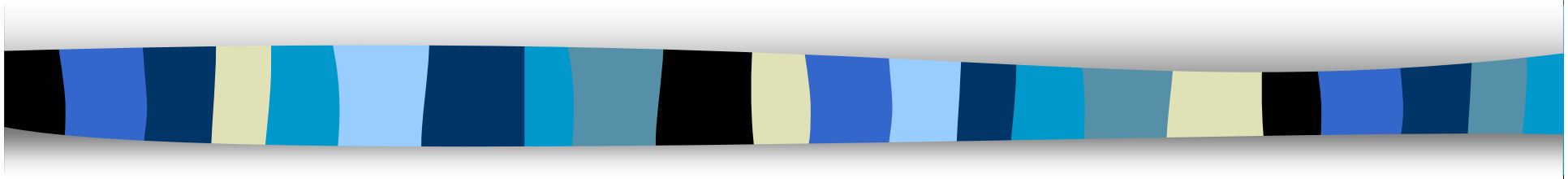
Fyziologie působení farmak a toxických látek



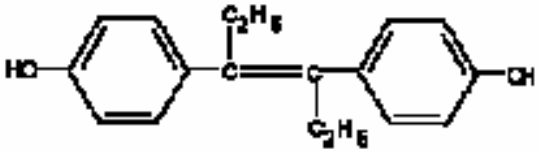
Přednáška č.4
Endokrinní disrupce u obratlovců I.
ER, AR, PR, GR

Endocrine disruptor:

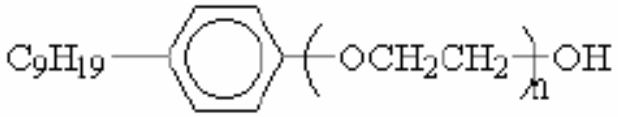
A chemical that interferes with the
synthesis,
secretion,
transport,
binding, action or
elimination
of any hormone in the body

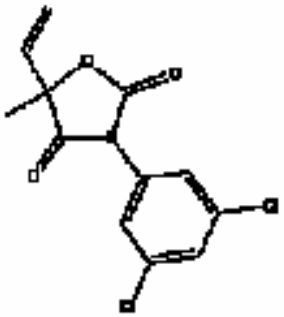
Endocrine Disrupting Chemicals

DES

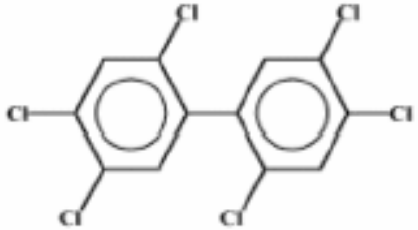


Nonylphenol

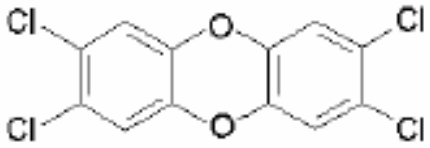




Vinclozolin



PCB-153



Dioxin

Výzkum endokrinní disrupce je soustředěn do dvou oblastí:

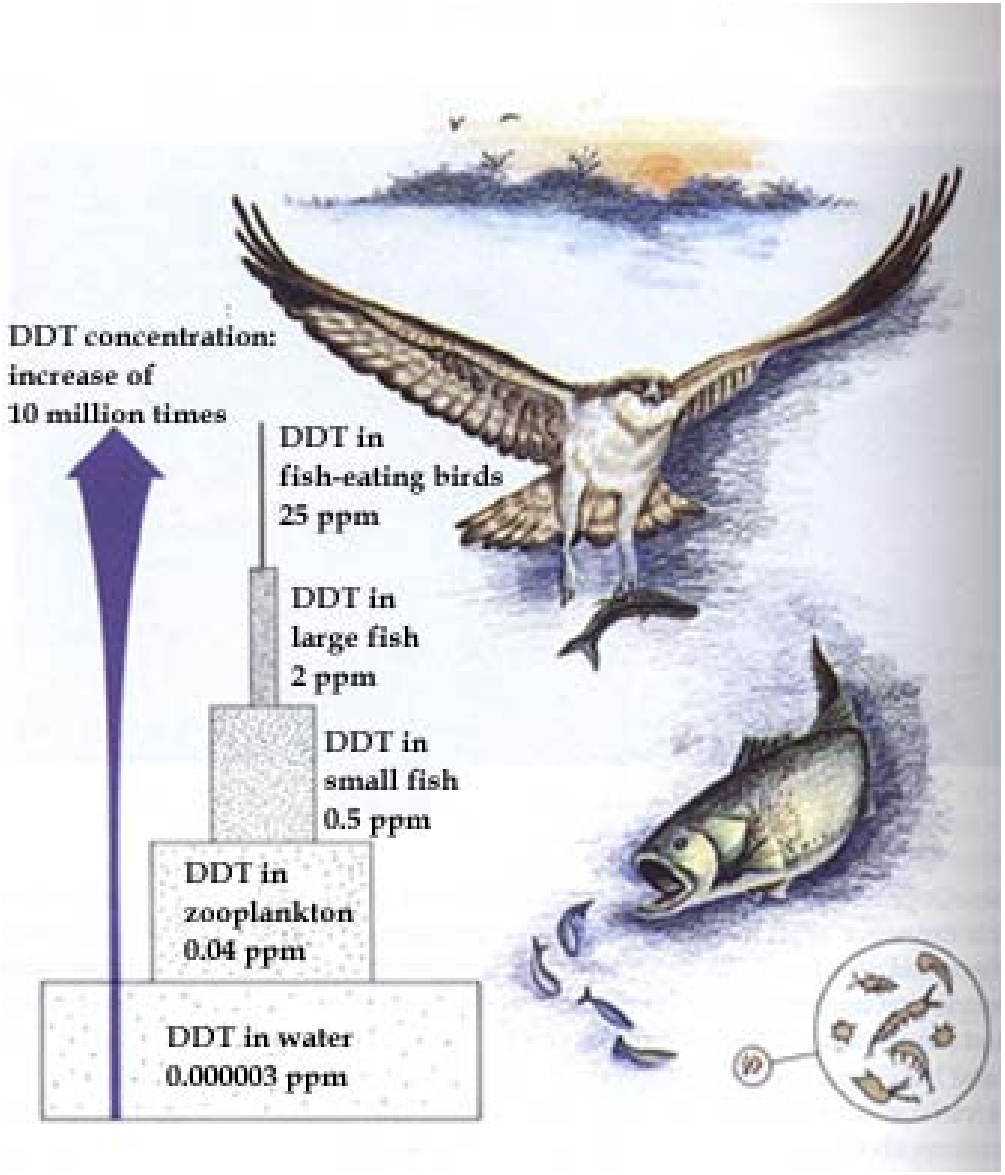
- obratlovci, kteří alespoň část svého životního cyklu tráví ve vodném prostředí – ryby, obojživelníci – expozice vodou, potravou;

- terestričtí obratlovci – expozice především v rámci potravního řetězce;

Nejohroženější skupina – vrcholoví konzumenti – dravci.

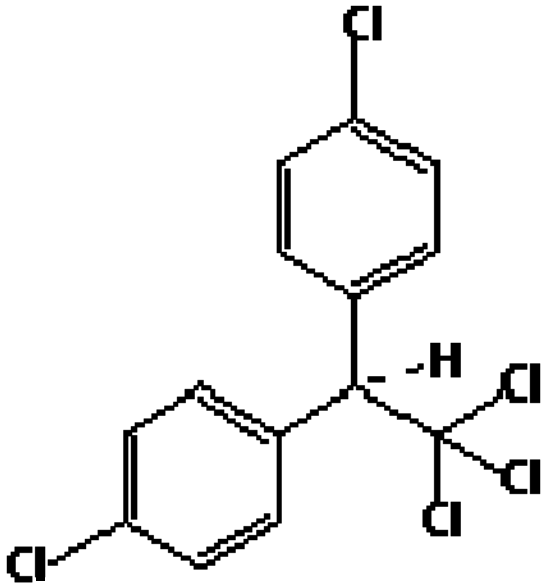
http://www.epa.gov/endo/

Biomagnifikace a bioakumulace



Effects of DDT
(Dichlorodiphenyltrichloroethane)

- Invertebrates
- Fish
- Birds
- Mammals



Endocrine Disruption in Wildlife

- Eggshell thinning in raptors from DDT
- Beak, skeletal, reproductive abnormalities from PCBs (bald eagles, gulls, cormorants)
- Intersex fish below UK sewage effluents from estradiol, alkylphenols

- Decreased plasma sex steroids, egg and gonadal size; delayed sexual maturity from dioxin below paper mills (Great Lakes white suckers)
- Poorly developed testes, small penises, low testosterone; abnormal ovaries; males with high estradiol; poor hatchling success from DDE (Lake Apopka alligators)

Endocrine Disruption in Lab

- Masculinization of females by kepone, DDT, methoxychlor
- Disruption of estrous cycle by atrazine, choroquine
- Hypospadias, vaginal pouches, reduced sperm production in males exposed to vinclozolin *in utero*
- Impaired testosterone synthesis, and spermatogenesis; decreased anogenital distance, delayed testis decent, impaired and feminized behavior of rats by dioxin
- Acceleration of puberty and loss of fertility in females by many estrogenic chemicals
- Delay of puberty, binding to androgen receptor; nipple retention in males by many estrogenic chemicals
- Atrophy of the thymus by PCBs and dioxin

Evidence for ED in Humans

- Genital malformation (boys), vaginal cancer, infertility (girls) exposed in utero to DES
- Neurological effects, decreased growth, developmental abnormalities (e.g., penis size) in children exposed in utero to PCBs
- Altered girl/boy ratio after population exposure to dioxin (Saveso, Italy)
- Shortened lactation associated with DDE
- Decreased sperm count and quality
- Increased prostate, testicular, breast cancer

Human Breast Cancer

- Breast cancer has increased

but

- Epidemiological studies are conflicting - It is not possible to assign a specific chemical or physical cause at this time

