Environmental effects of estrogens - xenoestrogens

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Athens, Greece, 18 March 2017

Lecture Diagram

- Definitions
- Environmental Sources
- Actions
- Main Male Problems:

1. Hypospadias
2. Cryptorchidism
3. Testicular cancer
4. Semen quality
5. Prostate Cancer

- Conclusion

Environmental estrogens - xenoestrogens

- Molecules with identified estrogenic activity (estrogen mimics), primarily through Estrogen Receptor activation:

1. Natural chemicals found in human and animal food.
2. Synthetic chemicals used as industrial products and their byproducts.

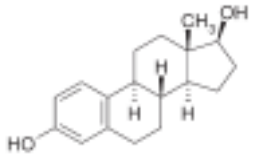
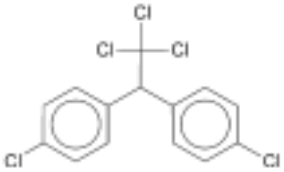
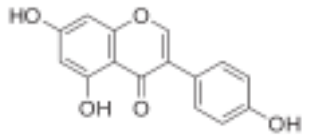
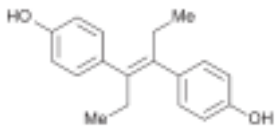
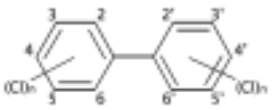
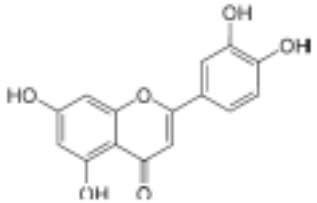
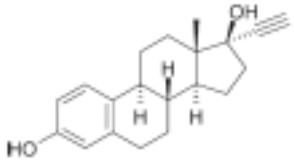
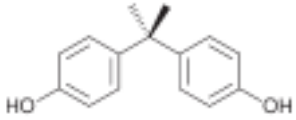
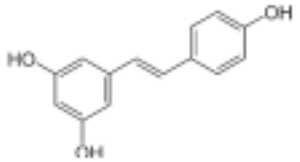
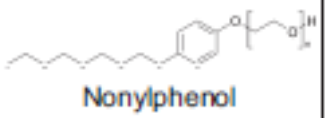
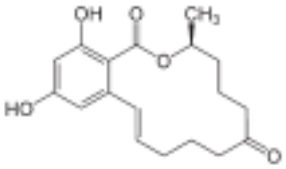
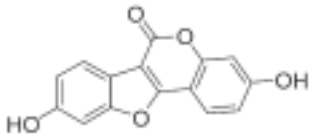
Diamanti-Kandarakis E et al, Endocrine Reviews 2009

Part of the broader Endocrine Disruptors Group

"An endocrine-disrupting compound" is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that is responsible for the maintenance of homeostasis, reproduction, development and/or behavior"

U.S. Environmental Protection Agency (EPA)

Estrogens - Xenoestrogens

Steroids	Pollutants	Plant Products
 17β-Estradiol	 DDT	 Genistein (isoflavone)
Pharmaceuticals		
 Diethylstilbestrol	 PCB	 Luteolin (flavone)
 Ethynyl estradiol	 Bisphenol A	 Resveratol (stilbene)
Fungal Products	 Nonylphenol	
 Zearalenone	 Kepone	 Coumestrol (coumarin)

McLachlan JA et al, Endocrine Reviews 2001

Environmental Sources

1. *Phytoestrogens:* soya, cereals, nuts
2. *BPA:* polycarbonate plastics, plastic toys and bottles, lining of food cans
3. *DDT:* contaminated water, soil crops, fish
4. *DES:* pharmaceutical
5. *EE2:* oral contraceptives, contaminated water and food
6. *PCBs:* contaminated air and food, skin contact with old electrical equipment
7. *Phthalates:* contaminated food, PVC plastics and flooring, personal care products, medical devices and tubing

U.S. Environmental Protection Agency (EPA)

Everywhere!

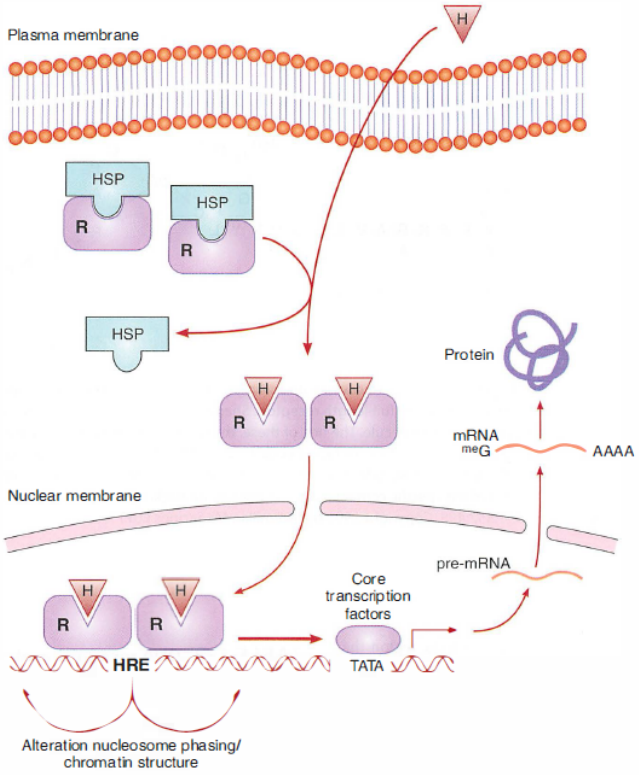

SAMSUNG
12:45

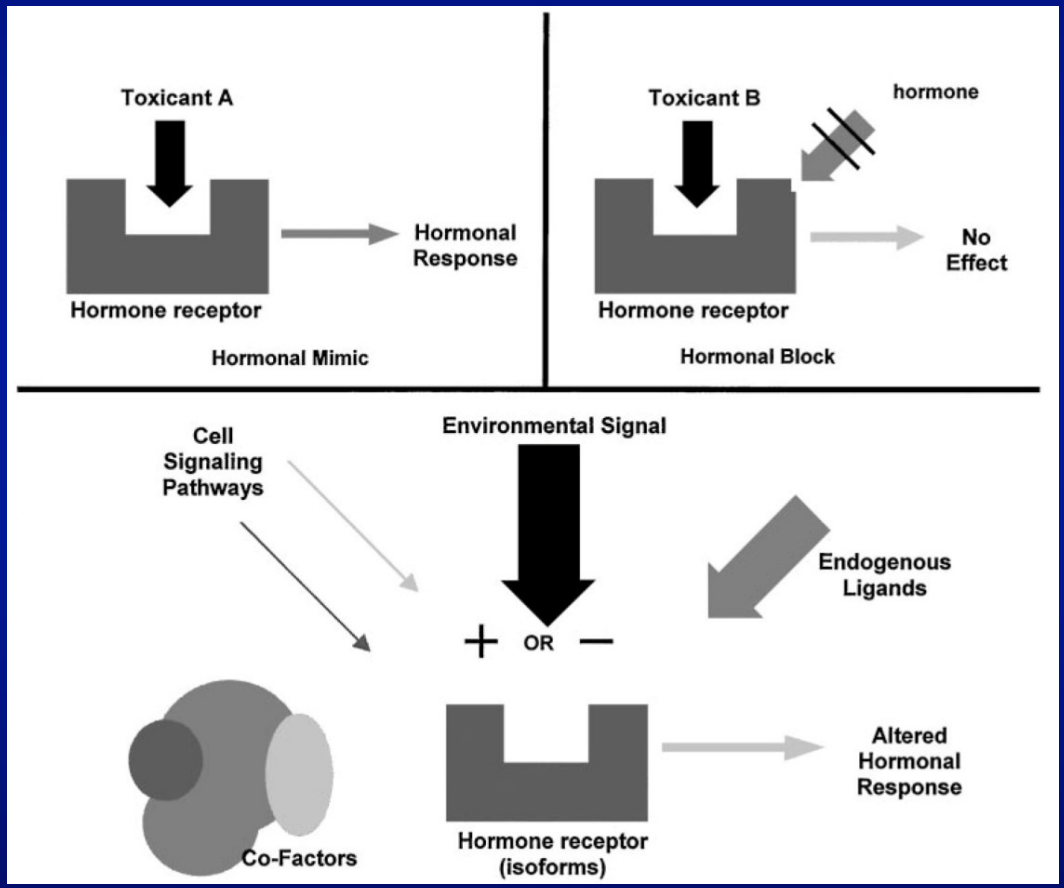

SOY
DREAM
SOYMILK
USDA
ORGANIC
VANILLA
Enriched
VITAMINS A, D, E & CALCIUM

General mechanisms of action

A. Binding to and activation of a receptor
B. Binding to and non-activation of a receptor (anti-hormone)
C. Binding to other receptors
D. Modification of the receptor number
E. Modification of hormones metabolism
F. Interactions with transport proteins

