Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

09 – Mechanisms
Nuclear Receptors

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www.recetox.cz

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Content of the presentation - 2023

- **Section 1 -** Slides in the beginning (**No. 1-36**) were discussed during the lecture to illustrate
  - Mechanism of action of NRs
  - Effects of endocrine disrupters on various levels of hormone action (synthesis, transport, action at receptor, metabolism/clearance)
  - Examples of the most studied and well recognized outcomes related to ED – i.e. feminization (caused by estrogens and antiandrogens)

- Section 2 - Other slides
  - Not presented during the lecture but kept for illustration of other examples

SECTION 1
SLIDES PRESENTED & DISCUSSED

Various signalling types … now focus on nuclear receptors

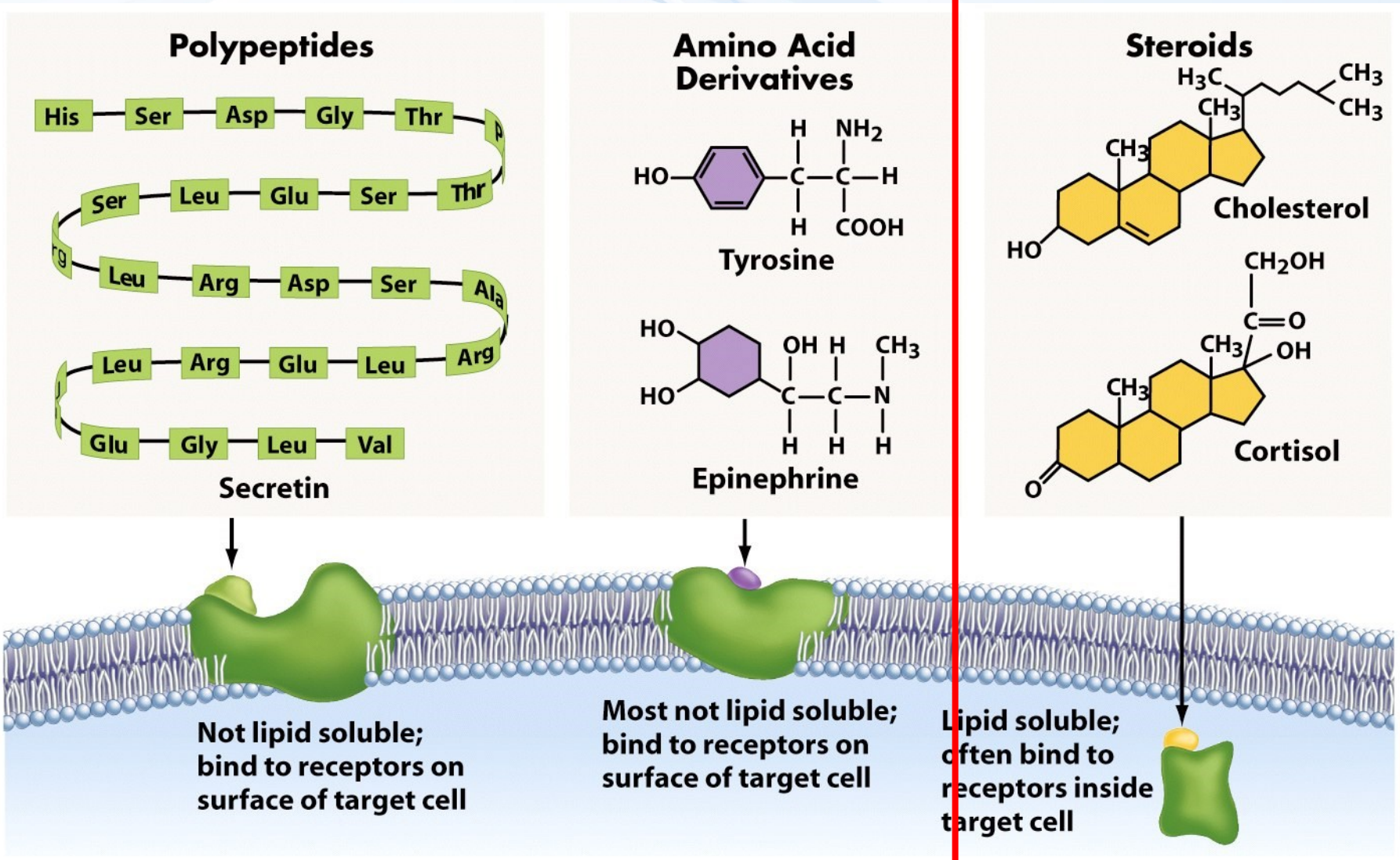


Figure 47-3 Biological Science, 2/e

NUCLEAR (Intracellular) RECEPTORS in summary

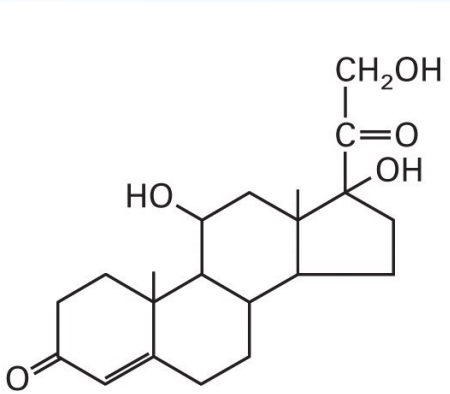
- Important physiological functions, and
- Important roles in pathologies and chemical toxicity
  - Endocrine disruption
  - Dioxin-like toxicity,etc.

- All NRs share similar structure and mechanisms of action
  - Act as **direct transcription factors on DNA**

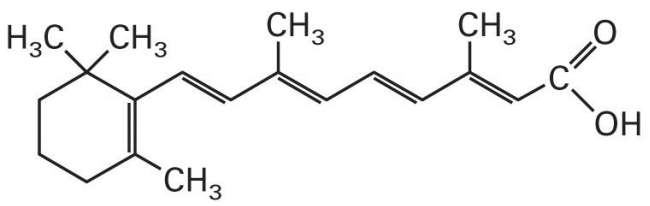
- Natural **ligands are small lipophilic** hormones (steroids, thyroids, retinoids)
  - Role in toxicity – NR are modulated (activated/inhibited) by structurally close xenobiotics

Natural ligands of NR

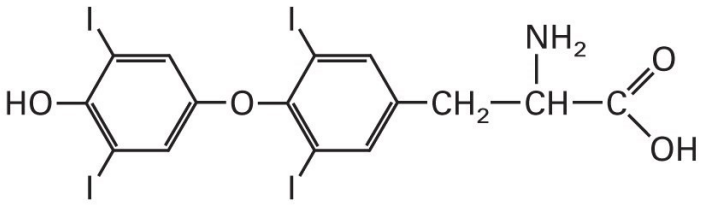
- **Small, lipid-soluble molecules**
  - Diffuse through plasma and nuclear membranes and interact directly with the transcription factors they control.
  - **STEROID HORMONES:**
    - sex steroids (estrogen, progesterone, testosterone)
    - corticosteroids (glucocorticoids and mineralcorticoids)
  - **OTHER HORMONES and ligands**
    Thyroid hormone, vitamin D3, retinoic acid, ligands of AhR
  - **Small molecules - gases**
    e.g. NO (signaling for immune reactions)