- **Better animal models are needed to predict human risk**

Human Sperm Counts

Carlsen et al, 1992 meta-analysis: 61 studies

- Suggests 50% decline in count, volume
- Decline seen in both Europe and US

but

- Large geographic variation among studies
- Potential selection bias, other confounders

A large, carefully controlled prospective study is needed for confirmation

Testicular Cancer

- Increase in testicular cancer observed in most countries
- Affects mostly ages 15-45
- Year of birth, birth weight, genital tract abnormalities are risk factors
- Evidence suggests high estrogen environment during fetal life may be involved

but

- No increase in testicular cancer in DES sons

TDS = testicular dysgenesis syndrome

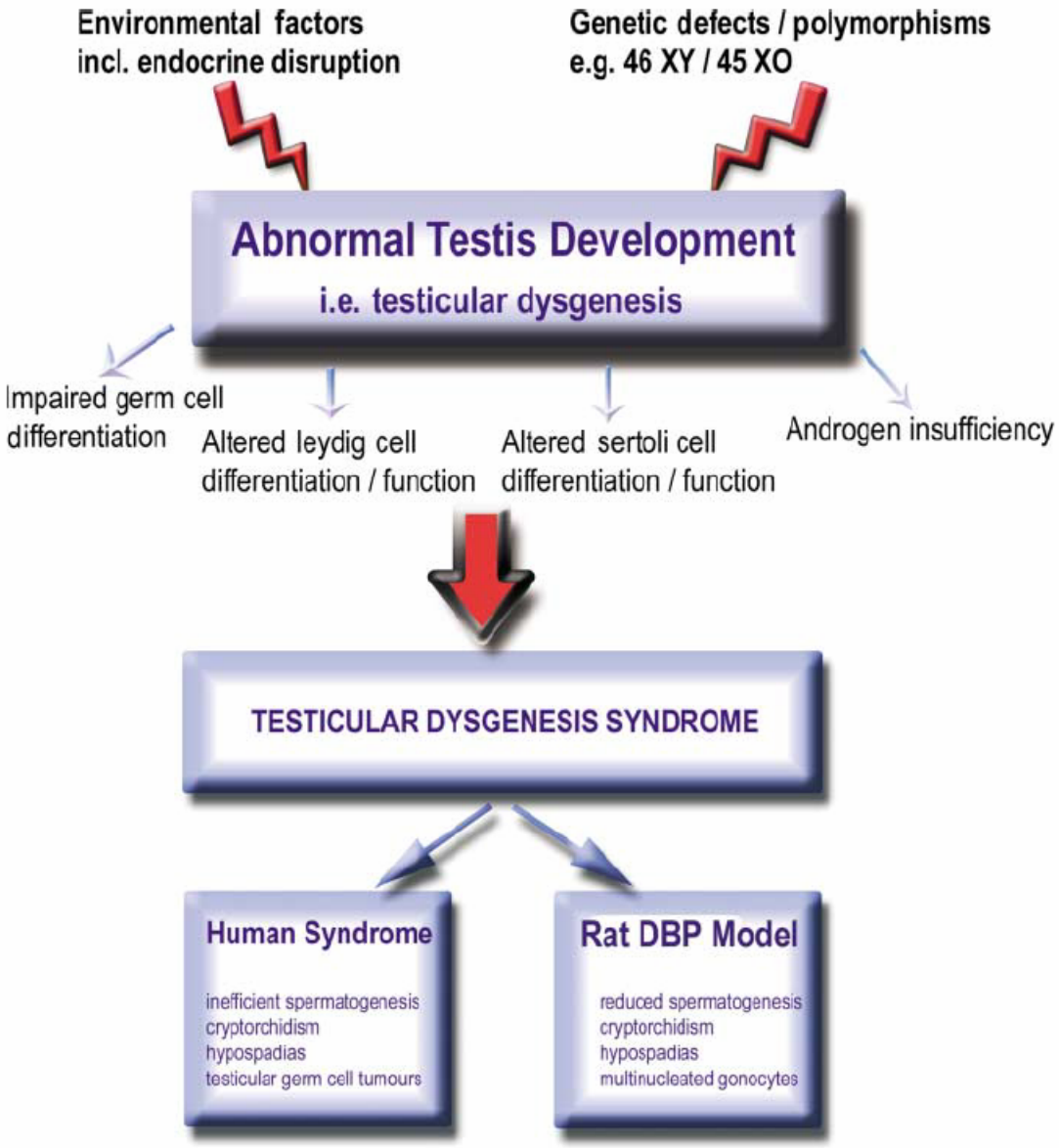
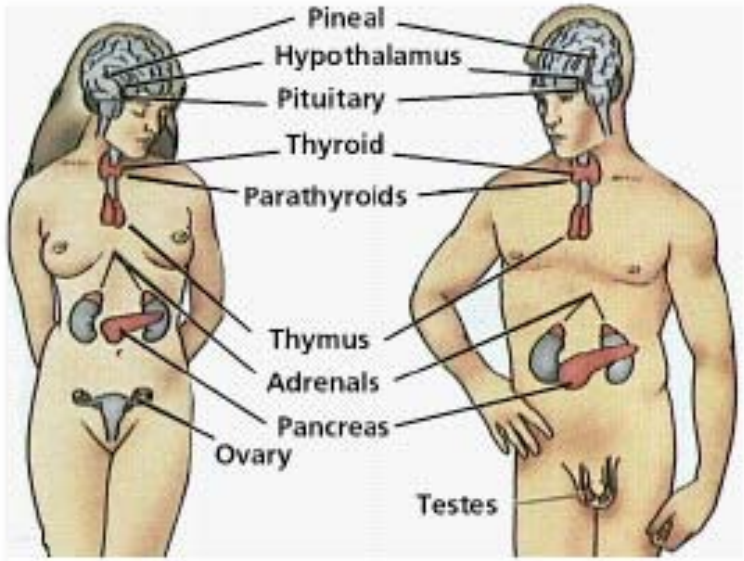


Figure 1 Schematic representation of the potential pathogenic links between testis development and the clinical manifestations of testicular dysgenesis syndrome (TDS). The similarities in the pathologies induced by *in utero* dibutyl phthalate (DBP) administration and human TDS are compared.

Endocrine (hormonal) system regulates

- **Metabolic function and equilibrium**
- **Reproduction**
- **Growth/development**

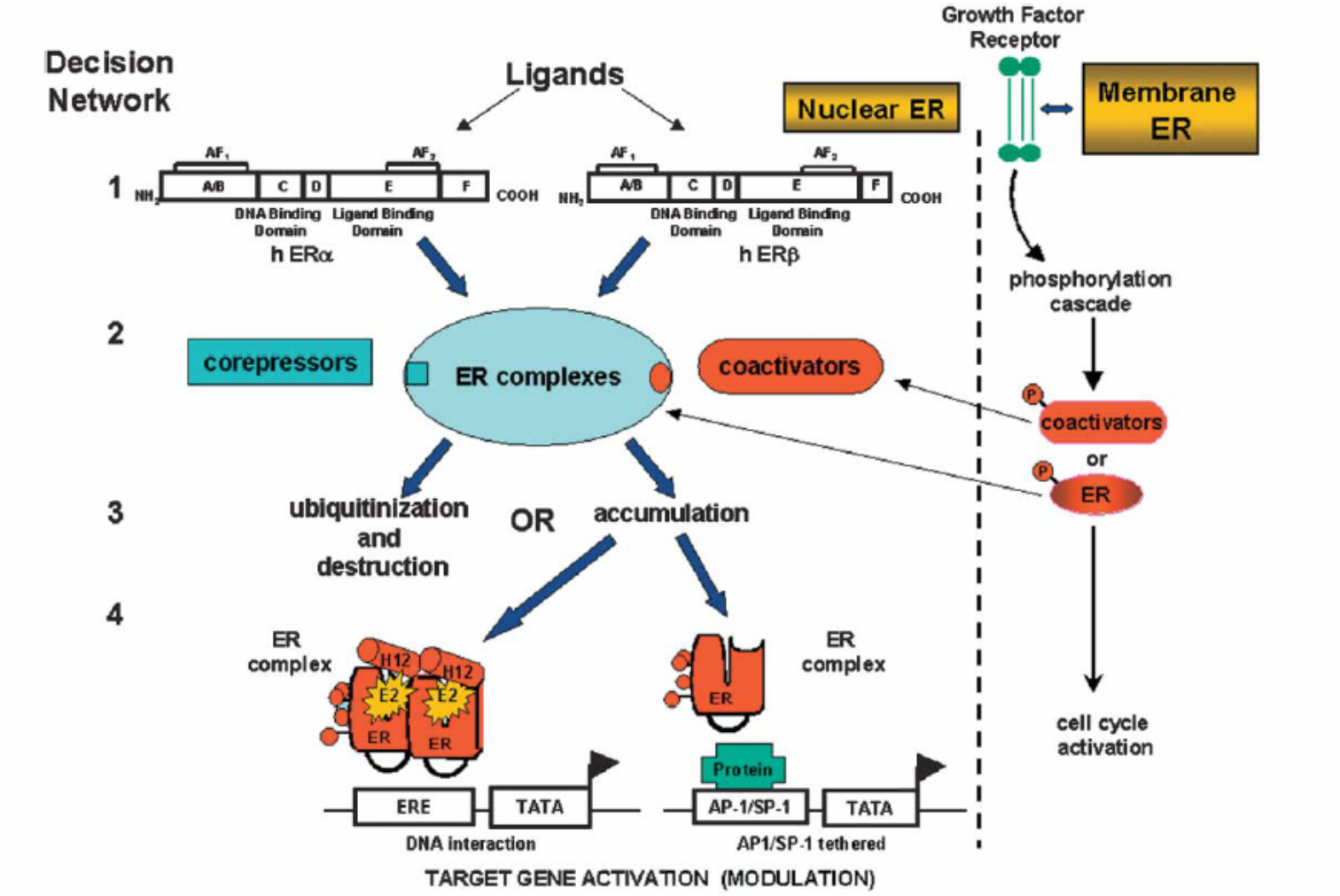


There are over 50 different hormones

Environmental estrogens (xenoestrogens)