Mastorakos G et al, Endocrine 2007

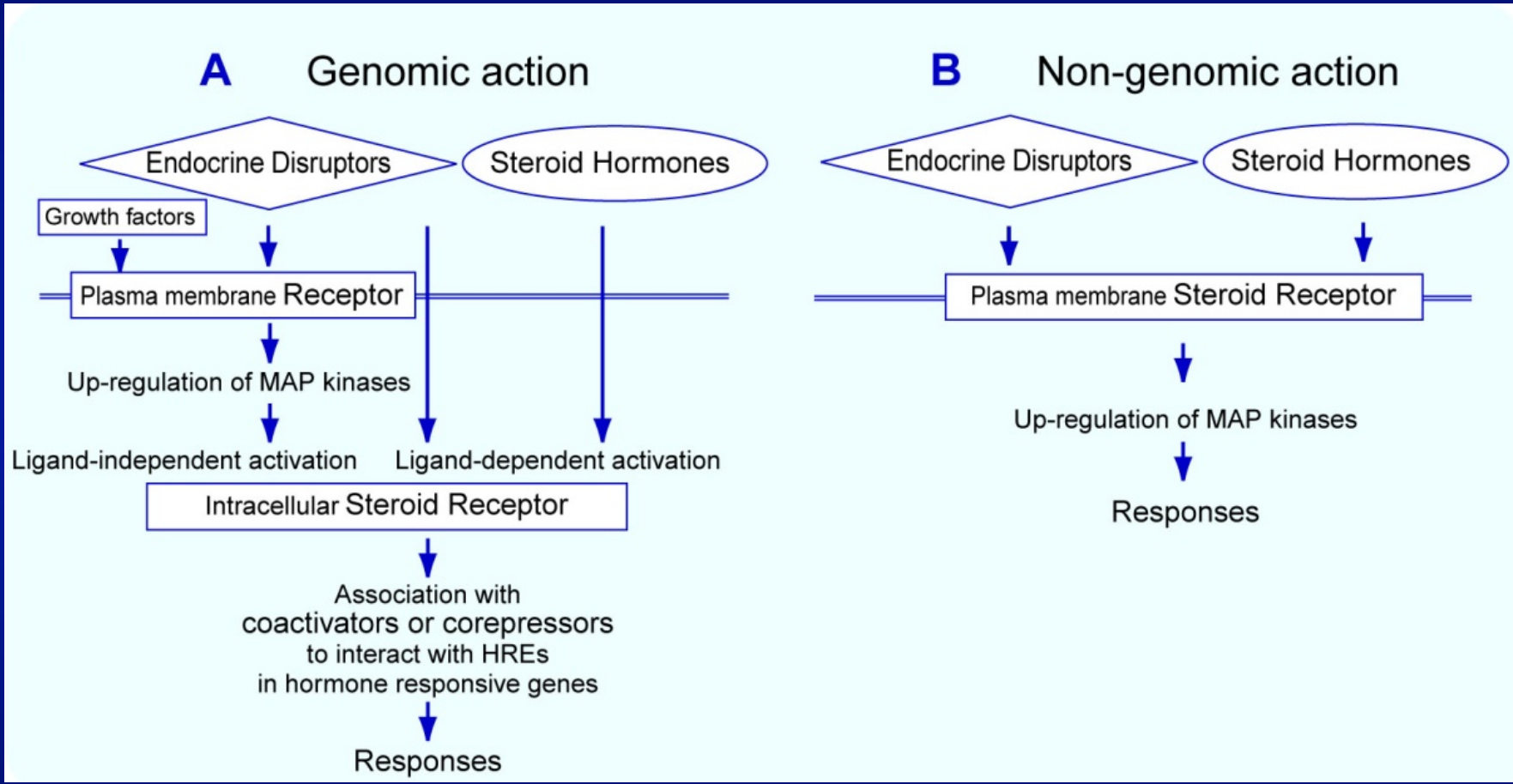




Greenspan's 9th Edition

McLachlan JA, et al Endocrine Reviews 2001

Genomic and non-genomic actions



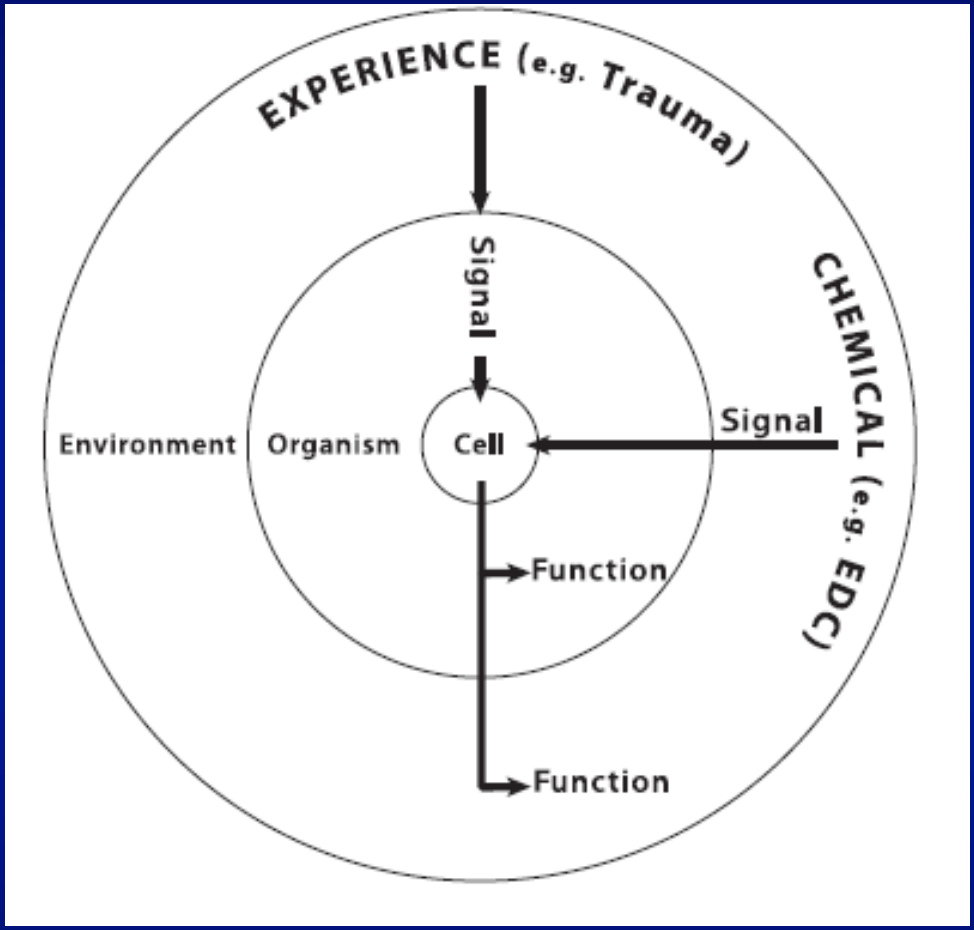
Epigenetic Effects

Heritable changes in gene expression that are not due to changes in DNA sequence:

- ✓ Methylation of cytosine residues on DNA
- ✓ Post-translational modification of histones
- ✓ Altered microRNA expression

- ➢ When EDCs introduce epigenetic changes during early development, they permanently alter the epigenome in the germline and the changes can be transmitted to subsequent generations.
- ➢ When EDCs introduces epigenetic changes during adulthood, the changes within an individual occur in somatic cells and are not permanent or transmitted to subsequent generations.

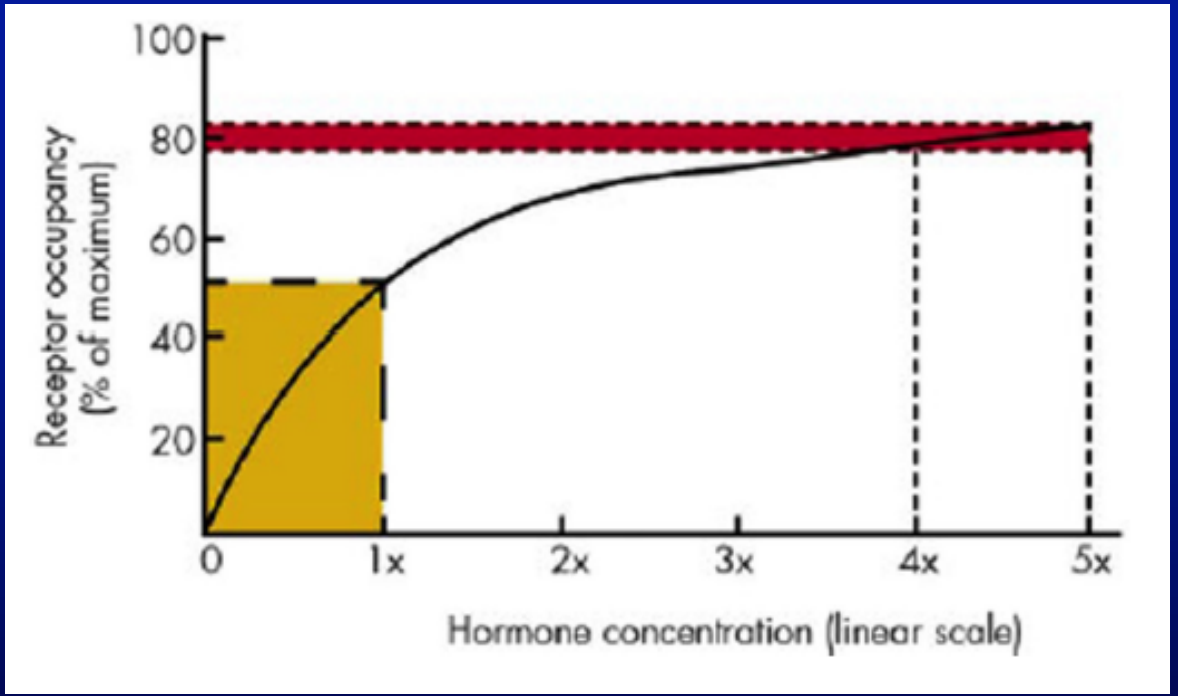
- ✓ In some cases, the environmental chemical is itself the signaling molecule.
- ✓ In others, an environmental factor stimulates internal signaling systems.
- ✓ In either cases, the signal elicits a functional change in the cell or organism.



