Relative bioavailable estrogen	1	<100

Human vs. Murine Gestation

- Basic physiological differences
  - Role of corpus luteum
  - Pathways of estrogen production
  - Types of estrogens produced
  - Levels of bioavailable estrogen (<100-fold higher in humans)
- In utero species differences suggest that humans might be resistant to adverse effects of environmental estrogens observed in murines.

Stress, A Potential Confounder in Endocrine Disruptor Assessment



Secretory Patterns of ACTH and Glucocorticoid

Basal (diurnal or circadian): Recurring increase followed by a decrease every 24 hrs as a function of subject's activity pattern.

Stress-induced: Abrupt or prolonged increase in response to homeostatic disruption (or "stressor").

Effects of Stress or Glucocorticoid Excess on Reproduction and Development

- Testicular dysfunction
  - impaired testosterone production and Leydig cell apoptosis
- Ovarian hypofunction, amenorrhea, and infertility in women and comparable effects in animals
  - a "multistage' effect on the hypothalamo-pituitary-ovarian axis.
- Fetotoxic effects and post-partum "programming"

Cooke, Holsberger, Witorsch et al., Toxicology and Applied Pharmacology 194: 309-335, 2004

Effects of Stress or GC Excess In Utero on Offspring

- Intrauterine growth retardation (IUGR)
- Suppression of testosterone "surge" from fetal testes
- Postnatal endocrine/reproductive and other effects (programming)
  - Insulin resistance and hypertension
  - Feminization of sexually dimorphic areas of hypothalamus of male offspring
  - Behavioral changes (decreased copulatory behavior of adult males)
  - Decreased anogenital distance, delayed or abnormal testicular descent and decreased testicular weight in male offspring

How Stress Can Confound Endocrine Disruptor Assessment

- Effects attributable to endocrine disruptive chemicals (EDCs) might be due to direct effects of stress-induced elevation of glucocorticoids.
- Putative EDC might evoke a nonspecific stress response activating the hypothalamo-pituitary-adrenocortical (HPA) axis.
- Putative EDC might activate element of HPA axis.
- Procedures performed during an assay (e.g. oral gavage, restraint) might activate HPA axis.
- Activation of HPA can produce false positive or false negative effects.

Summary and Conclusions

- A brief overview of the essentials of endocrine physiology was presented.
- Endocrine disruption was discussed with regard to the following considerations:
  - Diversity of biological effects produced by ER ligands;
  - Species differences (murine vs. human gestation);
  - The influence of stress (glucocorticoids) on reproductive function and development of offspring.
- The above are important considerations in screening for endocrine disruptors.

Thank You