- Sources
  - pesticides
  - plastics
  - pharmaceuticals
  - some cleansers
  - contraception
- vs. phytoestrogens
  - antiherbivore compounds in many plant species
  - lignans (many fruits, vegetables), isoflavones (soy)

Možnosti účinků environmentálních estrogenů na buněčné úrovni



Environmentální estrogeny:

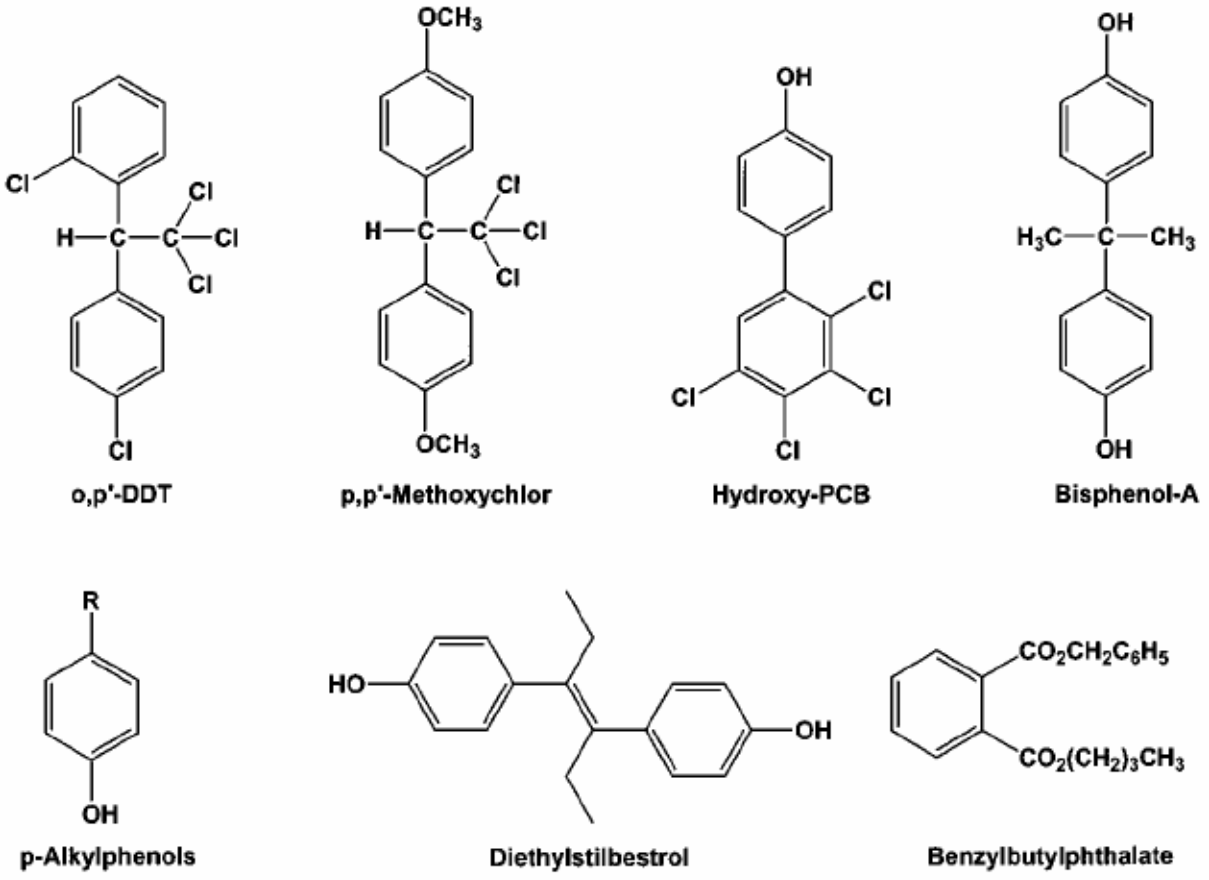


Figure 2 Structures of some xenoestrogens.

Environmentální antiandrogeny:

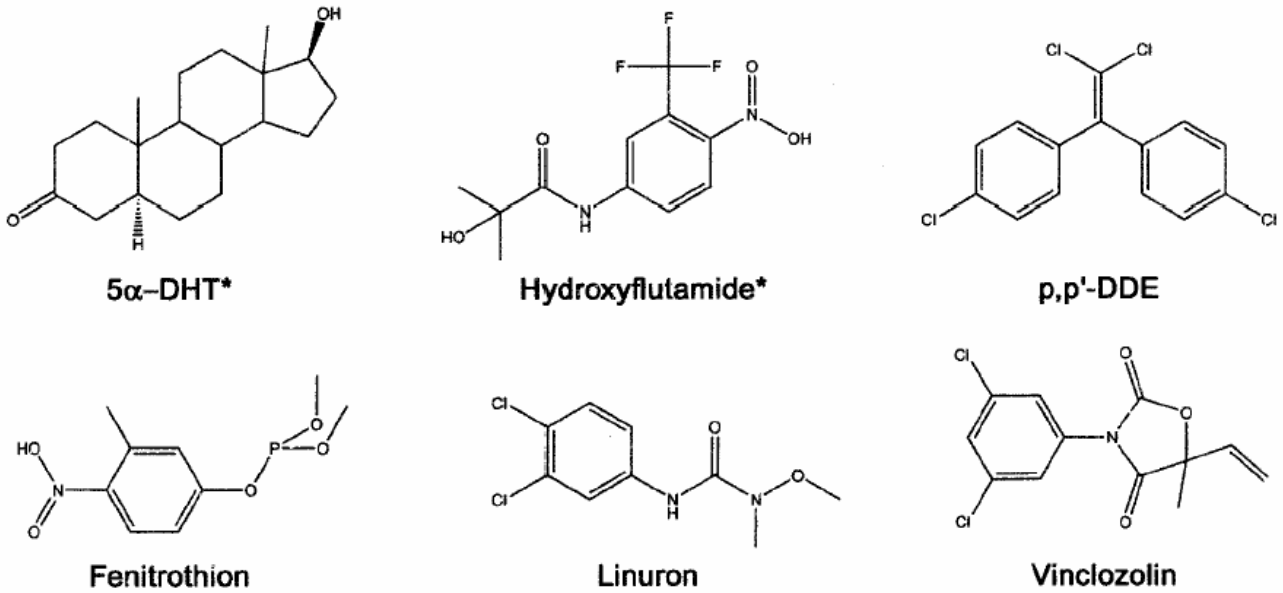


FIG. 2. Structural diversity among environmental chemicals reported to be antiandrogenic. The steroidal androgen, 5α-dihydroxytestosterone (5α-DHT) and its pharmaceutical antagonist, hydroxyflutamide, are shown for comparison. *p,p′*-DDE is a persistent contaminant, while the remaining are currently used pesticides: fenitrothion, an insecticide; linuron, an herbicide; and vinclozolin, a fungicide.

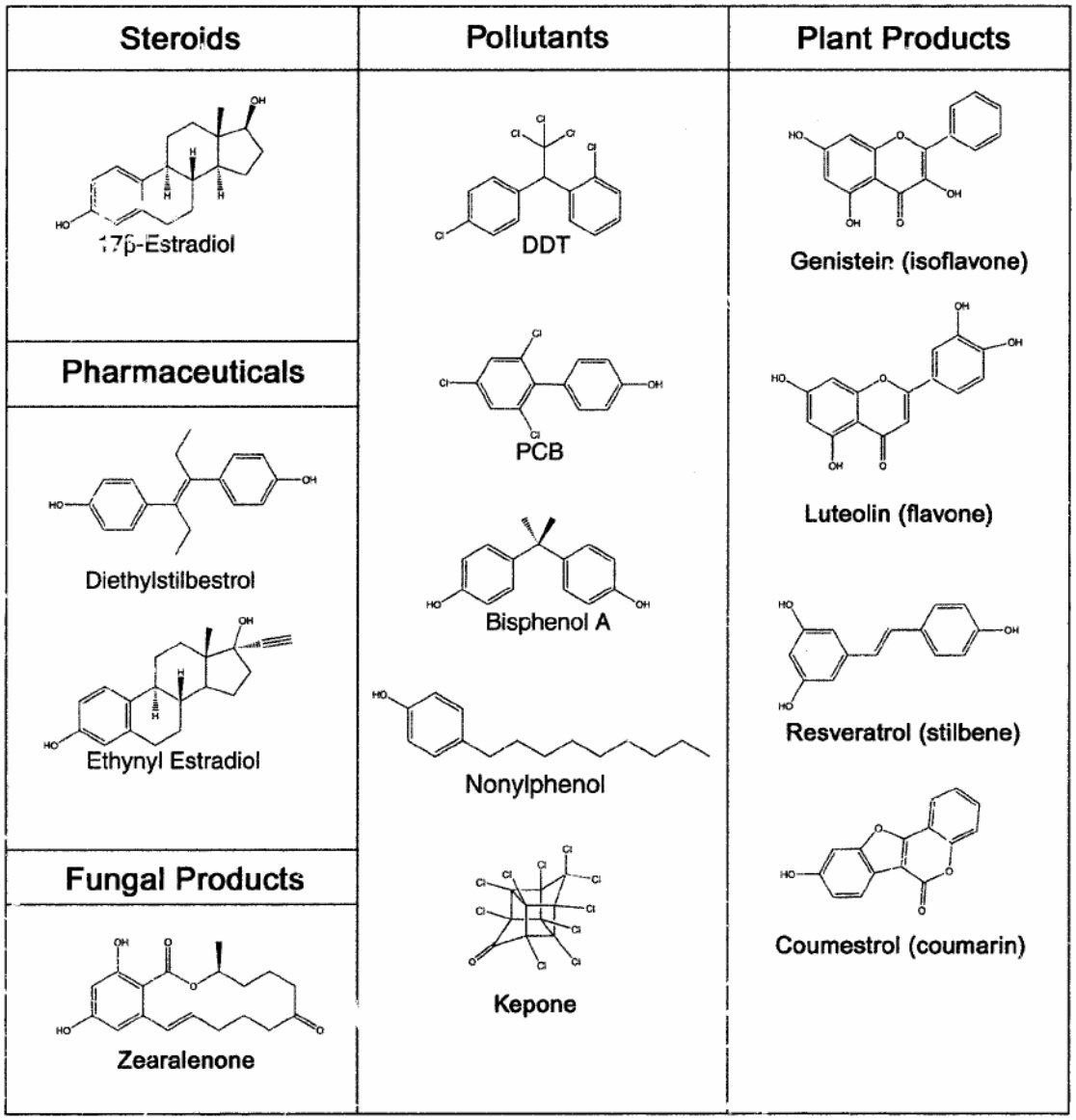
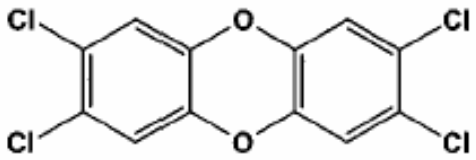


FIG. 5. Chemicals found in the environment reported to be estrogenic. This list is not comprehensive, but illustrates representative structures of estrogenic compounds from various sources. Information on these compounds is contained in the text.