McLachlan JA, Andrology 2016

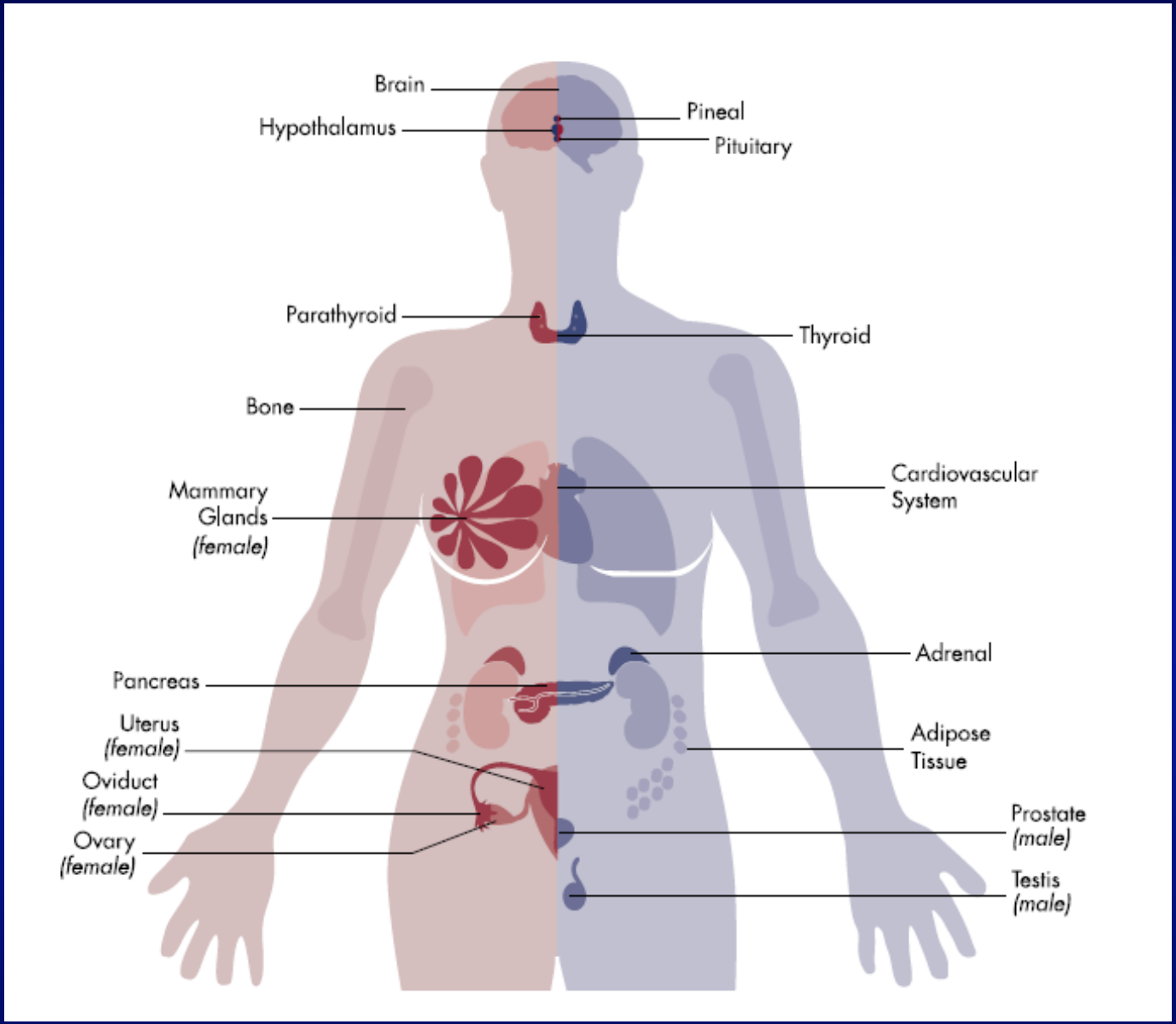
Dose-response characteristics of EDCs

- ✓ Receptor characteristics
- ✓ Ligand characteristics
- ✓ Non-linear response pattern
- ✓ Differences in tissues/cells sensitivity
- ✓ Differences between:
  - toxicology studies
  - human studies
  - animal studies
- ✓ Difficulties:
  - potency
  - thresholds
  - kinetics



Key issues to consider

- ✓ Diverse sources of exposure, which vary widely around the world.
- ✓ Some EDCs have long half-lives, do not decay easily, they may not be metabolized, or may be metabolized into more toxic compounds.
- ✓ EDCs are usually mixtures with different effects, occasionally additive or even synergistic.
- ✓ Susceptibility to EDCs may vary according to genetic polymorphisms.
- ✓ Latency periods
- ✓ Trans-generational effects



Gore AC et al, Endocrine Reviews 2015

Epidemiological Observations in Humans

I. Decrease in male reproductive parameters such as semen quality and Leydig cell function (*Carlsen et al 1992, Giwercman et al 1993, Irvine et al 1996, Swan et al 2000, Jørgensen et al 2012, Andersson et al 2007, Clementi et al 2008*).

II. Increase in male disorders, such as hypospadias, cryptorchidism and testicular germ cell cancer *(Giwercman et al 2007, Skakkebaek et al 2001).*

III. Increase in exposure to EDs such as pesticides, insecticides, persistent organic pollutants (POPs), perfluorinated chemicals (PFCs), polychlorinated biphenyls (PCBs), phthalates, bisphenol A (BPA) and heavy metals *(Clementi et al 2008).*

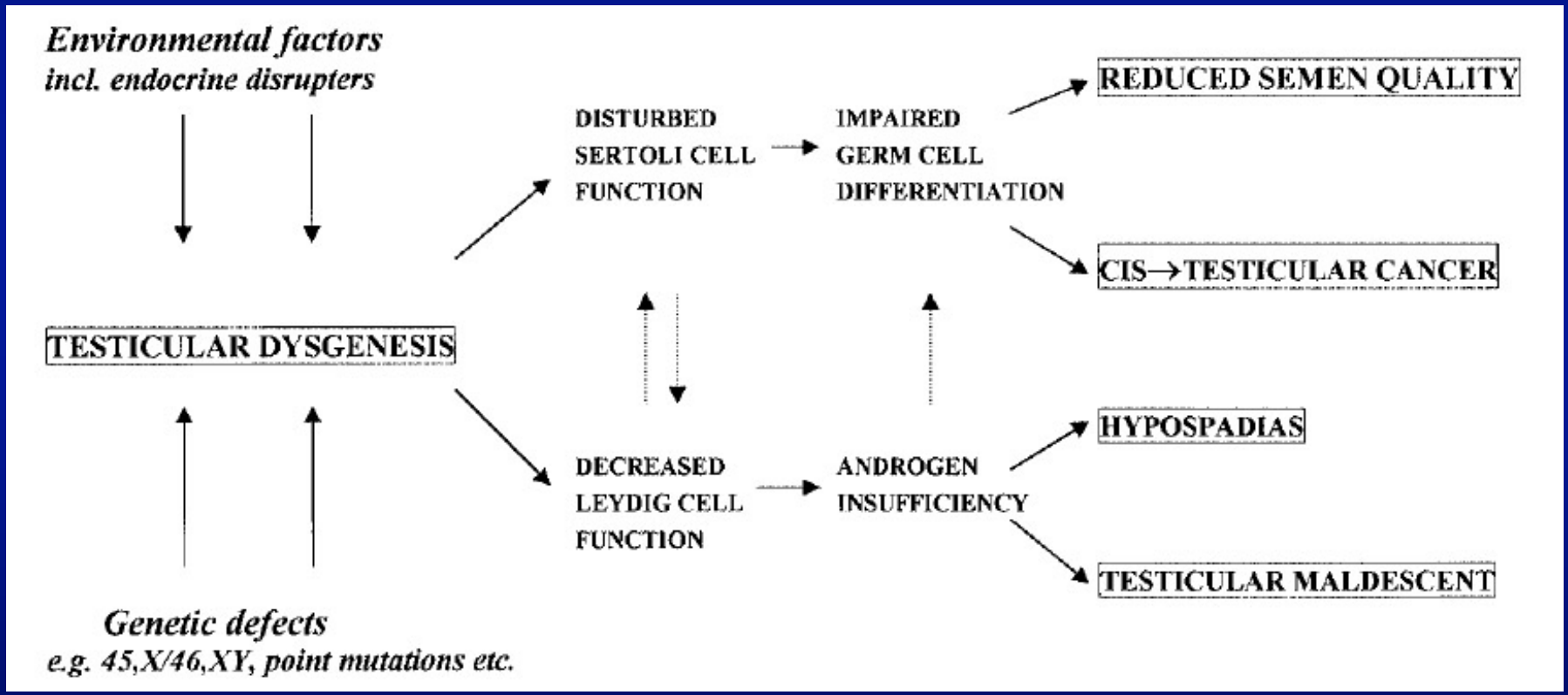
Experimental Studies in Rodents - Exposure to BPA, phthalates and alkylphenols

I. Developmental genitourinary abnormalies, cryptorchidism, shorter anogenital distance, smaller testicular and penile size, decreased epididymal weight, increased prostate weight (Williams et al 2001, Richter et al 2007, Salian et al 2009, Foster et al 2006, vom Saal et al 1998, Hossaini et al 2001, Nagel et a 1997, Talsness et al 2000).