Cortisol



Retinoic acid



Thyroxine

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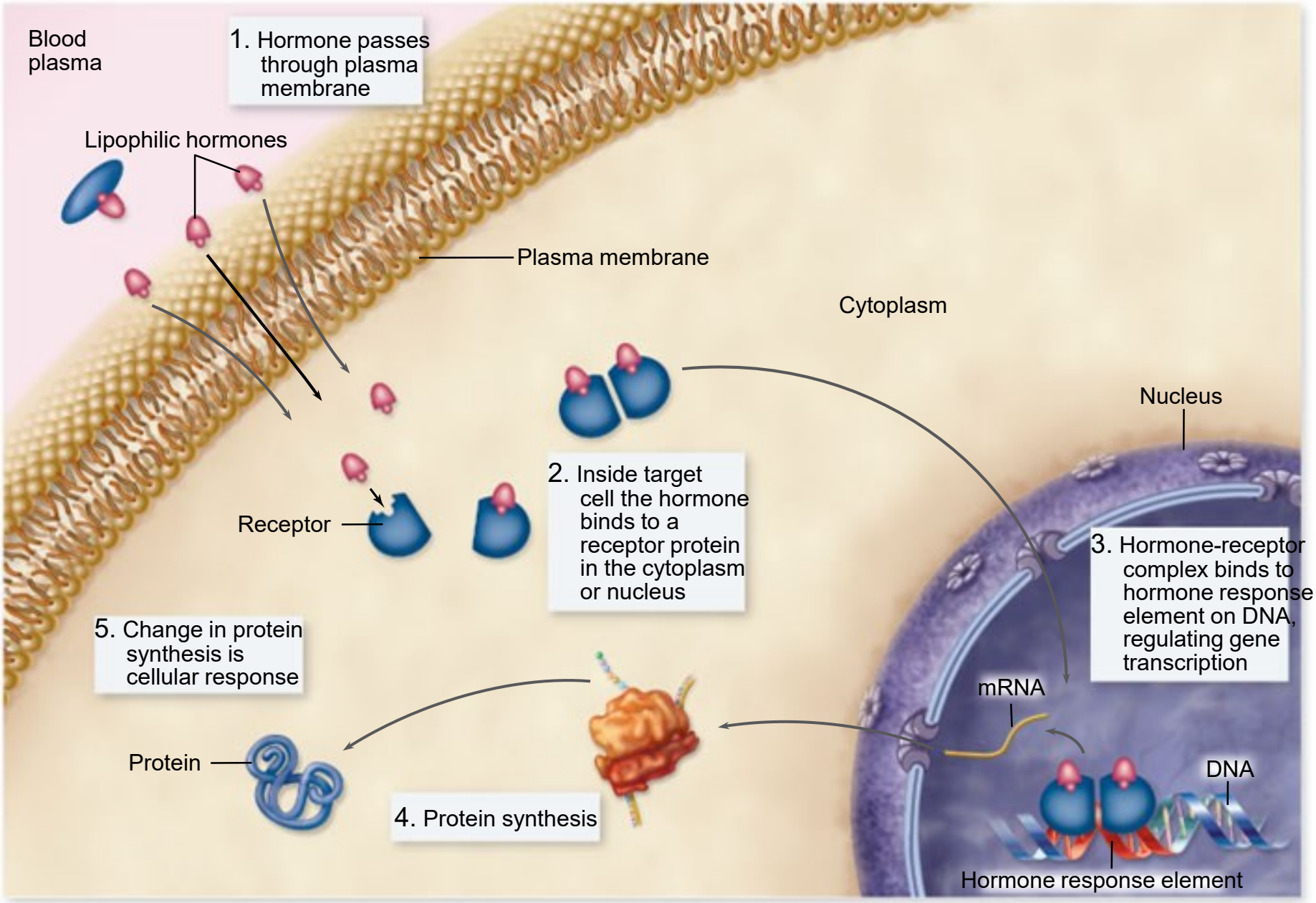
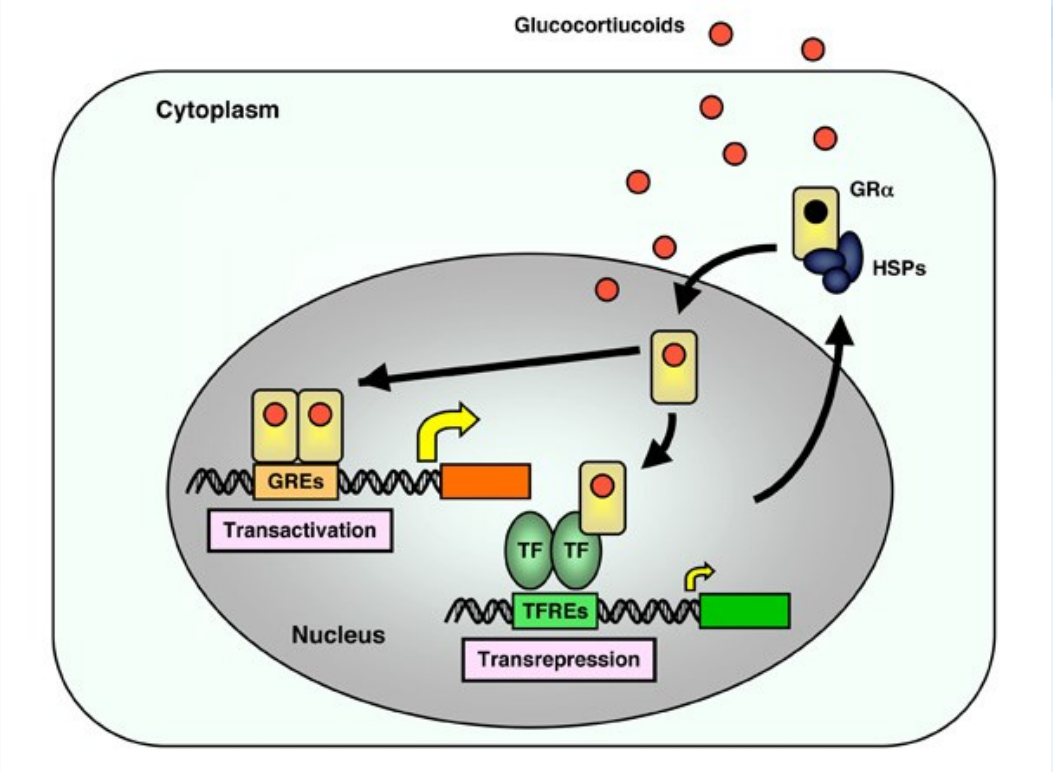


NR signalling is complex … examples of complexity (1)

1. Receptor activation is dependent not only on „ligand“ (**glucocorticoid**) but also on „inhibitor“ protein (Heat Shock Proteints - **HSPs**)

2. Dimerization (after the activation) is often needed for proper action (binding to **GREs** – *glucocorticoid responsive elements)*

3. Receptor with ligand can activate its own targets (GREs) as well as „repress“ other binding sites (**TFREs**)



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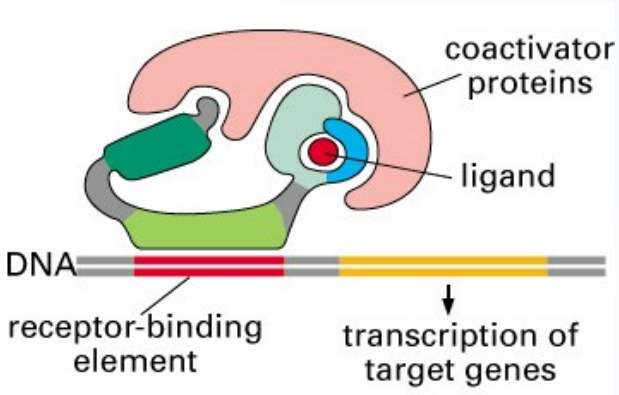
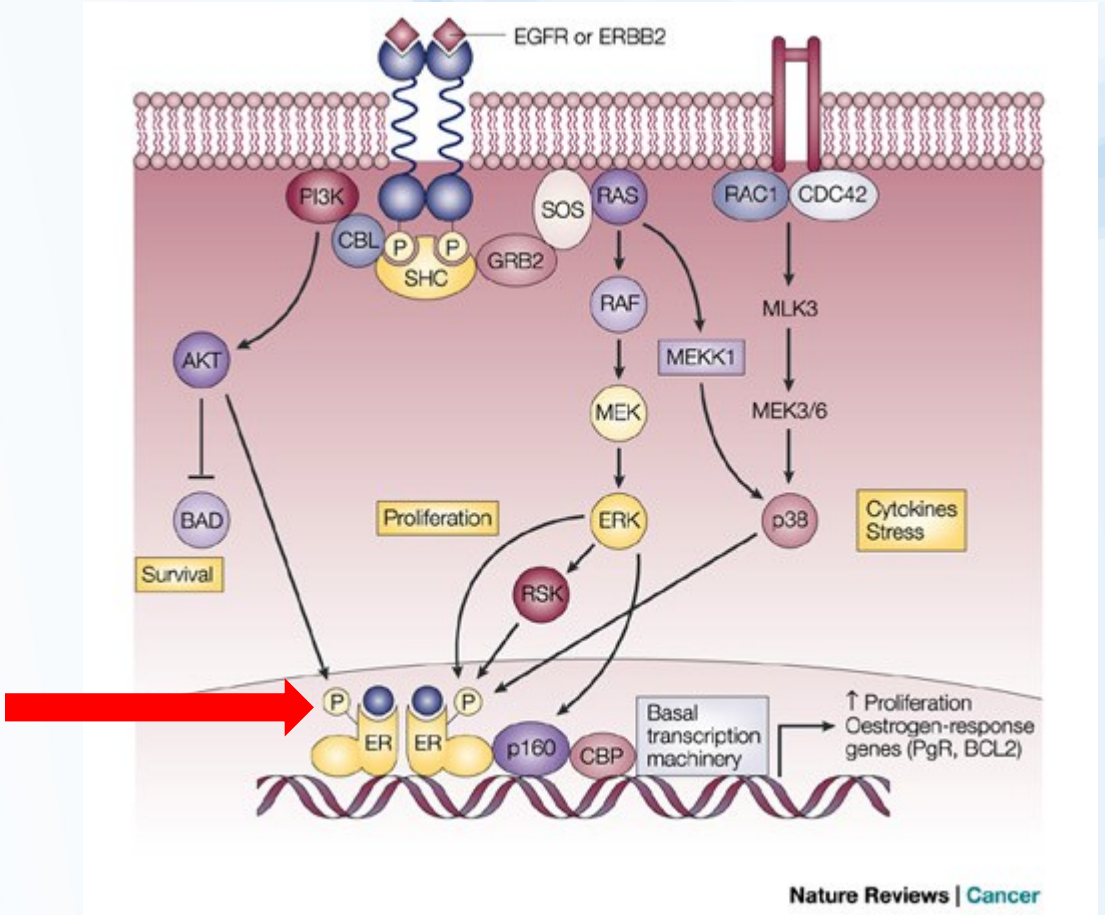
NR signalling is complex … examples of complexity 2