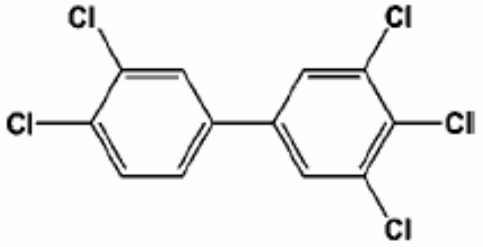
Xenoestrogens and xenoandrogens can:

- Mimic or partly mimic the sex steroid hormones estrogens and androgens (the male sex hormone) by binding to hormone receptors or influencing cell signaling pathways. Those that act like estrogen are called **environmental estrogens**.
- Modify the making and function of hormone receptors.
- Block, prevent and alter hormonal binding to hormone receptors or influencing cell signaling pathways. Chemicals that block or antagonize hormones are labeled **anti-estrogens** or **anti-androgens**.
- Alter production and breakdown of natural hormones.

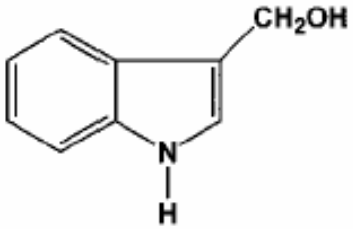
Interakce AhR a ER:



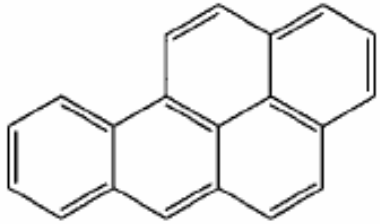
2,3,7,8-TCDD



3,3',4,4',5-pentaCB



I3C



BaP

Figure 5 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds that bind to the AhR.

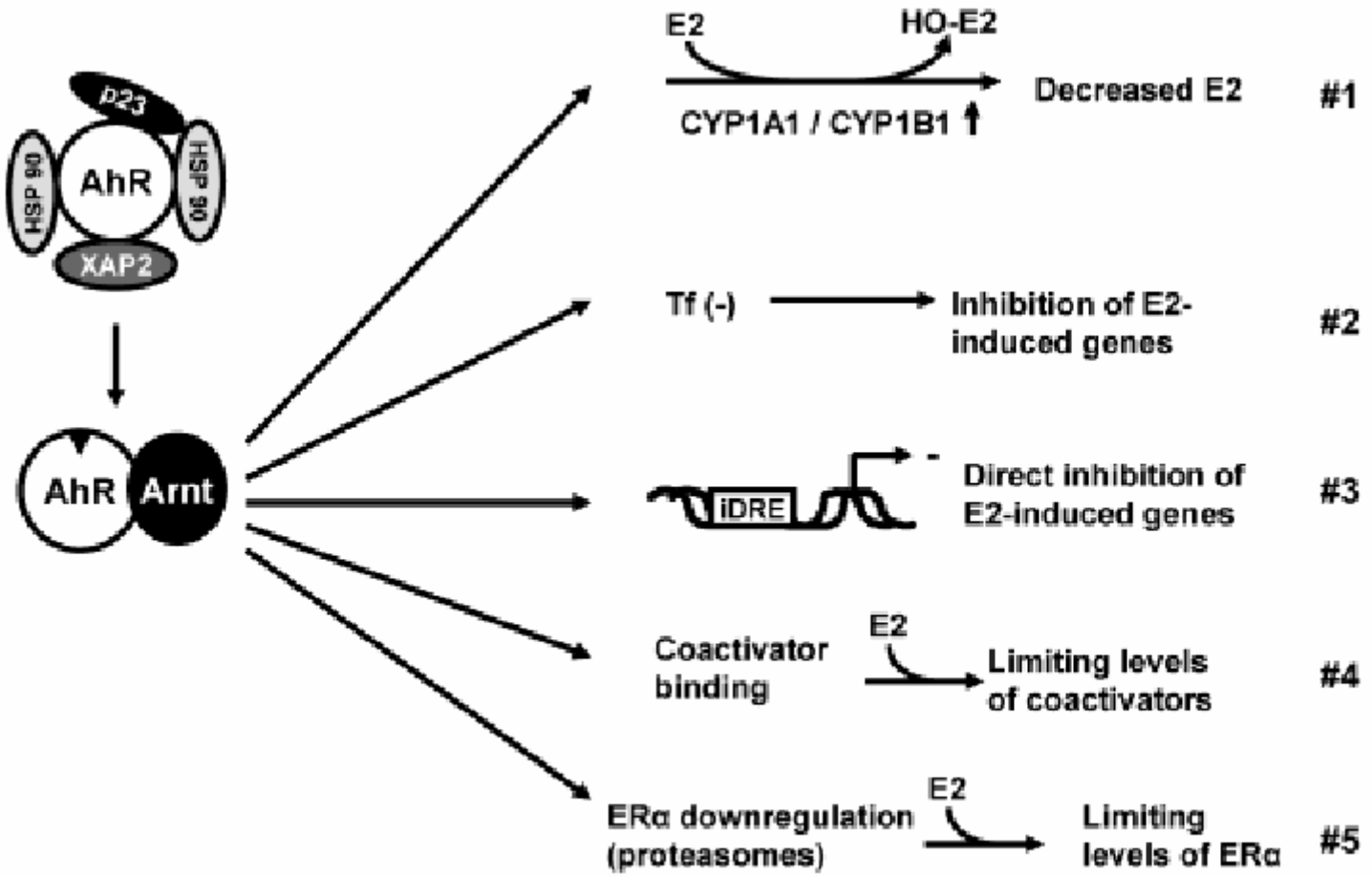
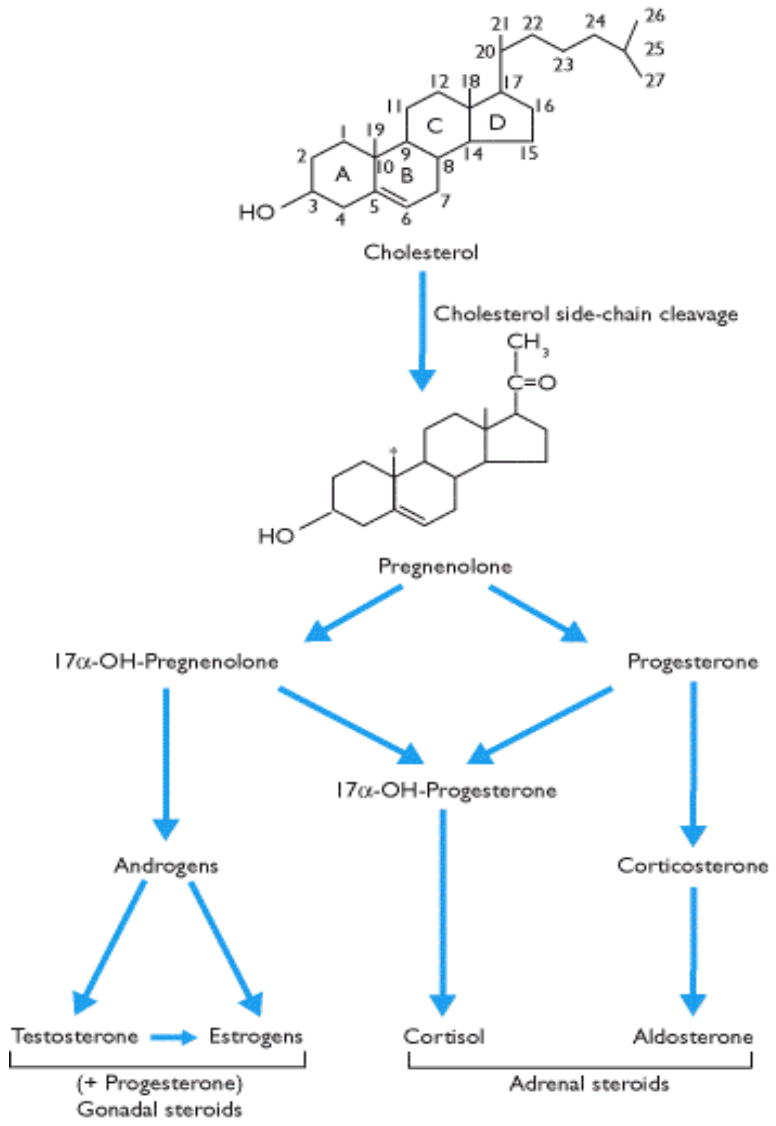