II. Lower daily sperm production, decreased sperm motility and morphology *(Talsness et al 2000, Aikawa et al 2004).*

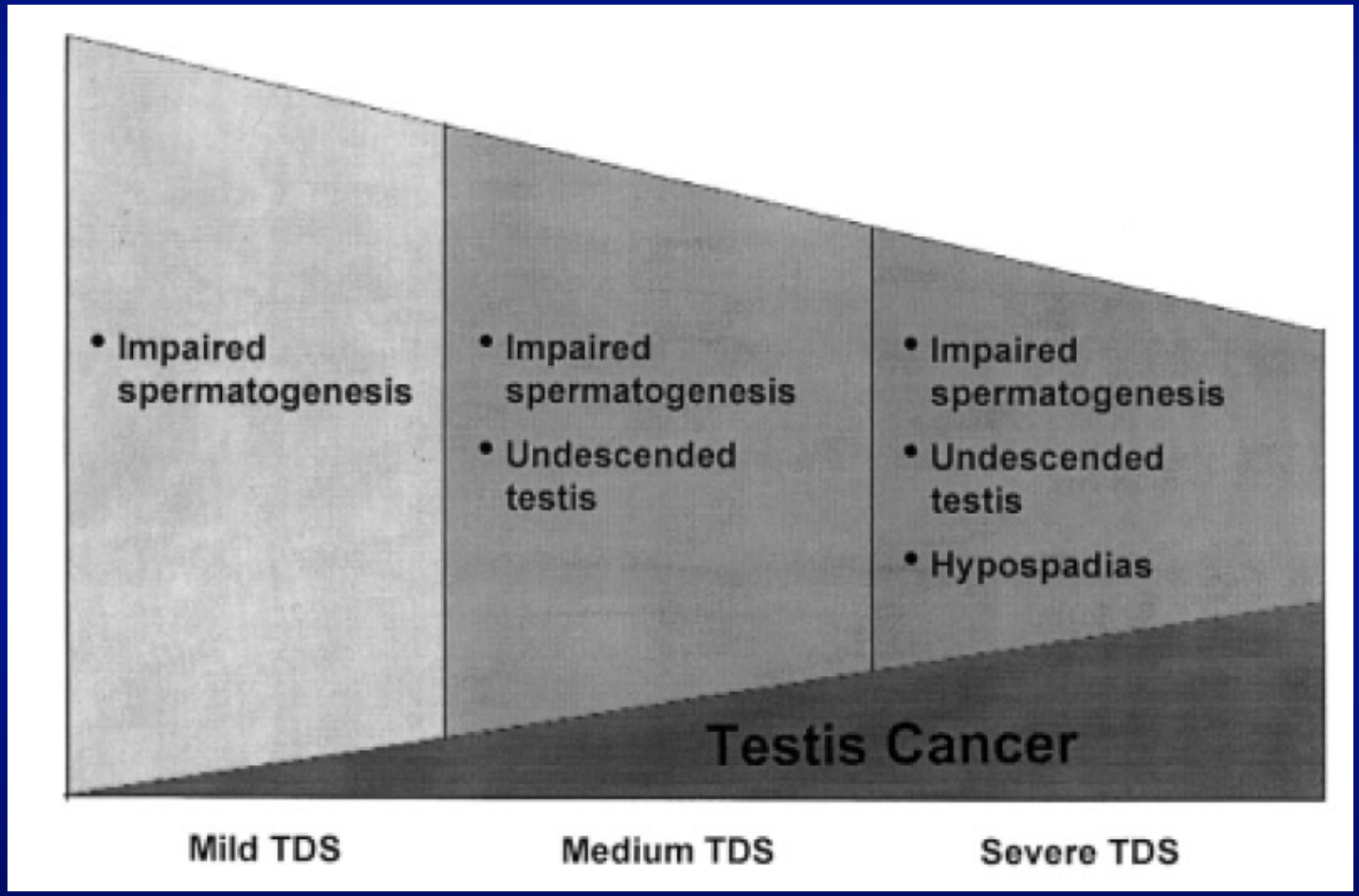
III. Abnormalities in the acrosomal granule and nucleus in older spermatids and spermatozoa, lower height of the seminiferous epithelium, lower testosterone concentrations *(Akingbemi et al 2001, Herath et al 2004, Richter et al 2007, Toyama & Yuasa 2004, Fisher et al 1999).*

Testicular Dysgenesis Syndrome: diminished semen quality, testicular germ cell cancer and male urogenital tract anomalies



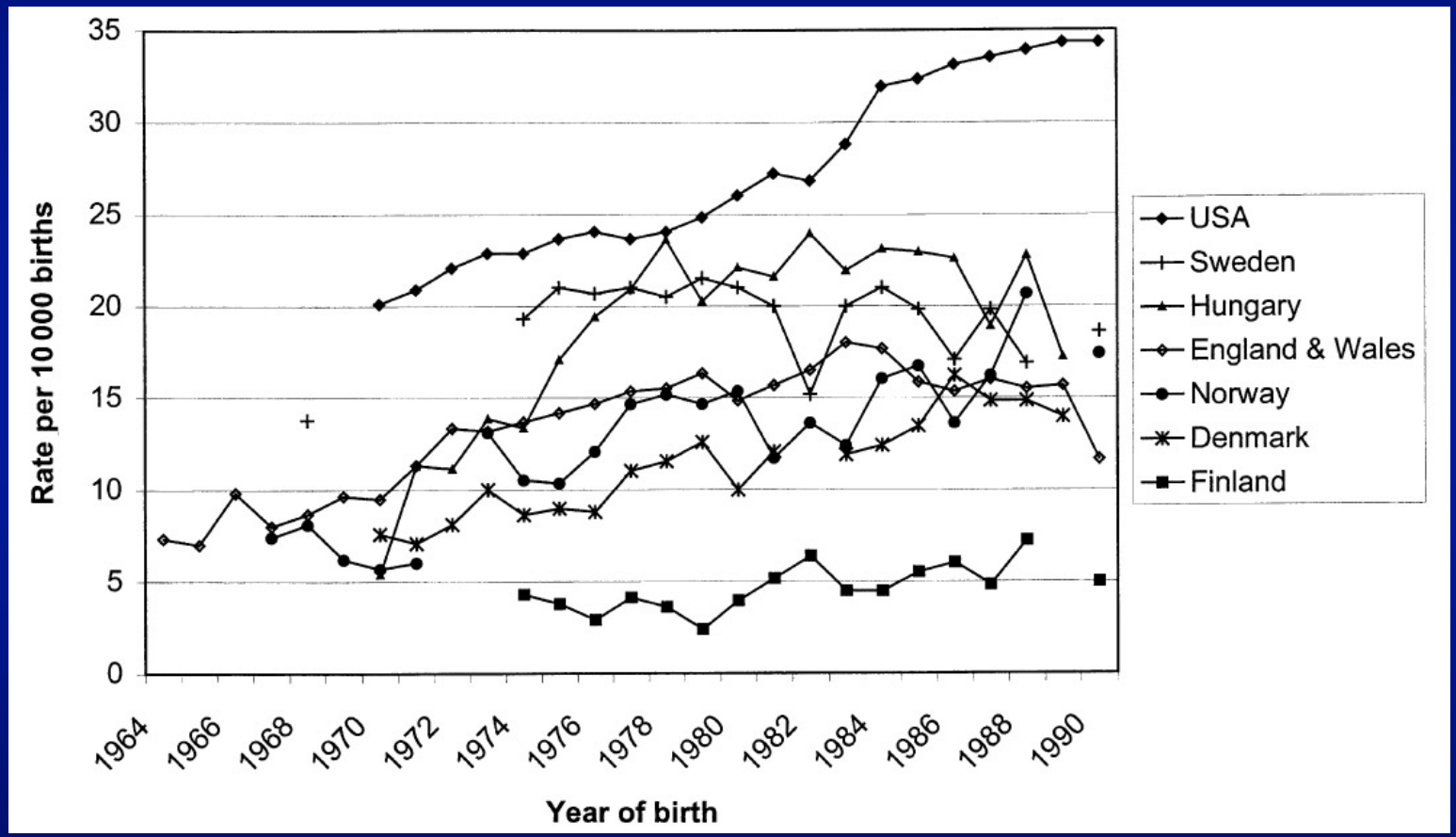
Skakkebaek et al Environ Health Perspect 2003

TDS was reproduced entirely or partly in rodent studies by employing phthalates and PCBs.



Moller and Skakkebaek. BMJ 1999

Hypospadias: the urethral folds do not fuse properly, and the urethra is exteriorized on the ventral side rather than the tip of the penis.