4. **„Co-activator“ proteins** are needed for proper action on DNA



5. Nuclear receptor action are (also) controlled - stimulated / suppressed - by **other signalling pathways** (e.g. phosphorylation by protein kinases)



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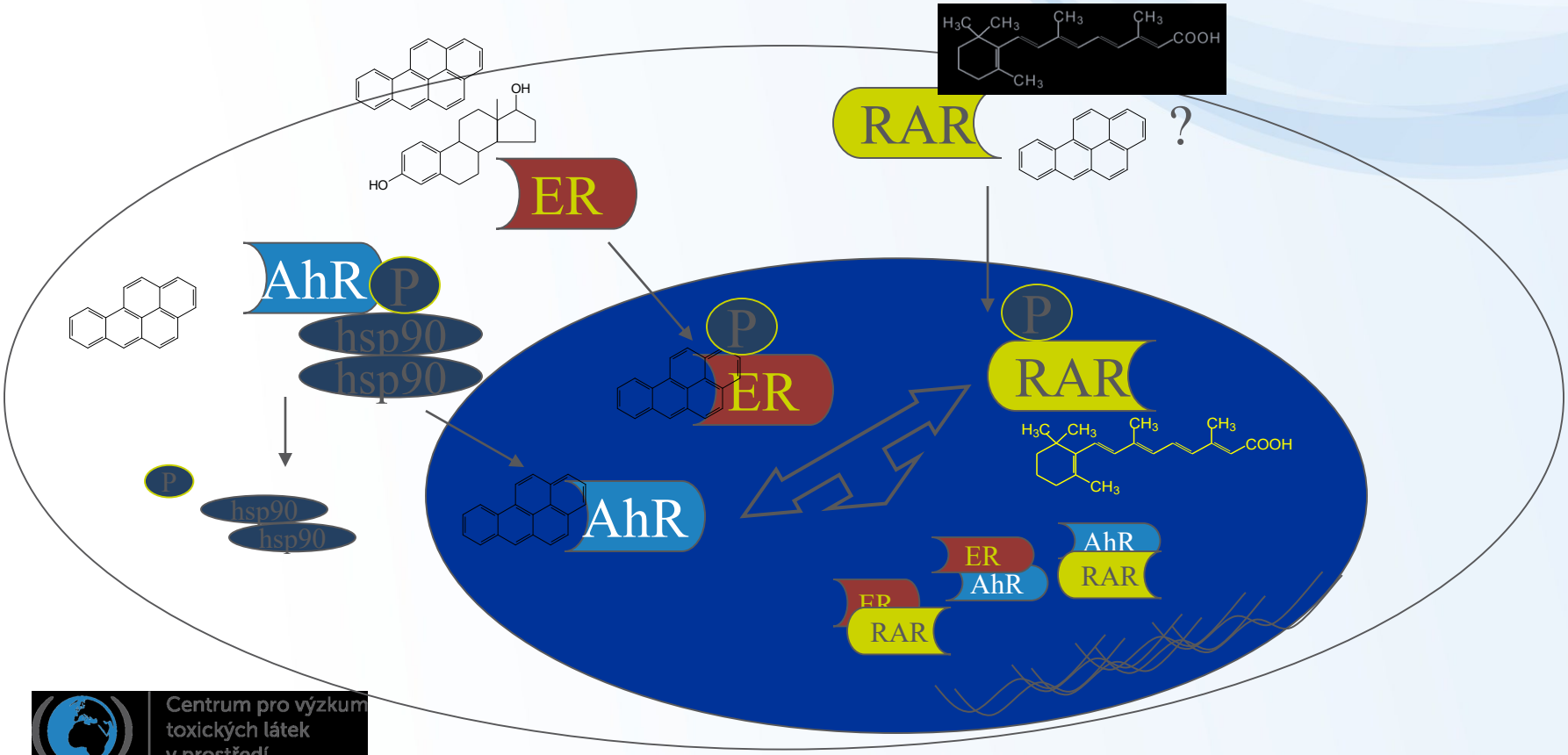
NR signalling is complex … examples of complexity 3

6. Interaction (crosstalk) among various NRs

- "antiestrogenicity" of AhR ligands
- fast clearance of retinoids after AhR activation
- Immunosuprresions after ER activations



NR signalling is complex … examples of complexity 4

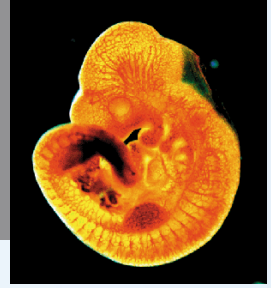
- Regulation of transcription activity - mechanisms may vary
  - Steroid receptors often **dimerize** with a partner to activate gene transcription
  - Receptors for vitamin D, retinoic acid and thyroid hormone form **heterodimers** and then bind to responsive elements on DNA
    - Second component of the heterodimer is RXR monomer (i.e, RXR-RAR; RXR-VDR)

- **NR dimers**
  - Heterodimeric receptors - exclusively nuclear;
    - without ligand represses transcription (by binding to their cognate sites in DNA)
  - Homodimeric receptors
    - mostly cytoplasmic without ligands → hormone binding leads to nuclear translocation of receptors

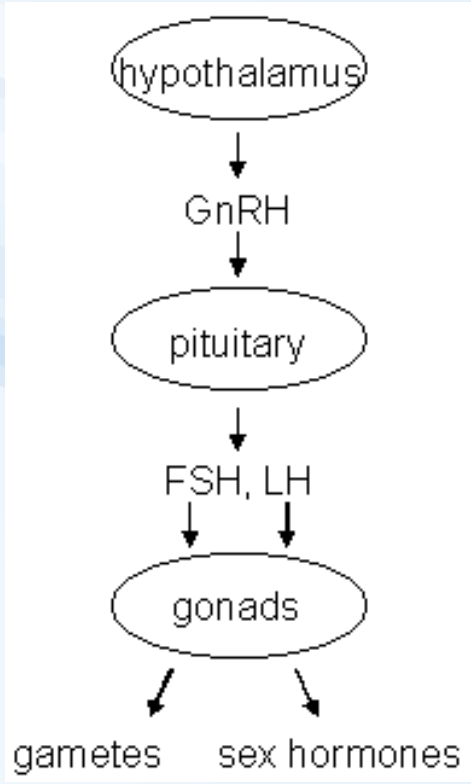
Why are NR important?

→ common mediators of Endocrine Disruption

Endocrine disruption



- **Interference of xenobiotics with normal functioning of hormonal system**



Known consequences

→ Disruption of homeostasis, reproduction, development, and/or behavior, and all other hormone-controlled processes:

- Shift in sex ratio, defective sexual development
- Low fecundity/fertility
- Hypo-immunity, carcinogenesis
- Malformations
- etc.

Toxicants interact with hormonal system at different levels

Synthesis

Transport

Interaction with receptors

Metabolization

Consequences (both negative!)