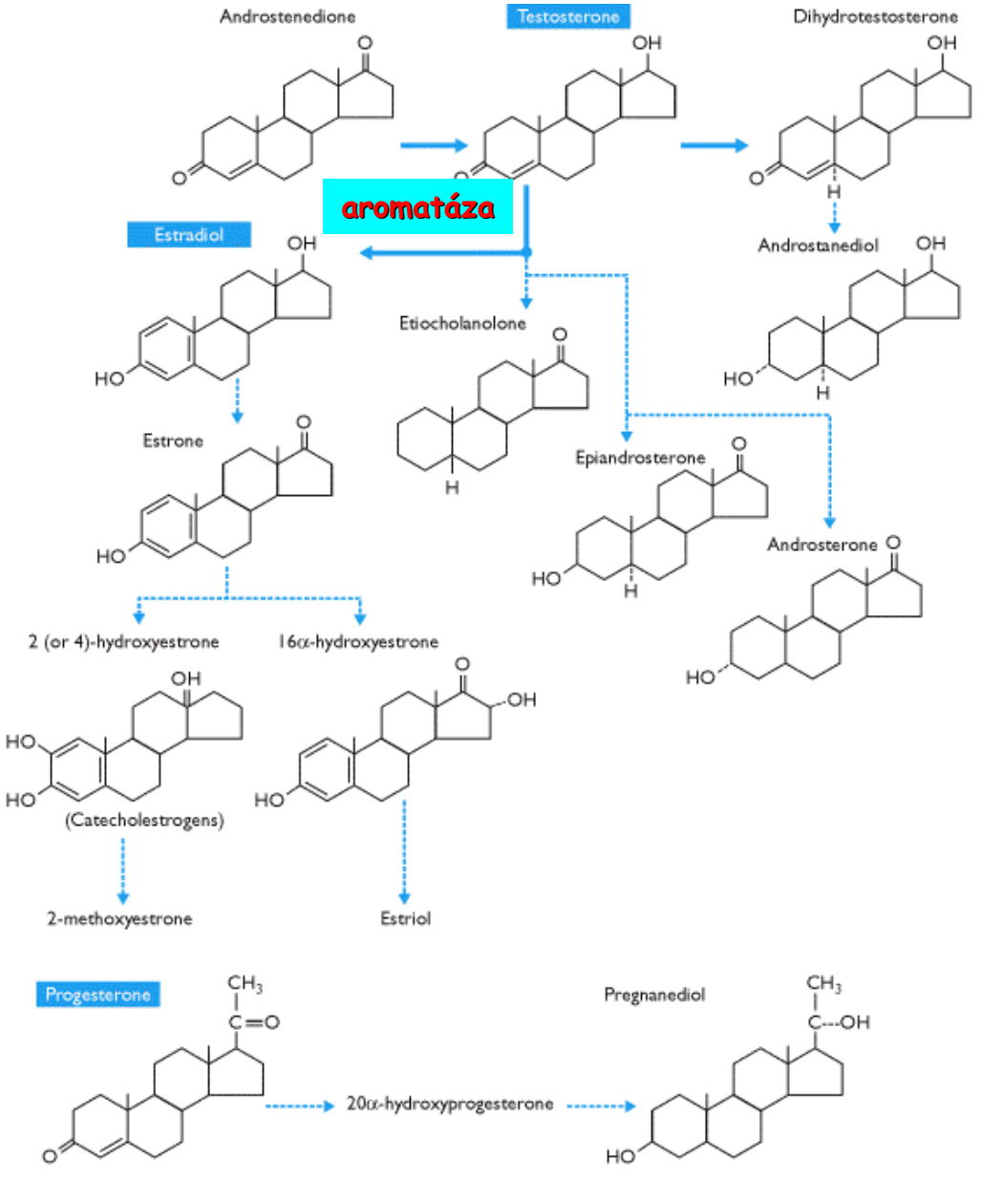
Figure 3. Proposed mechanisms of inhibitory AhR–ERα cross-talk (*123–126*).

Hormonální přípravky jako EDs - ethinylestradiol

- Male fish living near municipal sewage outlets in England had both male and female sex characteristics and their livers produced vitellogenin, a female egg-yolk protein not normally found in males
- cancers of the female and male reproductive tract
- malformed Fallopian tubes, uterus and cervix
- altered bone density and structure
- abnormal blood hormone levels
- reduced fertility
- altered sexual behavior
- modified immune system

Biosyntéza steroidních hormonů a endokrinní disrupce:



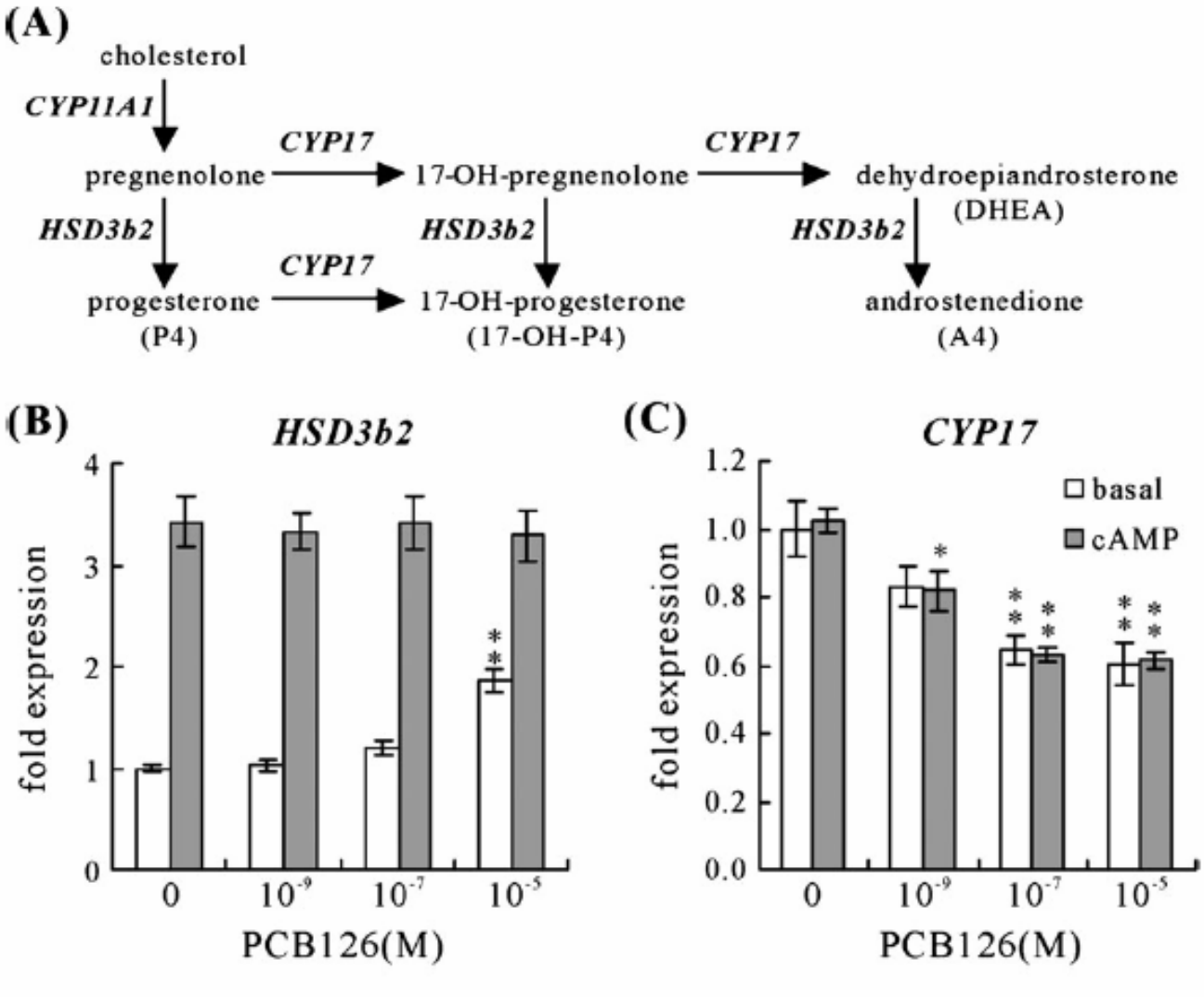

Androstenedione
Testosterone
Dihydrotestosterone
aromatáza
Estradiol
Androstanediol
Etiocholanolone
Estrone
Epiandrosterone
Androsterone
2 (or 4)-hydroxyestrone
16α-hydroxyestrone
(Catecholestrogens)
2-methoxyestrone
Estriol
Progesterone
20α-hydroxyprogesterone
Pregnanediol