Toppari et al, Human Reproduction Update 2001

Pesticides and hypospadias: A meta-analysis

Carissa M. Rocheleau, Paul A. Romitti*, Leslie K. Dennis

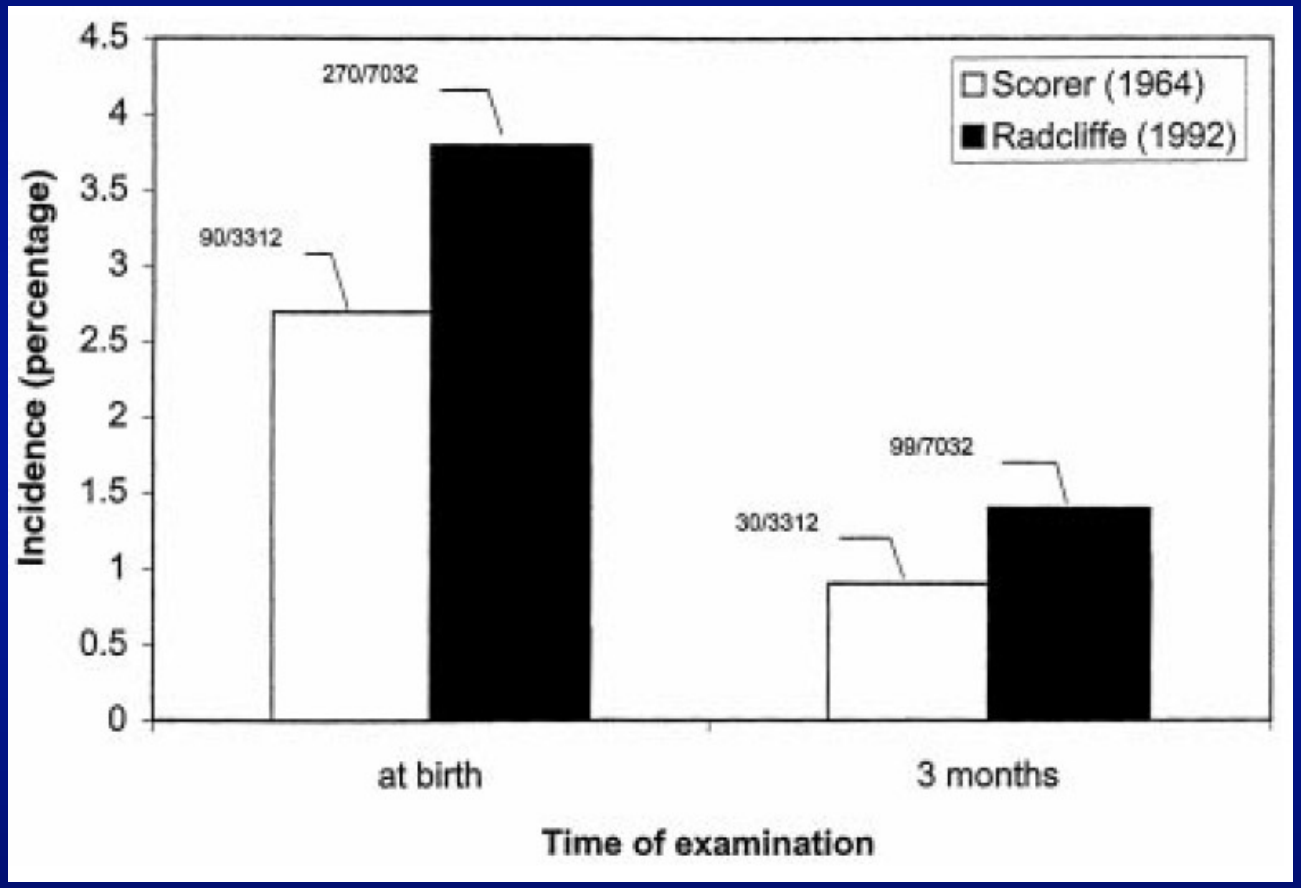
Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA, USA

- 9 studies included
- Pooled risk ratios were *1.36 (95% CI, 1.04–1.77)* and *1.19 (95% CI, 1.00–1.41)* for maternal and paternal exposures, respectively.

Rocheleau CM et al, Journal of Pediatric Urology 2001

Cryptorchidism: the failure of the testis to fully descend to the bottom of the scrotum

Incidence in full-term boys at birth and at 3 months during 1950s and 1980s in UK.



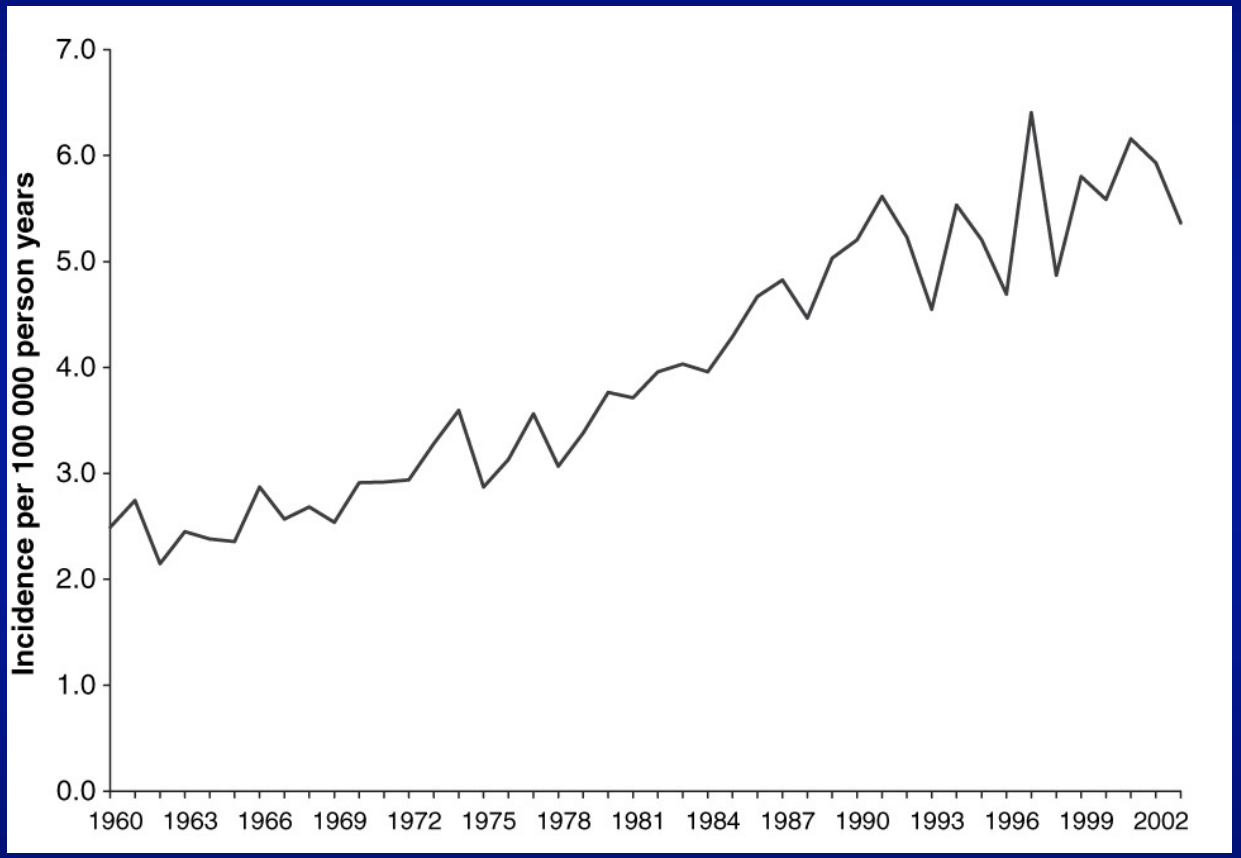
Toppari et al, Human Reproduction Update 2001

Cryptorchidism

- ✓ *California* children in the two higher quartiles of DDT exposure had a higher incidence of cryptorchidism (*Bhatia R et al, Environ Health Perspect. 2005).*
- ✓ *A Spanish study* that assessed total estrogenic burden in placenta samples linked cryptorchidism with xenoestrogen exposure (*Fernandez MF et al, Environ Health Perspect. 2007).*
- ✓ *A French case-control study* on cryptorchidism showed that boys in the high exposure group for DDE and PCBs had a higher risk of cryptorchidism than boys in the lower exposure group *(Krysiak-Baltyn K et al, Int J Androl 2012).*
- ✓ *In a Finnish-Danish study,* PCBs and PBDEs were measured in mother's milk and placenta, with no positive association with cryptorchidism *(Main KM et al, Environ Health Perspect. 2007).*

Age standardized incidence of testicular cancer in Sweden 1960-2003

- First generation of immigrants follows the pattern of their home country.
- Second generation (born in Sweden) adopts the Swedish risk profile.



Hardell et al, International Journal of Andrology 2006

- ✓ Maternal serum concentrations of PCBs (collected when the men were adults) were higher in the TGCC group than in controls, whereas no association was found between TGCC and PCB levels in the adult men themselves.