Suppression

Stimulation

Possible mechanisms of endocrine disruption

- Disruption of the „master“ hormones (FSH/LH)
- Decrease of HR cellular levels
- Nonphysiological activation of hormone receptor (HR)
- Binding to HR without activation
- Changes in hormone metabolism (clearance)

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Fate and action of HORMONES activating NRs

- **Circulation** in the blood bound to transport proteins
- **Dissociation from carrier** at target cells
- Passing **through cell membrane**
- **Binding** to an intracellular receptor (either in the cytoplasm or the nucleus)
- Hormone-receptor com**plex binds to hormone responsive elements in DNA**

  → Regulation of gene expression

→ De-regulation at any level described above = TOXICITY

Mechanisms of toxicant effects in detail

→ various MoAs of endocrine disruption

biosynthesis and release of hormones

e.g. steroidogenesis

↓

e.g. modulation of CYP11A and/or CYP19 activities

binding to plasmatic transport proteins

↓

e.g. down-regulation of receptor levels

binding to nuclear hormonal receptor (HR)

↓

Direct interference (activation / inhibition)

activation of HR
(dissociation of associated heat shock proteins, formation of homodimers)

↓

e.g. modulation of other nuclear receptors
(PPAR/RXR, RXR/TR)

binding of the activated receptor complex to specific DNA motifs - HREs

↓

chromatin rearrangement and transcription of estrogen-inducible genes

↓

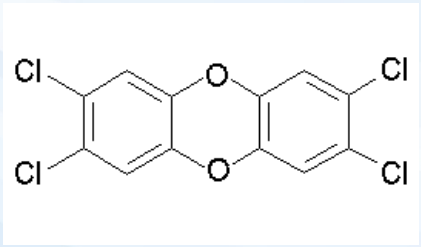
effects at the cellular, tissue, organ, organism, and/or population level

Endocrine disrupters in the environment?

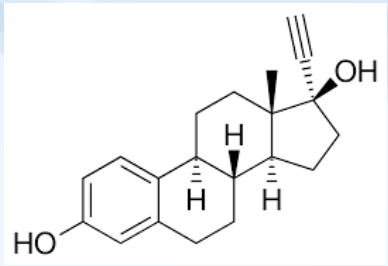
EDCs…

- Persistent Organic Compounds (POPs and their metabolites)
- steroid hormones and their derivatives from contraception pills
- alkylphenols
- organometallics (butyltins)
- pharmaceuticals
- Pesticides
- + number of unknowns …

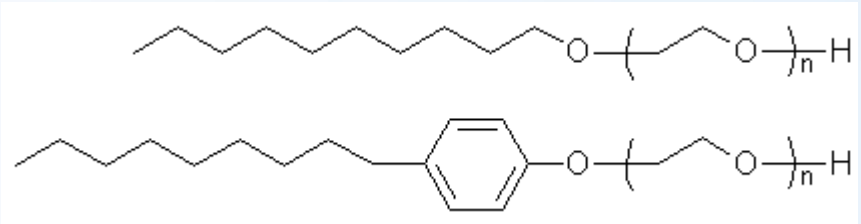
2,3,7,8-TCDD



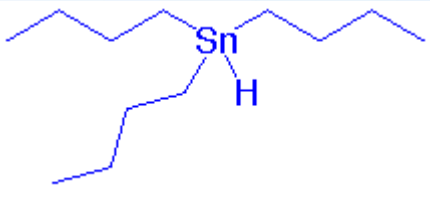
ethinylestradiol



alkylphenols

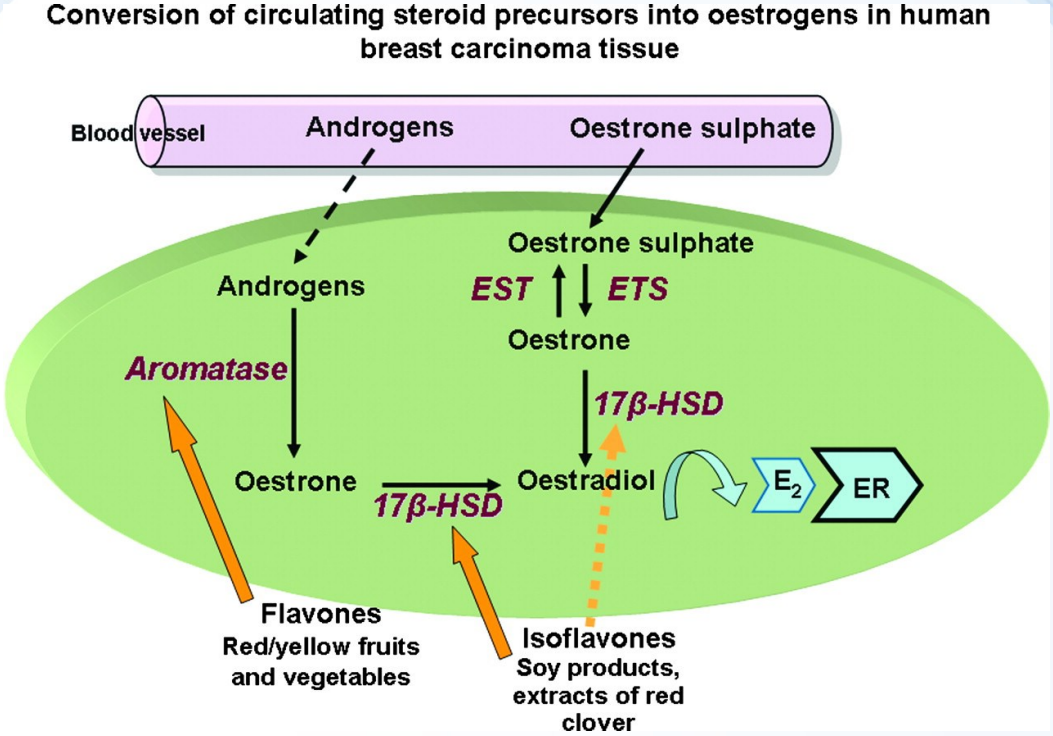


Tributyl-tin

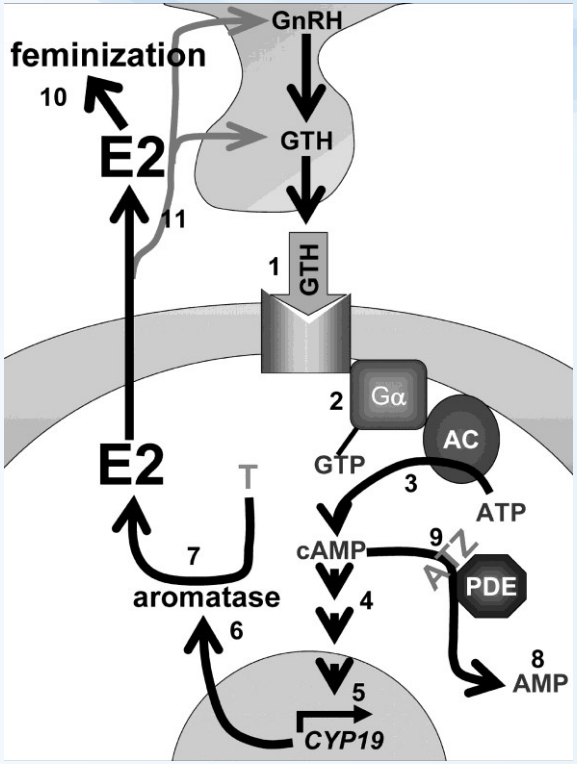


Examples – modulations of (synthetic) enzyme activities

Phytoestrogens promote **synthesis of estrogens**
→ **feminization**



Crosstalk with other signalling pathways (such as **cAMP**), which can be target to toxicants



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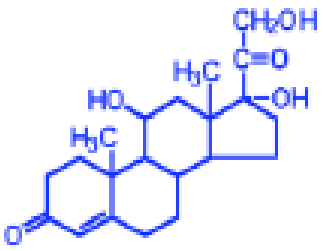
STEROIDs - most studied ligands detailed view

Steroid hormones - a review

Steroid hormones are derived from cholesterol metabolism in mitochondria

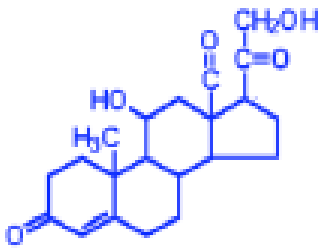
Cortisol

The dominant glucocorticoid in humans. Synthesized from progesterone in the *zona fasciculata* of the adrenal cortex. Involved in stress adaptation, elevates blood pressure and Na^+ uptake. Immunomodulation.