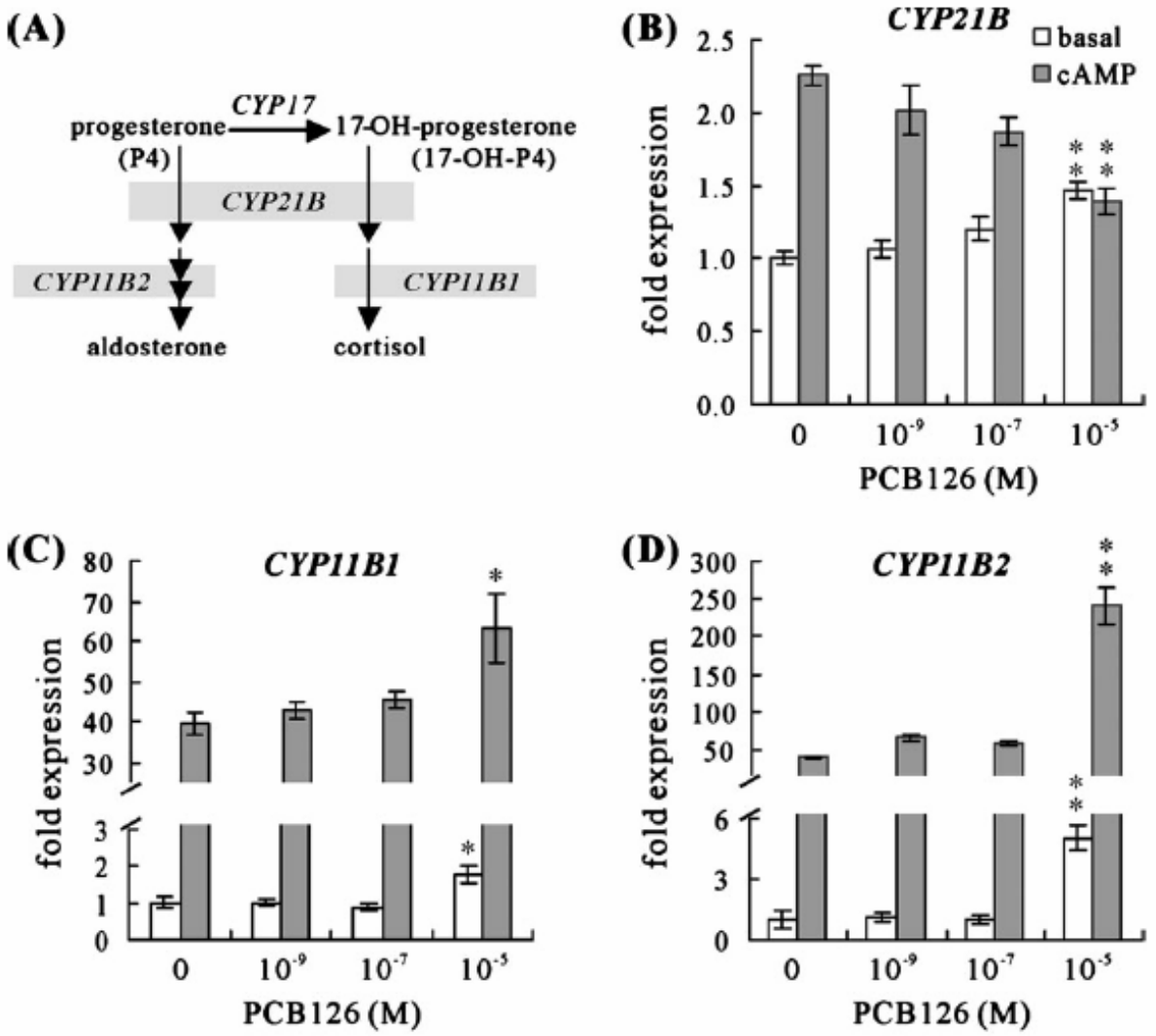
In vitro model:

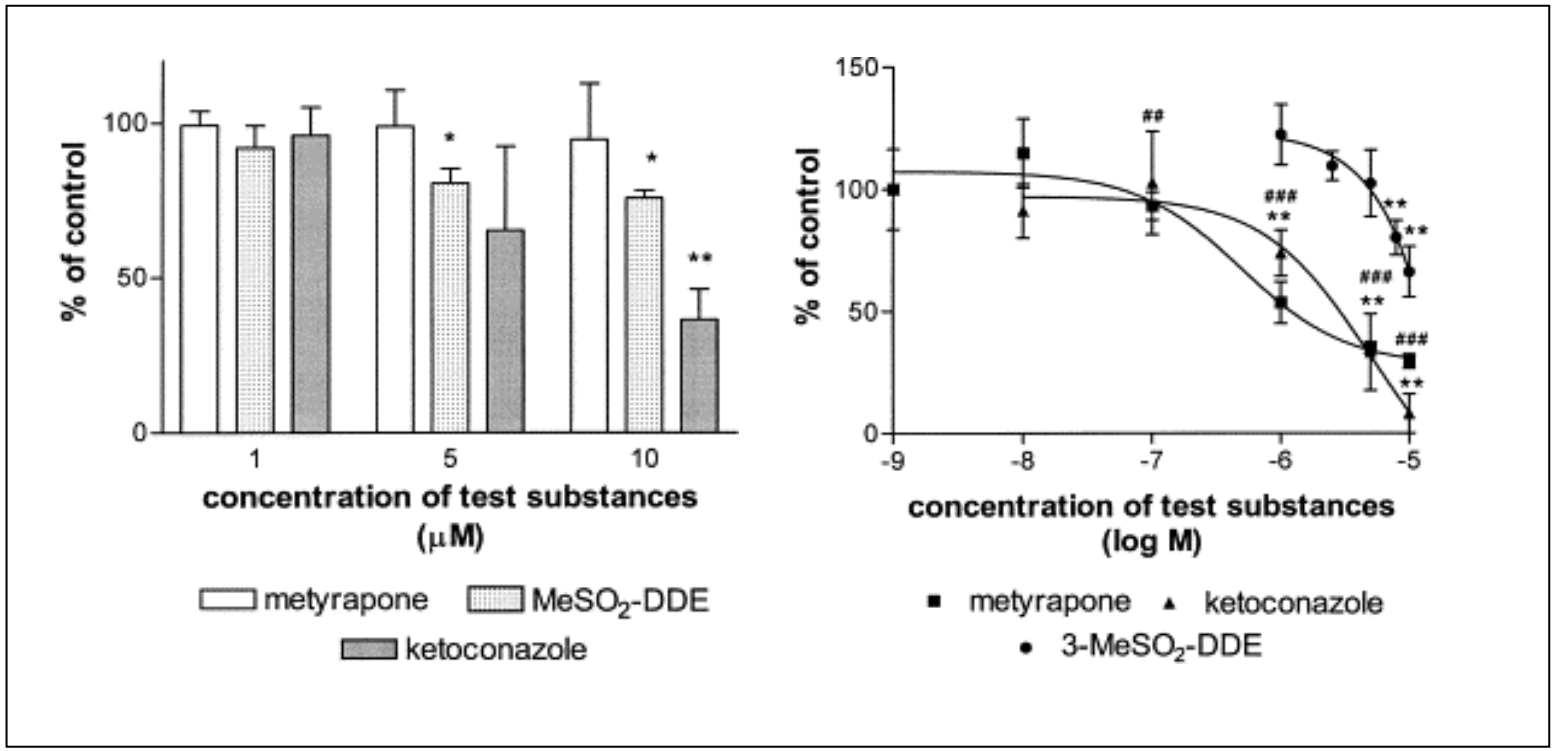
buňky H295R

Buněčná linie odvozená od karcinomu kůry nadledvinek, která je schopna in vitro produkovat většinu steroidogenních enzymů:

- aktivita enzymů;
- exprese enzymů na úrovni mRNA a proteinu


(A)
cholesterol
CYP11A1
pregnenolone
CYP17
17-OH-pregnenolone
CYP17
dehydroepiandrosterone
(DHEA)
HSD3b2
HSD3b2
HSD3b2
progesterone
(P4)
CYP17
17-OH-progesterone
(17-OH-P4)
androstenedione
(A4)
(B)
HSD3b2
fold expression
PCB126(M)
(C)
CYP17
basal
cAMP
fold expression
PCB126(M)


(A)
progesterone
(P4)
CYP17
17-OH-progesterone
(17-OH-P4)
CYP21B
CYP11B2
CYP11B1
aldosterone
cortisol
(B)
CYP21B
basal
cAMP
fold expression
PCB126 (M)
(C)
CYP11B1
fold expression
PCB126 (M)
(D)
CYP11B2
fold expression
PCB126 (M)



Effects of test substances on cortisol and 11-deoxycortisol formation in H295R cells, assumed to represent CYP11B and CYP21 activity.

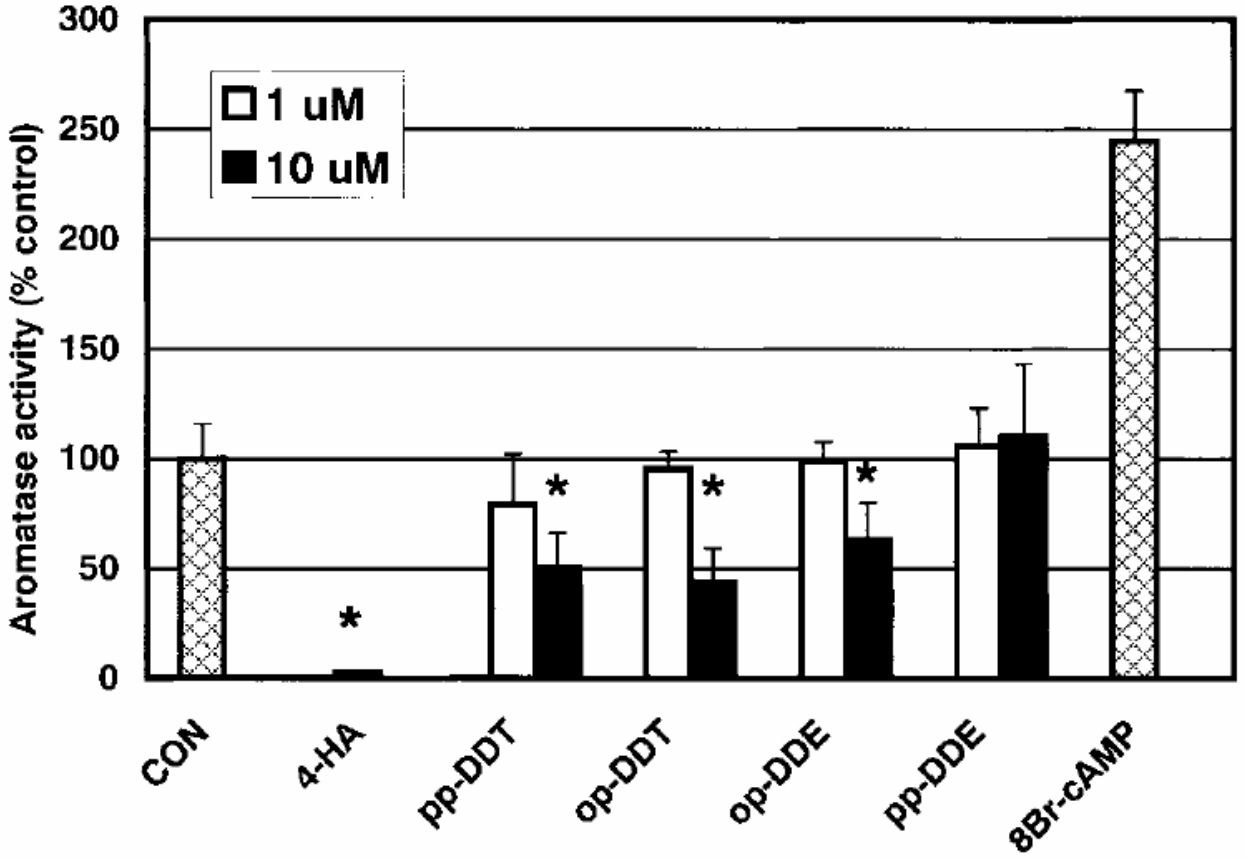


FIG. 2. Effect of 4-hydroxyandrostenedione (4-HA; 1 μM), DDT, three of its metabolites (1 or 10 μM) or 8-bromo-cyclic adenosine monophosphate (8Br-cAMP; 300 μM) on aromatase activity in H295R cells. Exposures were for 24 h, in quadruplicate. *Significantly lower than control.