Table 6. OR (95% CI) for mothers of cases with testicular cancer, all types combined.[a]

	Cases/controls	OR (95% CI)
Sum of PCBs[b]	34/20	3.8 (1.4–10)
HCB	35/22	4.4 (1.7–12)
p,p´-DDE	22/22	1.3 (0.5–3.0)
cis-Heptachlordane	27/21	2.1 (0.8–5.0)
cis-Chlordane	22/15	2.5 (0.99–6.1)
Oxychlordane	28/22	2.6 (0.9–7.1)
MC6	25/22	1.3 (0.5–3.2)
trans-Nonachlordane	34/22	4.1 (1.5–11)
cis-Nonachlordane	32/22	3.1 (1.2–7.8)
Sum of chlordanes	27/22	1.9 (0.7–5.0)

Hardell et al, Environ Health Perspect 2002

OR for mothers of cases with testicular cancer

Table 1 Odds ratio (OR) and 95% confidence interval (CI) for mothers of cases with testicular cancer, all types combined. The median concentration of the chemicals in mothers of controls was used as cut-off value. Numbers greater than median (expressed in nanogram per gram lipid) are shown. Adjustment was made for age and BMI

	All			≤55 years old			>55 years old		
	Cases/controls	OR	95% CI	Cases/controls	OR	95% CI	Cases/controls	OR	95% CI
Sum of PCBs	34/20	3.8	1.4–10	18/11	3.1	0.7–14	16/9	5.3	1.1–25
HCB	35/22	4.4	1.7–12	19/9	14	2.8–75	16/13	2.2	0.5–9.6
p,p′-DDE	22/22	1.3	0.5–3.0	11/11	2.3	0.6–9.5	11/11	0.9	0.3–3.4
Sum of chlordanes	27/22	1.9	0.7–5.0	12/7	3.9	0.9–16	15/15	0.9	0.2–4.1

Table 2 Odds ratio (OR) and 95% confidence interval (CI) for mothers of cases with testicular cancer. Grouping of PCB congeners was made according to structural and biological activity. The median concentration of the chemicals in mothers of controls was used as cut-off value. Numbers greater than median (expressed in nanogram per gram lipid) are shown. Adjustment was made for age and BMI

	Seminoma		Non-seminoma		All (n = 43)	
	Cases/controls	OR (CI)	Cases/controls	OR (CI)	Cases/controls	OR (CI)
Estrogenic PCBs[a]	10/20	2.3 (0.6–8.9)	20/20	2.4 (0.8–6.8)	30/20	2.4 (0.95–6.0)
Enzyme-inducing PCBs[b]	9/21	1.4 (0.4–5.3)	22/21	3.3 (1.1–9.7)	31/21	2.6 (1.03–6.5)
TEQ[b]	11/21	3.5 (0.8–15)	22/21	3.3 (1.1–9.8)	33/21	3.3 (1.3–8.4)
Sum of PCBs[a]	11/20	3.1 (0.7–14)	23/20	4.3 (1.3–14)	34/20	3.8 (1.4–10)

Hardell et al, International Journal of Andrology 2006

Semen Quality

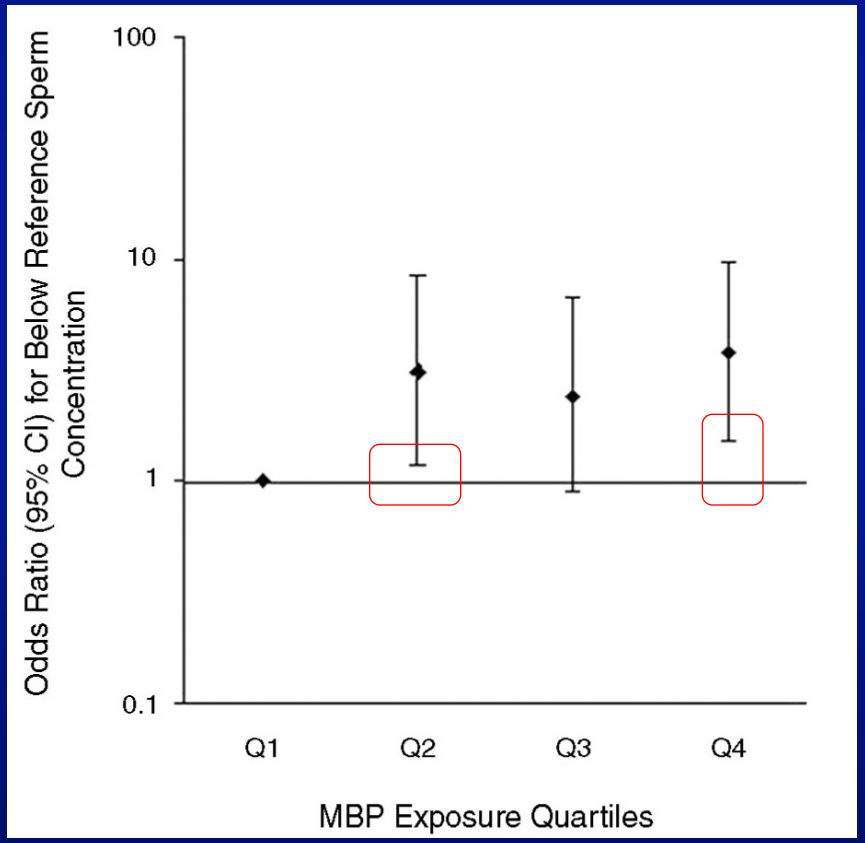
Association of below-reference value semen parameters (motility) with median phthalate monoester levels (MBP)

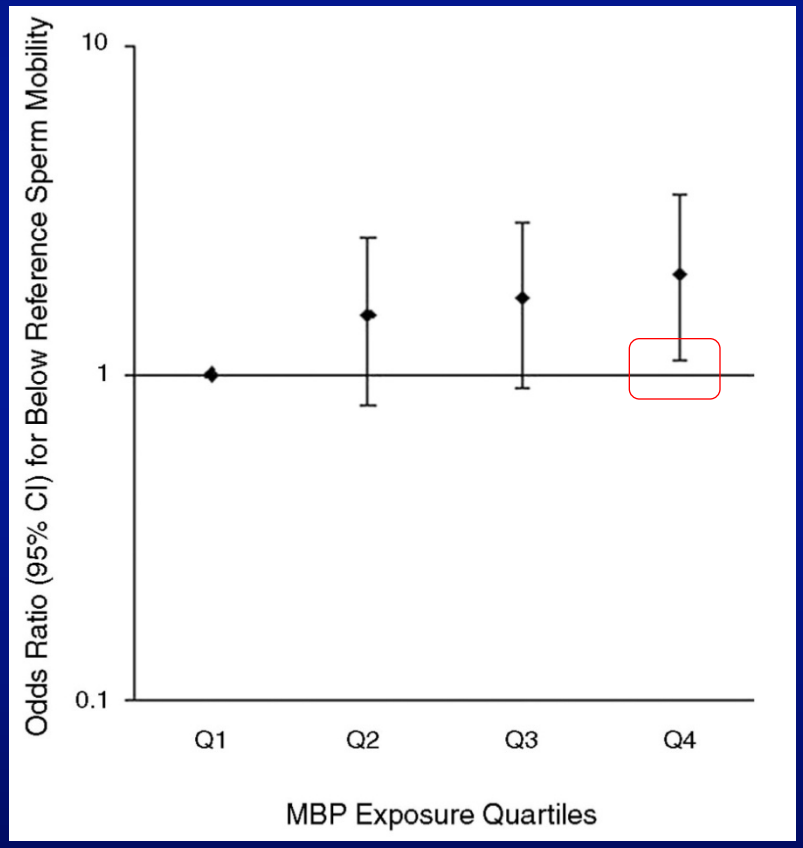
TABLE 3. Association of Below-Reference Value Semen Parameters with Median Phthalate Monoester Levels* (N = 143 subjects)†

	Semen Parameter					
	Sperm Concentration <20 Million/mL		Sperm Motility <50% Motile		Morphology <4% Normal	
Phthalate Monoester	Crude OR (CI)	Adjusted OR (CI)‡	Crude OR (CI)	Adjusted OR (CI)‡	Crude OR (CI)	Adjusted OR (CI)‡
Ethyl (MEP)	1.1 (0.4–2.9)	1.2 (0.4–3.4)	1.3 (0.6–2.6)	1.1 (0.6–2.4)	0.9 (0.4–2.1)	0.9 (0.4–2.2)
2-Ethylhexyl (MEHP)	1.1 (0.4–2.9)	1.0 (0.3–2.9)	1.4 (0.7–2.8)	1.4 (0.7–2.9)	1.2 (0.5–2.6)	1.2 (0.5–2.8)
Butyl (MBP)	2.2 (0.8–5.8)	2.4 (0.8–7.2)	2.3 (1.1–4.6)	2.4 (1.1–5.0)	1.6 (0.7–3.5)	1.7 (0.8–3.9)
Benzyl (MBzP)	1.8 (0.7–4.8)	2.7 (0.8–8.5)	1.6 (0.8–3.1)	1.8 (0.9–3.9)	1.8 (0.8–4.0)	2.1 (0.9–5.1)
Methyl (MMP)	2.3 (0.6–8.1)	1.7 (0.4–7.9)	1.3 (0.5–3.4)	1.1 (0.4–3.3)	2.9 (0.9–9.3)	3.2 (0.8–12.2)

Duty et al, Epidemiology 2003

Adjusted odds ratios for below reference sperm concentration and mobility associated with higher quartiles of monobutyl-phthalate (MBP) urine concentration.