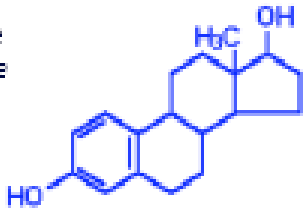
Aldosterone

Principal mineralocorticoid. Produced from progesterone in the *zona glomerulosa* of adrenal cortex, raises blood pressure and fluid volume, increases Na^+ uptake.



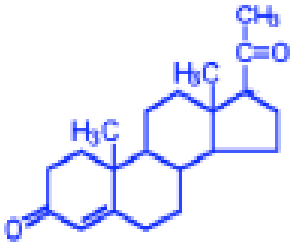
Estradiol

An estrogen, principal female sex hormone, produced in the ovary, responsible for secondary female sex characteristics. After menopause estrogen is produced from testosterone in the adrenal glands.



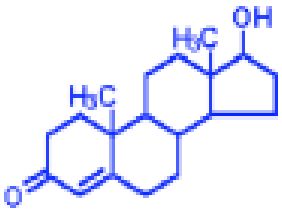
Progesterone

Produced from pregnenolone and secreted from the corpus luteum. Responsible for changes associated with luteral phase of the menstrual cycle, differentiation factor for mammary glands



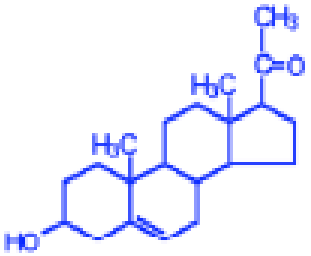
Testosterone

An androgen, male sex hormone synthesized in the testes from progesterone. Responsible for secondary male sex characteristics.

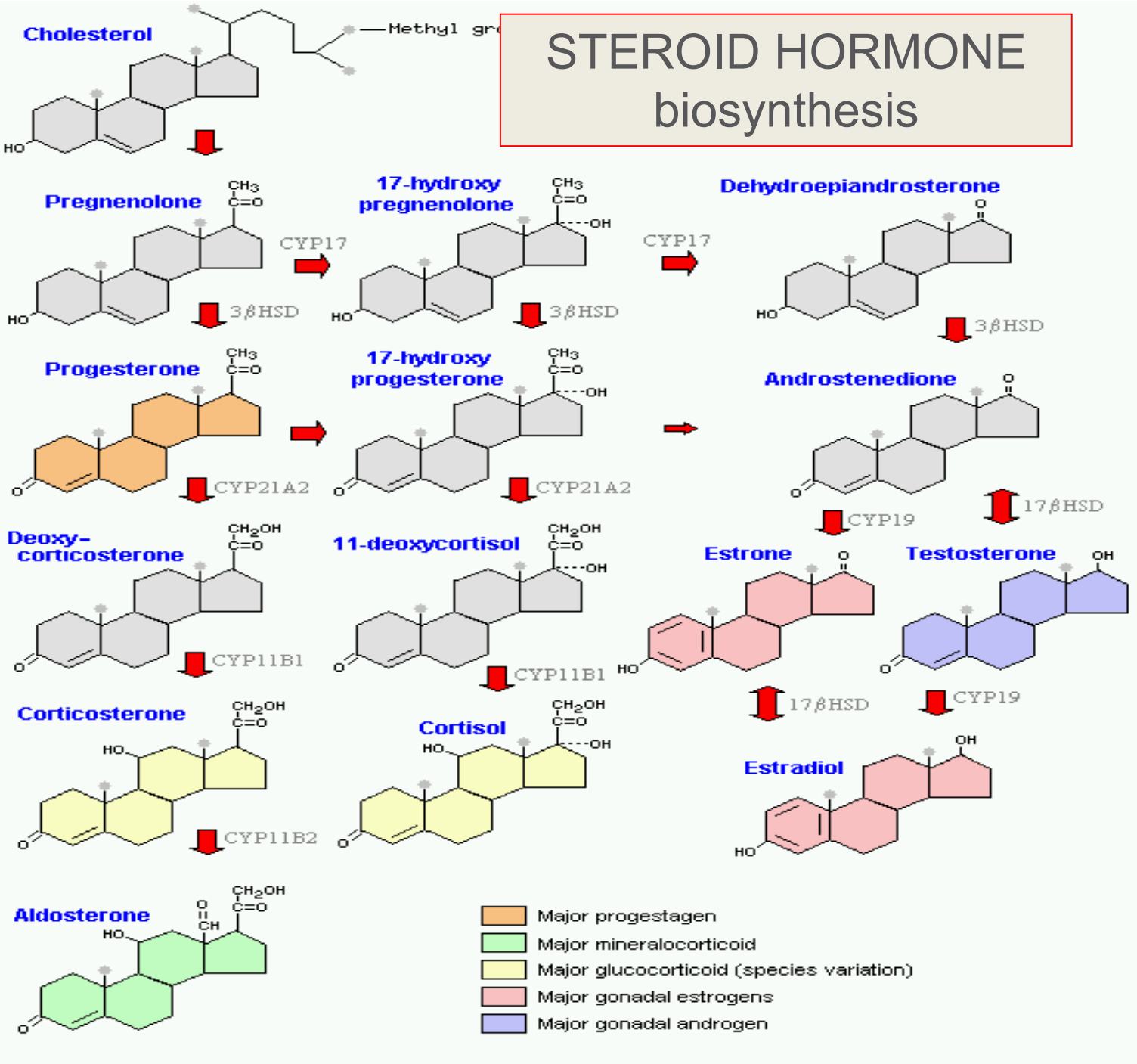


Pregnenolone

Made directly from cholesterol, the precusor molecule for all C_{18}, C_{19} and C_{21} steroids

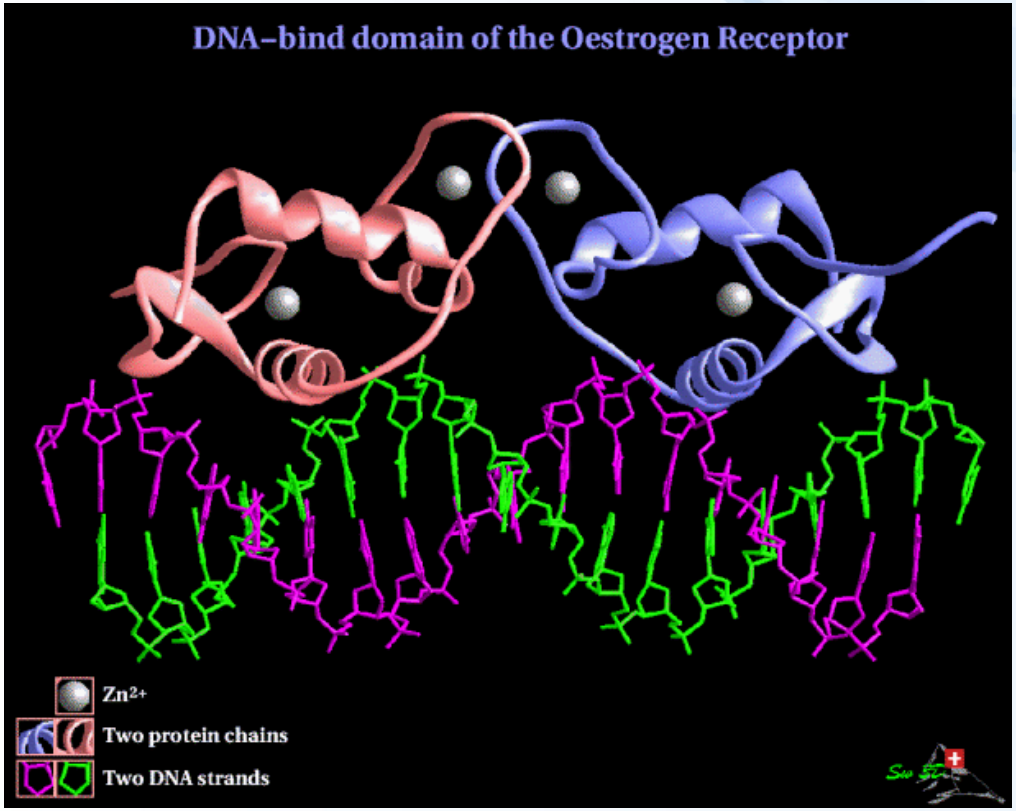


STEROID HORMONE biosynthesis



ESTROGEN RECEPTOR – ER

the most studied target of EDCs



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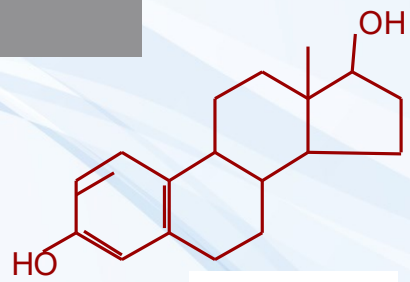
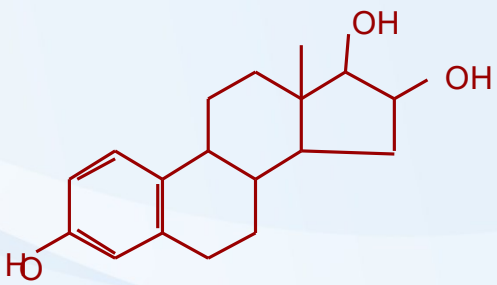
Estrogens