Testy estrogenity a antiestrogenity

In vitro assay	Measured endpoint	Advantages	Limitations
E-Screen	Proliferation of ERα-positive cells	Measures physiological endpoint of estrogen action, measures estrogens and antiestrogens	No defined ER expression, no mechanistic data
Ligand-binding (EDSTAC)a	Binding affinity to ERα or ERβ	Simple, high-throughput method	Does not measure ER activation, does not measure physiological response
ER-binding to ERE	Binding affinity of Erα or ERβ to ERE	High-throughput method, various EREs can be used	Does not measure ER activation, low sensitivity, does not measure physiological response
GST pull-down/FRET/ two-hybrid assay	Ligand-dependent association of ERα or ERβ with co-activators	Analysis of molecular interaction, defined ER subtype or ER domain as well as co-activators can be used, measures estrogens and antiestrogens	Does not measure direct ER activation, low throughput, does not measure physiological response
Transactivation assay in yeast or mammalian cells (EDSTAC)a	ERα or ERβ mediated activation of reporter	High-throughput method, measures estrogens and antiestrogens, can be done in metabolic competent cells to account for (anti)-estrogenic metabolites	Does not measure physiological response
Analysis of gene expression	Expression of ER-regulated genes	Analysis of physiological response, versatile, measures estrogens and antiestrogens	Low throughput
Analysis of enzyme activity	Activity of ER-regulated enzymes	Analysis of physiological response, measures estrogens and antiestrogens	Cell lines or primary cell cultures with active marker enzymes suitable only
Analysis of steroidogenesis (EDSTAC)a	Induction/inhibition of estrogen biosynthesis	Analysis of physiological response, measures ER-independent pathways	Cells with active steroidogenesis suitable only

Mikrobiální syntéza androgenů??

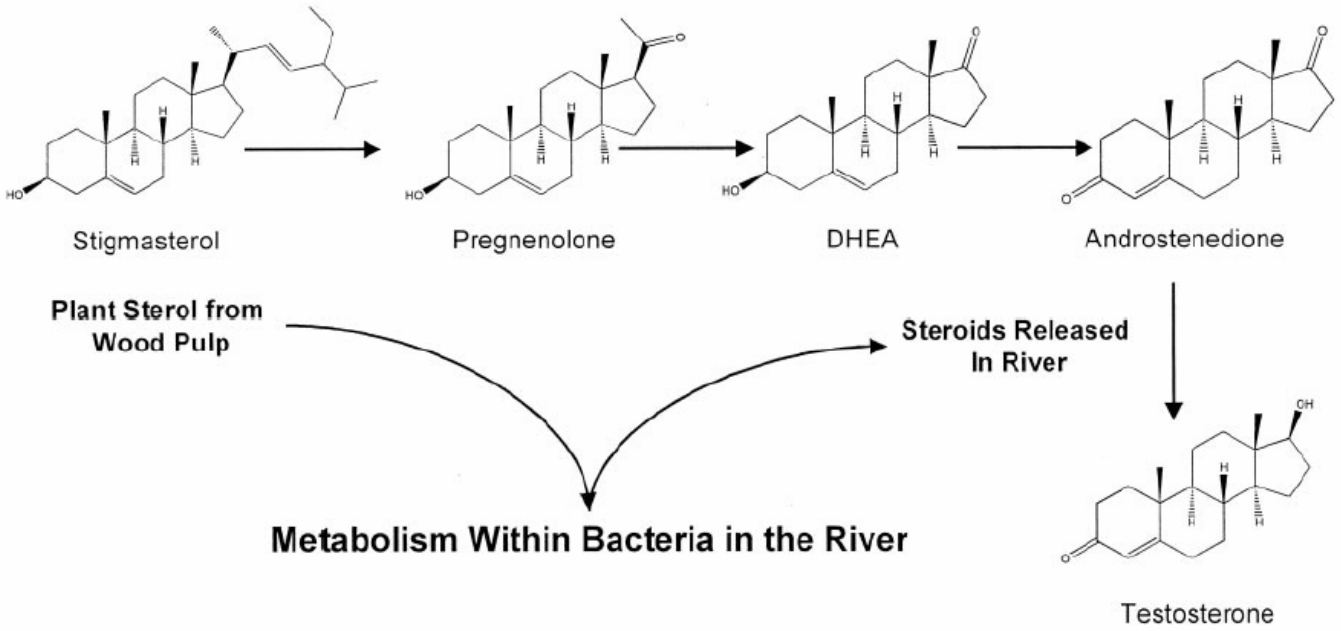


FIG. 3. The production of androgenic compounds by bacteria. Stigmasterol, a major plant sterol found in wood pulp, is efficiently metabolized to androgenic steroids such as androstenedione by the bacteria, *Mycobacterium smegmatis*. *M. smegmatis* form extensive colonies, or "bacterial mats," at the effluent site of pulp and paper mills. The natural plant sterol, stigmasterol, contained in the pulp effluent is converted by *M. smegmatis* into androstenedione, which is released into the river or stream. Female mosquito fish exposed to these androgens develop male structures. (See Refs. 34, 35, and 36 for details.)

Většina látek narušujících androgenní dráhy jsou antiandrogeny!!!!

Anti-androgenic compounds in the environment

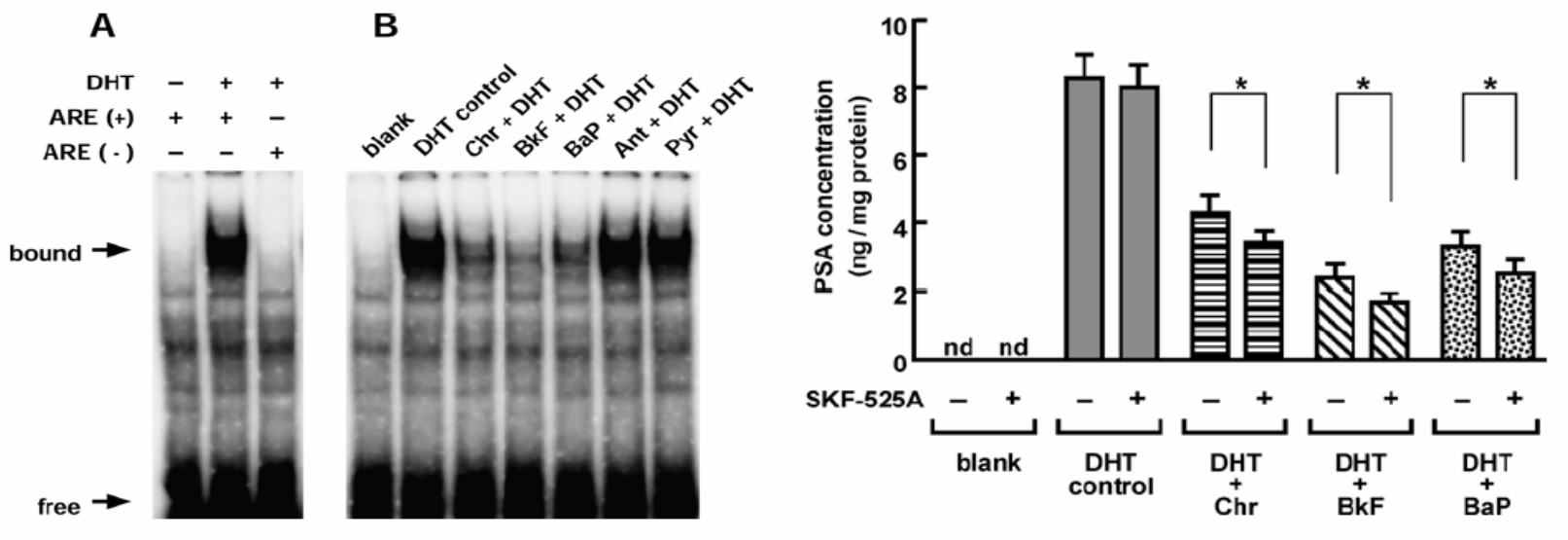
There are a number of commonly used environmental chemicals that have been identified as having anti-androgenic properties. These chemicals have been administered to pregnant rodents during the period of reproductive tract development. When the male pups were examined, they displayed many of the abnormalities associated with flutamide administration.

Some chemicals (vinclozolin, procymidone, linuron, p,p'-DDE (1,1,1-dichloro-2,2-bis(pchlorophenyl)ethane) act as androgen receptor antagonists, others (phthalate esters) reduce androgen synthesis, but it is likely that other modes of action are also involved in the toxicity induced by these compounds.