Hauser et al, Epidemiology 2006

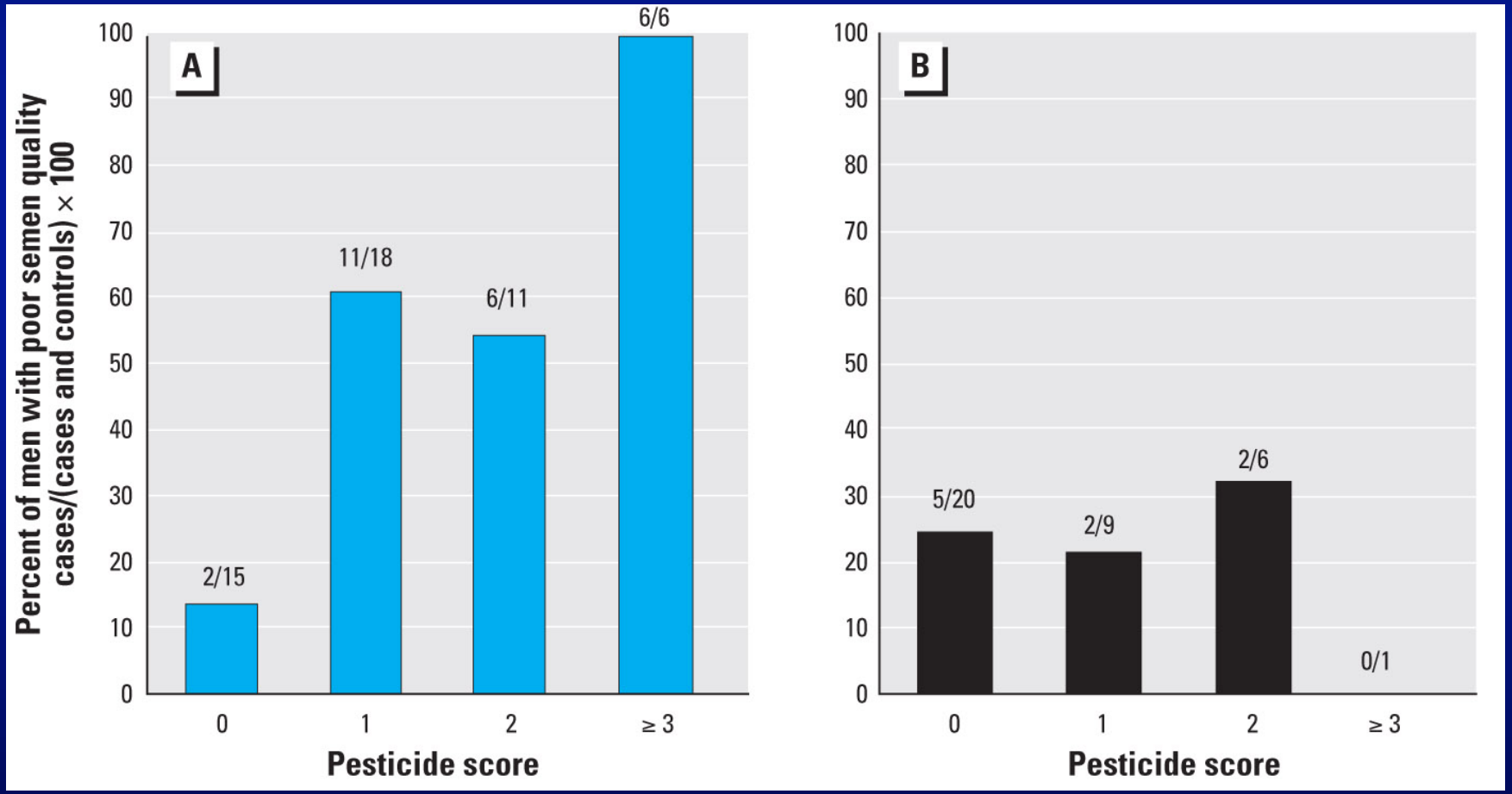
Odds ratios for low semen quality for men exposed to elevated pesticide levels

Table 4. ORs for low semen quality for men exposed to elevated pesticide levels.

	Missouri			
Pesticide	Level (μg/g creatinine)	Cases	Controls	OR (95% CI)
Alachlor	< 0.15	3	15	Reference
	0.15–0.7	10	8	6.3 (1.3–29.4)
	> 0.7	12	2	30.0 (4.3–210)
IMPY	< 0.1	6	20	Reference
	0.1–3.0	9	3	10.0 (2.0–49.2)
	> 3.0	10	2	16.7 (2.8–98.0)
Atrazine	< 0.1	17	24	Reference
	≥ 0.1	8	1	11.3 (1.3–98.9)
Metolachlor	< 0.15	5	11	Reference
	0.15–0.3	11	8	3.0 (0.7–12.2)
	> 0.3	9	6	3.3 (0.8–14.5)
2,4-D	< 0.1	20	19	Reference
	≥ 0.1	5	6	0.8 (0.2–3.0)
1-Naphthol	< 1.5	9	2	Reference
	> 1.5	12	1	2.7 (0.2–34.2)
3,5,6-Trichloropyridinol	< 0.5	5	2	Reference
	≥ 0.5	16	1	6.4 (0.5–86.3)
4-Nitrophenol	< 0.1	20	3	
	≥ 0.1	1	0	

Swan et al, Environ Health Perspect 2003

The proportion of men with poor semen quality in relation to pesticide score (the number of pesticides found at higher levels) (*A) Missouri (B) Minnesota*



Swan et al, Environ Health Perspect 2003

ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

OPINION ARTICLE

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Keywords:
endocrine disrupters, epidemiology, male reproductive system, methodology

Received: 10-Aug-2016
Revised: 12-Jan-2017
Accepted: 16-Jan-2017

Effect of endocrine disruptors on male reproduction in humans: why the evidence is still lacking?

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Methodological Problems

1. Exposed and non-exposed populations
2. Age
3. Insufficient control for confounders
4. ED assay and threshold
5. Time parameters of ED exposure
6. Study outcome

Bliatka D, Lymperi S, Mastorakos G, Goulis DG, Andrology 2017

Prostate Cancer

- ✓ Prostate gland is a hormone-dependent structure, and dysregulation of hormonal signaling is a known contributor to the high rates of prostate disease with aging.
- ✓ Epidemiological evidence indicates increased prostate cancer rates and mortality in men exposed to pesticides.
- ✓ EDC classes with known prostatic effects from animal studies include pesticides, PCBs, alkylphenols, BPA, and some heavy metals.
- ✓ Animal models and human cell-based studies provide evidence for elevated prostate cancer risk from BPA exposures, with increased sensitivity to BPA reprogramming during early-life developmental.

Bostwick DG et al, Scand J Urol Nephrol Suppl 2000
Alavanja MC et al, Am J Epidemiol 2003
Christensen CH et al, Environ Health Perspect 2010

Exposure to Bisphenol A Correlates with Early-Onset Prostate Cancer and Promotes Centrosome Amplification and Anchorage-Independent Growth *In Vitro*

Pheruza Tarapore[1,2,3], Jun Ying[1,2], Bin Ouyang[1,2,3], Barbara Burke[4], Bruce Bracken[4], Shuk-Mei Ho[1,2,3,5]*

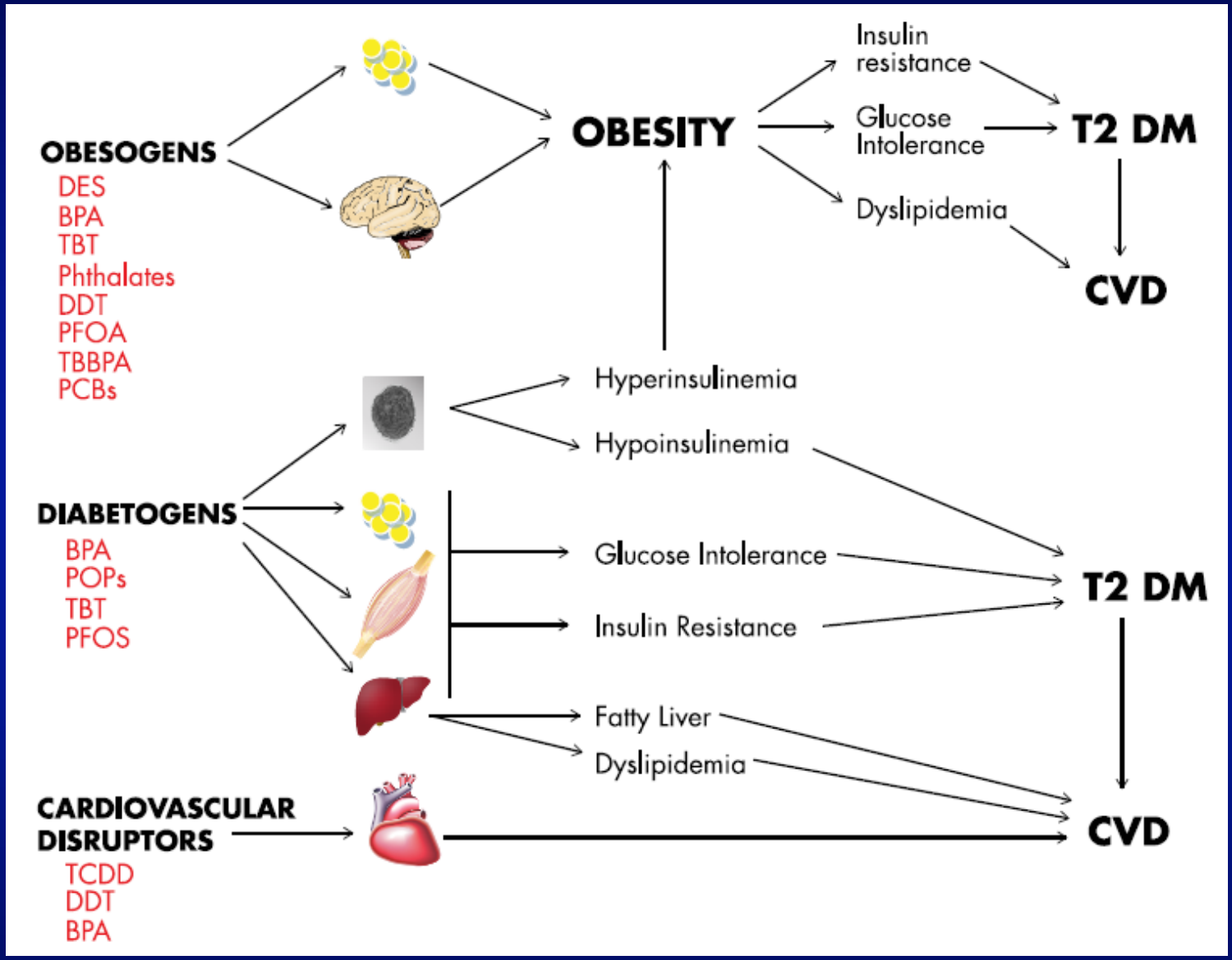
- ✓ The first direct clinical evidence
- ✓ Prospective study of 60 urological patients evaluated for potential prostate cancer due to elevated PSA levels
- ✓ Urinary BPA-glucuronide levels before prostate biopsy were significantly higher in the biopsy-confirmed prostate cancer patients than those with no diagnosis of cancer.
- ✓ Cancer-positive patients < 65 years old had higher urine BPA-glucuronide levels than non-cancer patients, whereas there was no difference in BPA levels in cancer vs non- cancer patients in men □> 65 years old.