17-β-estradiol



estriol

- **Synthesis in ovaries**

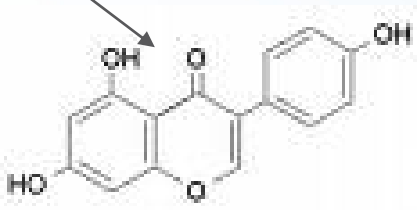
- **Functions**
  - key roles in female hormone regulation and signalling
  - responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
  - involved in the growth, development and homeostasis in a number of tissues
  - control the bone formation, regulation of homeostasis, cardiovascular system and behaviour
  - regulate **production, transport and concentration of testicular liquid and anabolic activity of androgens** in males

- **DISRUPTION OF ESTROGEN SIGNALLING**
  → many documented effects in aquatic biota & laboratory organisms

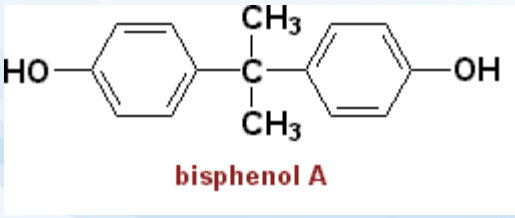
Environmental estrogens (xenoestrogens, exoestrogens)

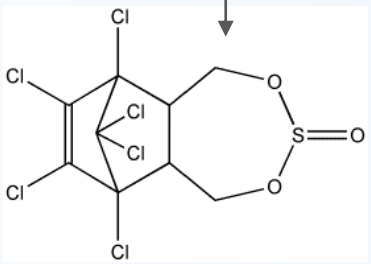
>> Highly diverse group of substances
>> Do not necessarily share structural similarity to the prototypical estrogen 17β-estradiol
>> may act as **AGONISTS** **and/or** **ANTAGONISTS** (depending on situation and concentration!)

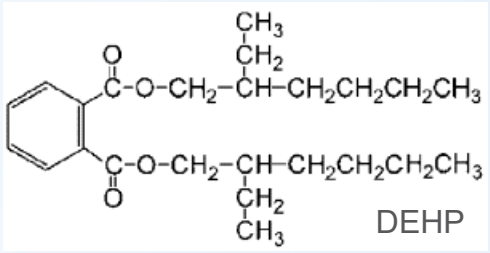
Natural products
genistein
naringenin
coumestrol
zearalenone



Industrial chemicals
Bisphenol A
Nonionic surfactants
Pthalate esters (eg. DEHP)
Endosulfan (pesticide)







Various POPs
DDT
kepone
PCBs/OH-PCBs
PAHs and dioxins

Pharmaceuticals
Ethinyl estradiol
Diethylstilbestrol
gestodene
norgestrel

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Exoestrogens - Relative Potencies to bind to ERa (REPs)

REP – a measure of toxic potency of a compound (similar also at other NRs)

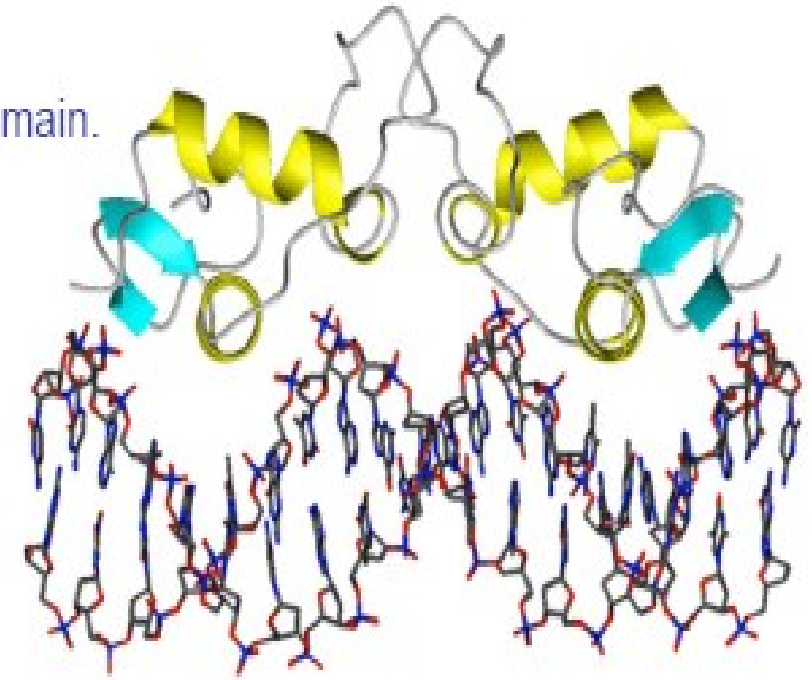
Chemical group	Substance	REP
Endogenous hormones	Estradiol	1
	Estriol	$6{,}3.10^{-3}$
	Testosteron	$9{,}6.10^{-6}$
Phytoestrogens	Cuomestrol	$6{,}8.10^{-3}$
	Genistein	$4{,}9.10^{-4}$
Pesticides	o,p´-DDT	$1{,}1.10^{-6}$
PCBs	2,4,6-trichlorbiphenyl-4´-ol	1.10^{-2}
	2,5-dichlorobiphenyl-4´-ol	$6{,}2.10^{-3}$
	3,3´,5,5´tetrachlorobiphenyl-4,4´-diol	$1{,}6.10^{-4}$
alkylphenoles	4-tert-oktylphenol	$3{,}6.10^{-6}$
phthalates	butylbenzylphthalate	4.10^{-6}

REP (RElative Potencies) of selected compounds related to 17-β-estradiol derived from reporter yeast assay

ANDROGEN RECEPTOR (AR)

role in toxicity confirmed ... but less explored than ER

Androgen receptor DNA binding domain.



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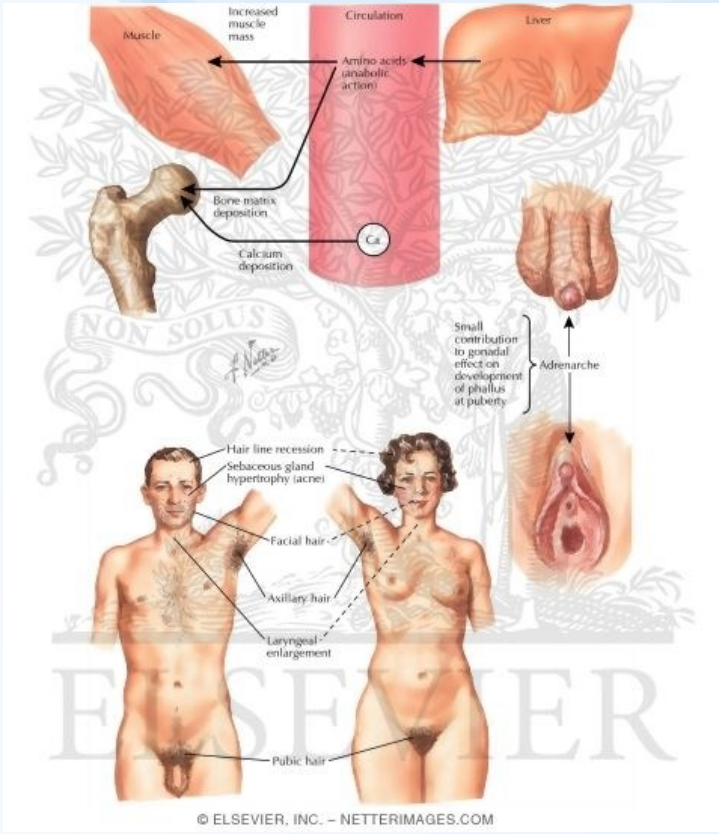
Androgens