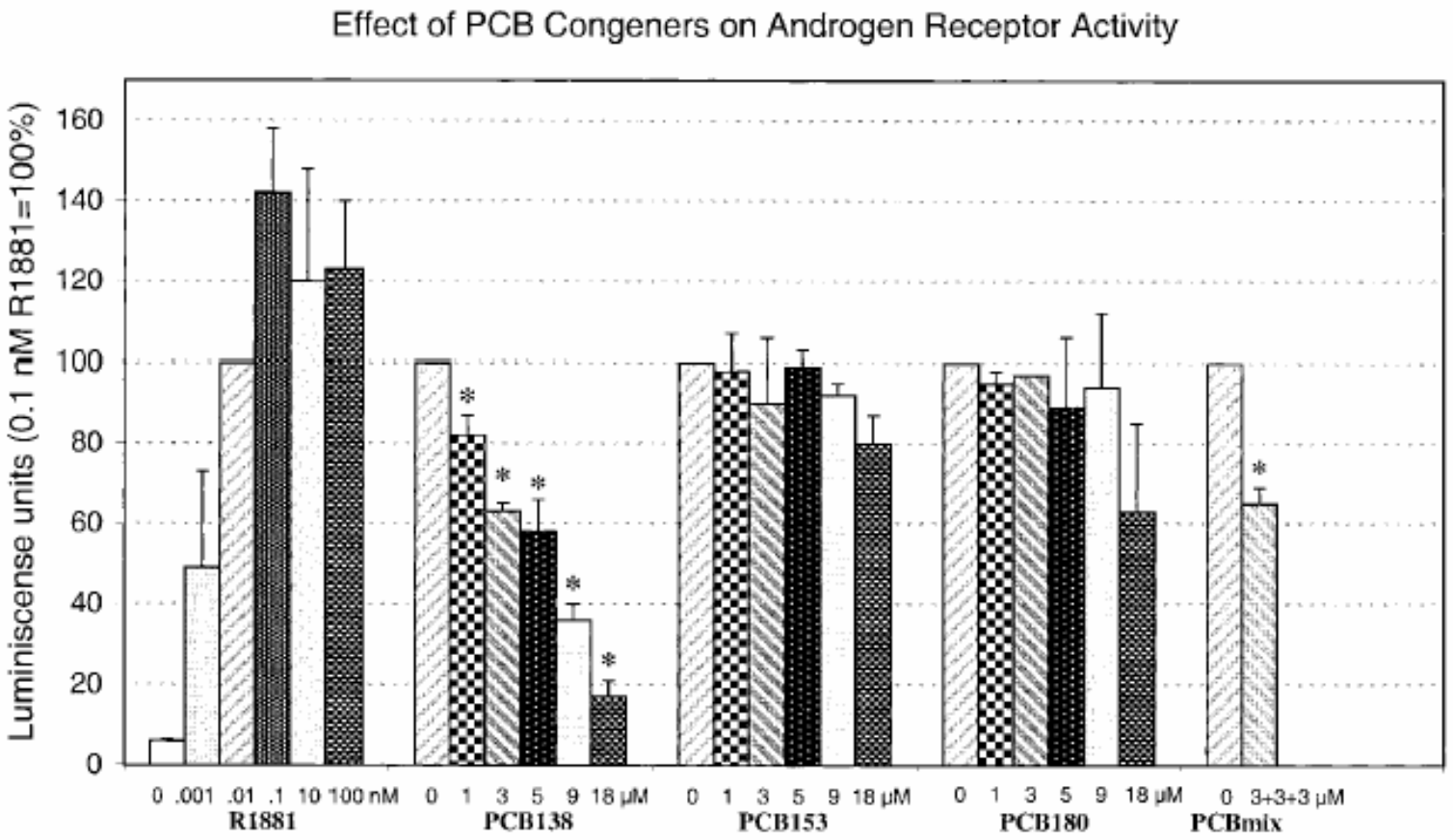
There are major problems in comparing the published studies of the effects of anti-androgenic compounds / inconsistent protocols.

Human impact????

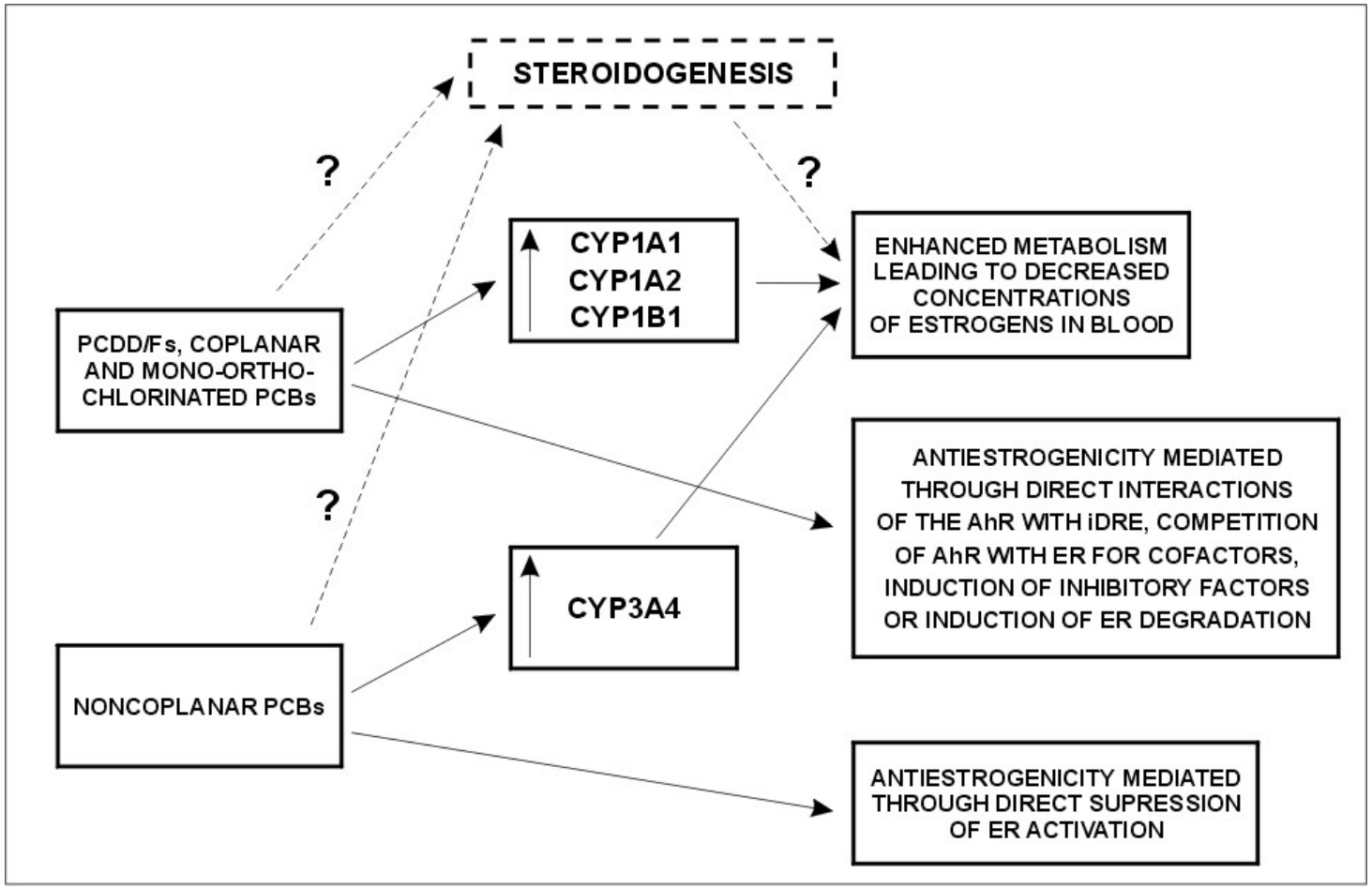
Polycyklické aromatické uhlovodíky mají antiandrogenní účinek:



Antiandrogenní účinky PCB:

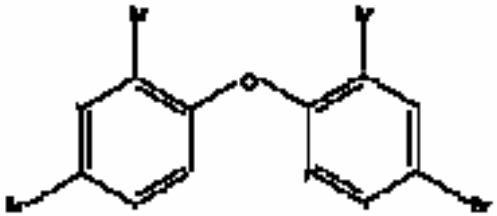


Interakce polutantů s endokrinní dráhou = velmi složitý proces:

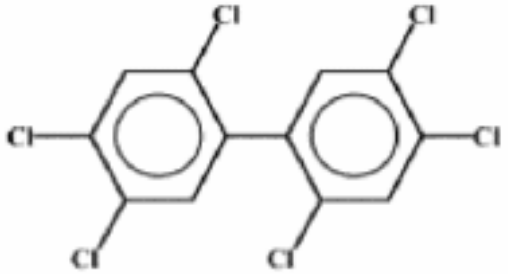


Bromované zpomalovače hoření – nový typ endokrinních disruptorů??

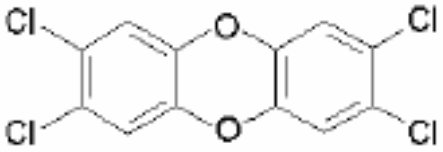
Structure compared to PCBs, dioxin, thyroxin



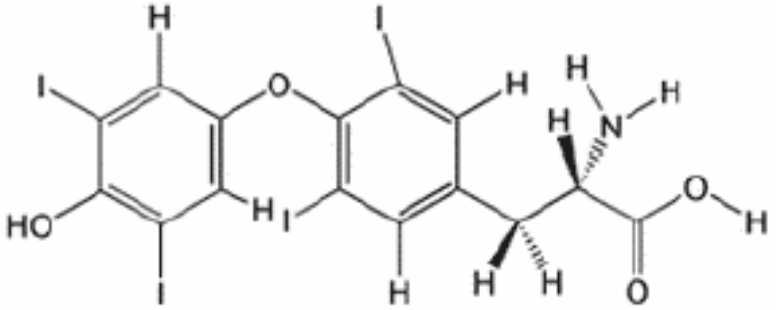
BDE-47



PCB



2,3,7,8-TCDD
(dioxin)



Thyroxin (T4)