Tarapore P et al, Plos One 2014

Reproductive health problems associated with environmental estrogens-xenoestrtogens through male life cycle

	Fatal/Neonatal	*Prepubertal*	*Pubertal*	*Adult*
Processes	Intrauterine growth	Adrenarche	Pubarche	Spermatogenesis
Disorders	IUGR	Premature Pubarche	Small Testes and high FSH	Oligospermia
	Cryptorchidism		Early Puberty	Testicular Cancer
	Hypospadias		Delayed Puberty	Prostate Hyperplasia

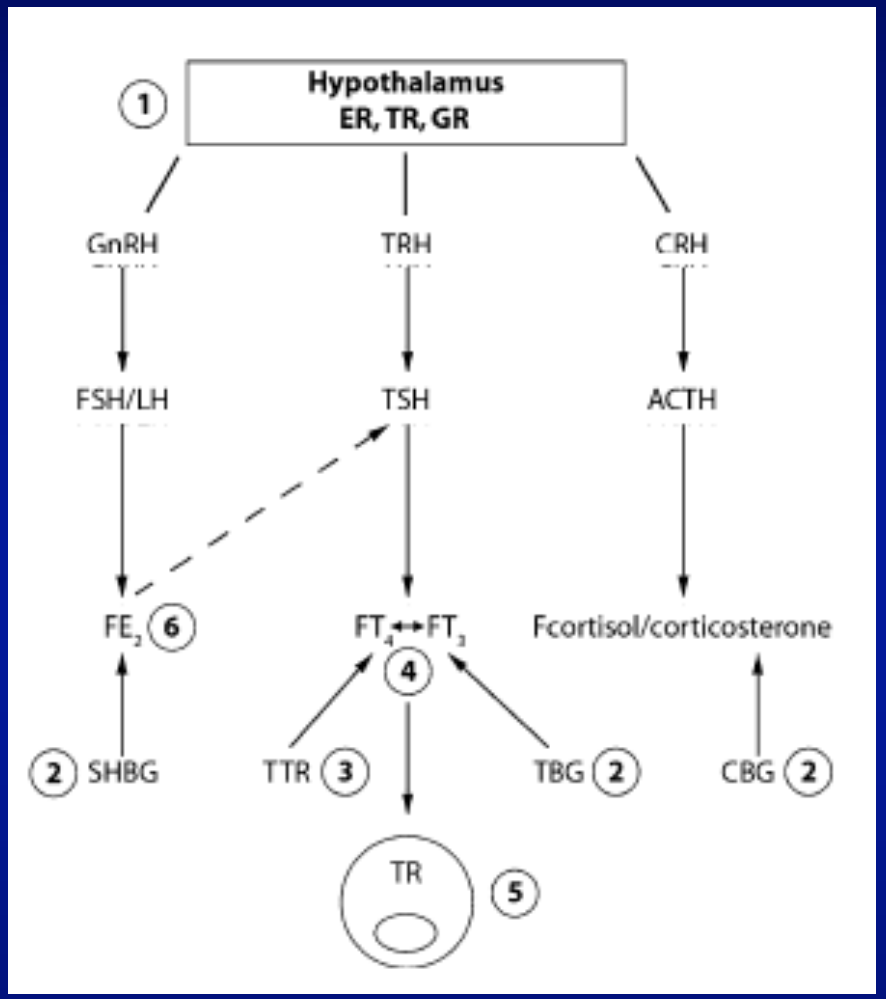
Diamanti-Kandarakis E et al, Endocrine Reviews 2009



Gore AC et al, Endocrine Reviews 2015

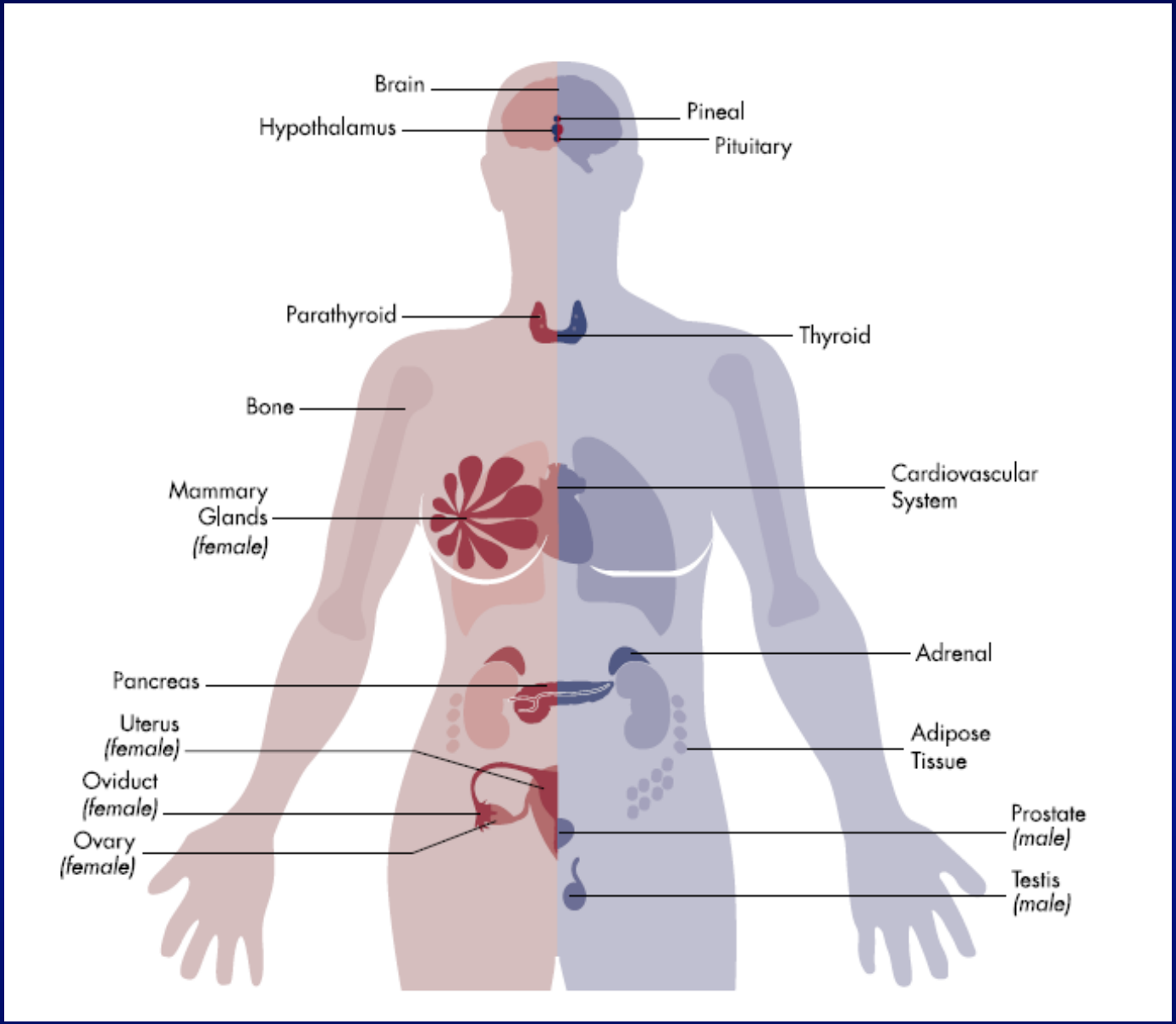
- ✓ Several key components of most hormones homeostasis are susceptible to the action of endocrine disruptors, including estrogens and xenoestrogens.

- ✓ Developmental disorders in the structure and functioning of the brain, leading possibly to behavioral changes.



Mastorakos G et al, Endocrine 2007

Environmental Sources



Gore AC et al, Endocrine Reviews 2015

Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement

Evanthia Diamanti-Kandarakis, Jean-Pierre Bourguignon, Linda C. Giudice, Russ Hauser, Gail S. Prins, Ana M. Soto, R. Thomas Zoeller, and Andrea C. Gore

Endocrine Reviews 2009

EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals

A. C. Gore, V. A. Chappell, S. E. Fenton, J. A. Flaws, A. Nadal, G. S. Prins, J. Toppari, and R. T. Zoeller

Endocrine Reviews 2015

"Until such time as conclusive scientific evidence exists to either prove or disprove harmful effects of substances, a precautionary approach should be taken in the formulation of EDC policy"

Endocrine Society 2009

"As we move forward, we believe that the evidence is sufficient to recommend greater regulation, more precaution, better communication between healthcare professionals and patients, and efforts to avoid introducing new EDCs, in a misguided effort to replace previous chemicals in the absence of proper testing"

Endocrine Society 2015