- **Role of androgens in males is similar to that of estrogens in females**
  - development of male sexual characteristics
  - stimulating protein synthesis, growth of bones
  - cell differenciation, spermatogenesis
  - male type of behaviour



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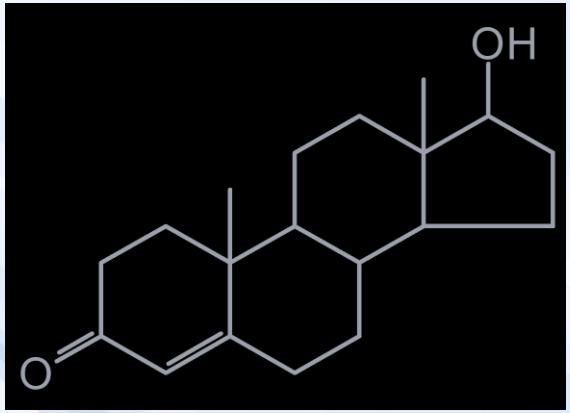
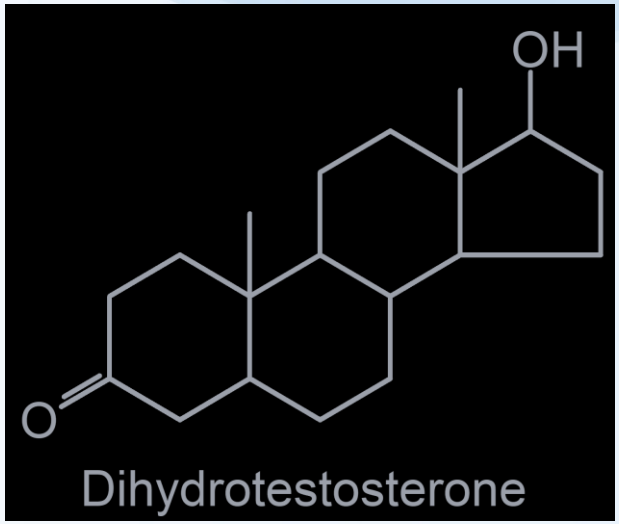
Androgens – endogenous ligands

- **Endogenous ligands – androgen hormones**
  - **Two key androgens**
    - **testosterone (T)**
    - **dihydrotestosterone (DHT)**
  - Other androgens – androstanediol, dehydroepiandrosterone, androstenedione



Testosterone



Dihydrotestosterone

- **T: synthesis in testis (Leydig cells)**
  - in lesser extent in adrenals
- **DHT**: Formed **extratesticulary** from T
  - In several tissues (seminal vesicles, prostate, skin) higher affinity to androgen receptor than T
  - Daily production 5-10% of testosterone

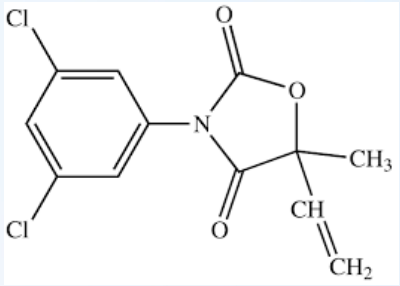
Several mechanisms how „xenoandrogens“ disrupt natural androgen signalling and action

1) Binding to AR

- Mostly competitive **inhibition** (xenobiotics mostly do not activate AR-dependent transcription)

- Only few compounds able to activate AR in the absence of androgen hormones but they are **anti-androgenic in the presence of strong androgens like T or DHT**
  - metabolites of **fungicide vinclozoline**
  - some PAHs

vinclozoline



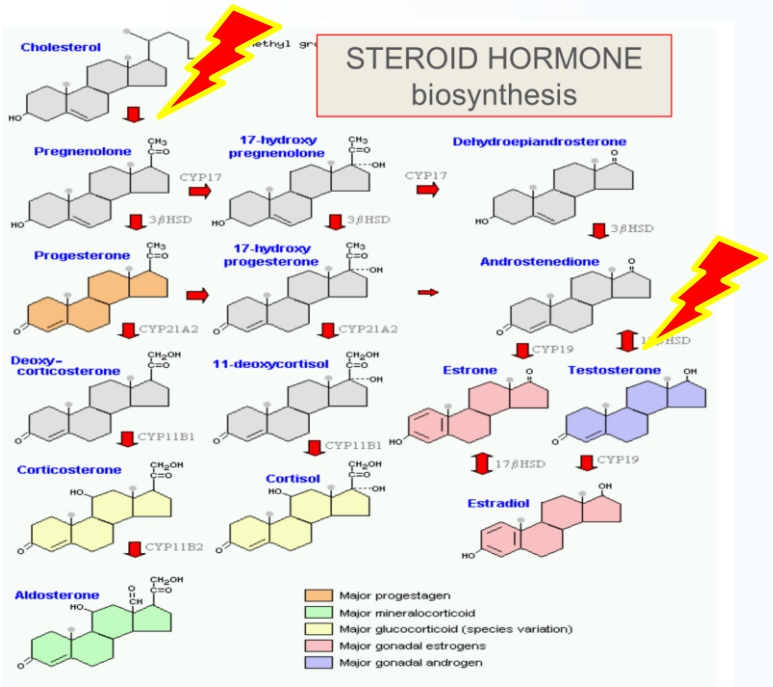
2) FSH/LH (gonadotropins) signalling disruption – less explored

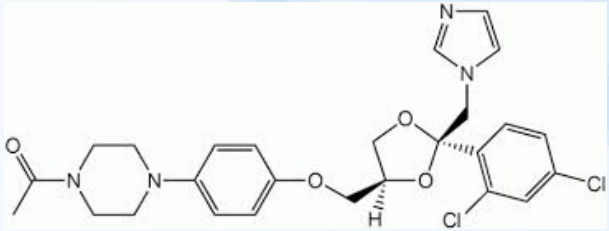
- *FSH/LH expression - regulation via negative feedback by testosterone*
- *Suppression → alterations of spermatogenesis*

Mechanisms of androgen signalling disruption

3) Alterations of *de novo* testosterone synthesis

- Inhibition of P450scc needed for side chain cleavage of cholesterol or inhibitions of 17-beta-hydroxylase and other CYPs
  - **fungicide ketoconazol**

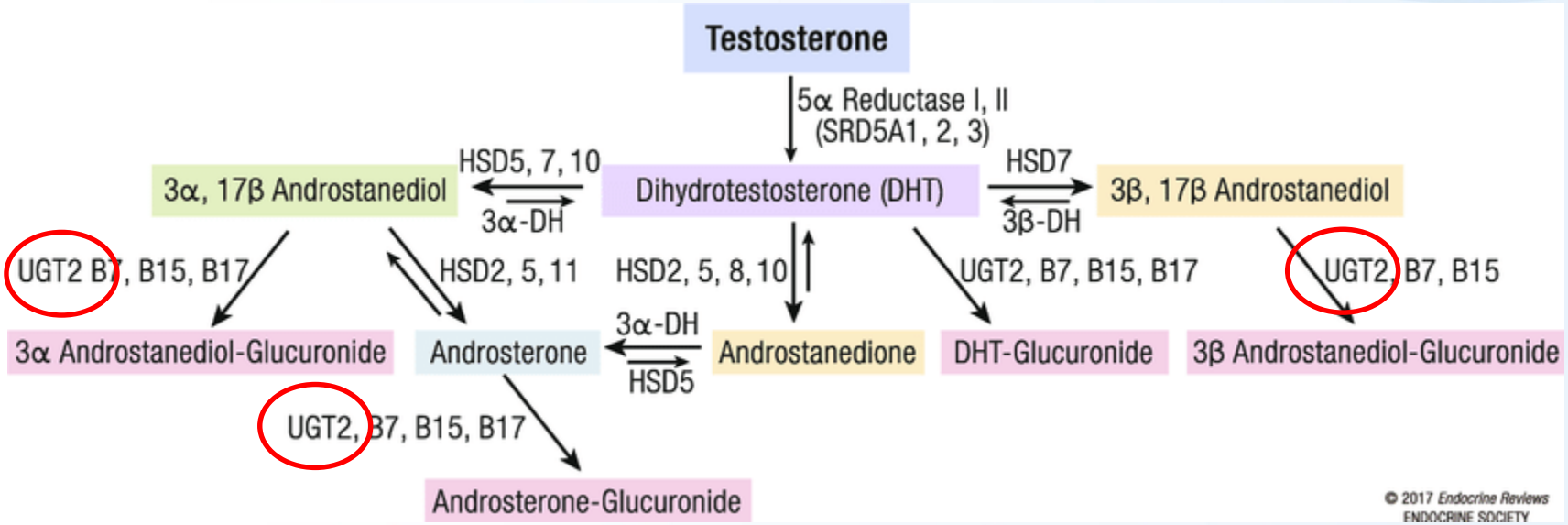




Mechanisms of androgen signalling disruption

4) Testosterone metabolic clearance

- Chemicals inducing detoxification enzymes – for Testosteron – most relevanat are UDP-glucuronosyltransferases (UGTs)
  - Documented e.g. for pesticides endosulfan, mirex, o-p´-DDT
  - *(degradation → lower T concentrations → anti-androgenicity)*

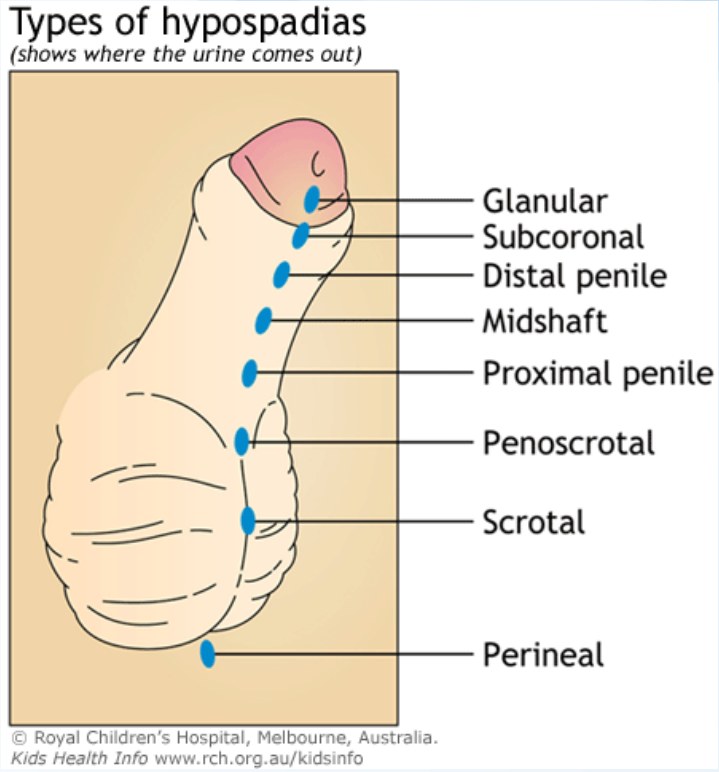


Effects of male exposures to **antiandrogens**

- **Exposure during prenatal development:**
  - malformations of the reproductive tract
    - reduced anogenital distance
    - **hypospadias** (abnormal position of the urethral opening on the penis)
    - vagina development
    - undescendent ectopic testes
    - atrophy of seminal vesicles and prostate gland

- **Exposure in prepubertal age:**
  - delayed puberty
  - reduced seminal vesicles
  - reduced prostate

- **Exposure in adult age:**
  - oligospermia
  - azoospermia
  - loss of sexual libido



Centrum pro výzkum toxických látek v prostředí

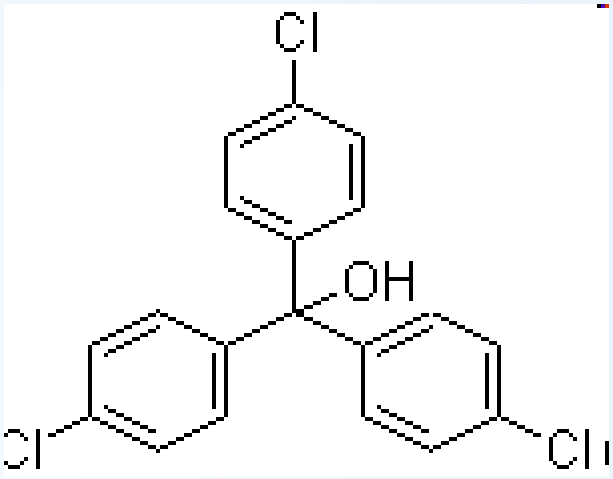
AR-binding – effective concentrations

Reference: active ligand dehydrotestosteron **DHT: EC50 ~ 0.1 μM**

Compound	IC_{50} (μM)
Benz[a]anthracene	3.2
Benzo[a]pyrene	3.9
Dimethylbenz[a]anthracene	10.4
Chrysene	10.3
Dibenzo[a,h]anthracene	activation in range 0.1-10μM
Bisphenol A	5
vinclozolin metabolites	9.7
hydroxyflutamide	5
Aroclor typical values	0.25-1.11
Individual PCBs typical values	64 - 87
***tris*-(4-chlorophenyl)-methanol**	**0.2**

Antiandrogenic compound

- **tris-(4-chlorophenyl)-methanol**
  - Ubiquitous contaminant of uncertain origin
  - Probable metabolite of DDT-mixtures
  - Levels in human blood serum cca. 50nM
    (antiAR effective EC50 – cca. 200nM)



Centrum pro výzkum toxických látek v prostředí

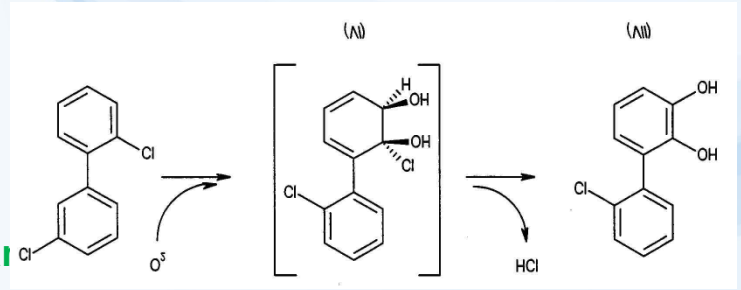
Disruption of transport of thyroid hormones in blood

- SPECIFIC TRANSPORTERS in blood
  - regulating free T4 and T3 levels
  - 3 types :
    - Thyroid-binding prealbunin (transthyretin) (20-25%)
    - Albumin (5-10%)
    - **Thyroid binding globulin (TBP, 75%)**

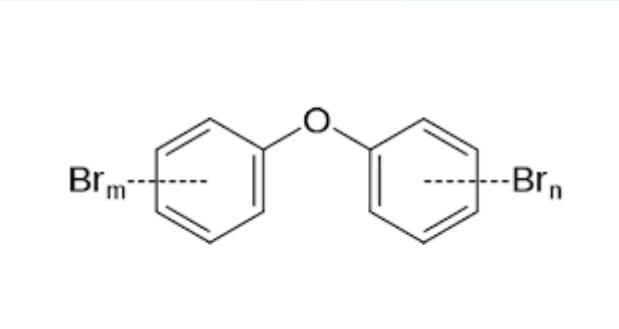
- **NUMBER OF EDCs → act on transport proteins**
  - OH-PCBs, **brominated and chlorinated flame retardan**
    DDT, dieldrin
  - **OH-PCBs** – equal affinity to **TBP** as T4 and T3 (!!!)

- Increased levels of "free T4" in blood
  - negative feedback to TSH release
    → increased depletion
    → increased weight, changes in thyroid gland
  - Documented after exposures to POPs in vertebrates

Hydroxylated PCB formation



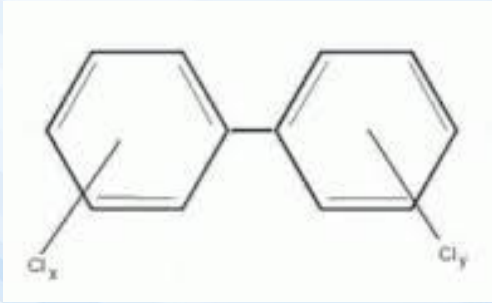
Polybrominated diphenyl ethers (PBDEs) – flame retardants



Centrum pro výzkum toxických látek v prostředí

Disruption of retinoid signalling by xenobiotics

- **Polluted areas**
  - **mostly decrease of retinoid levels**
    - Documented in aquatic birds, mammals and fish



- **Disruption of retinoid transport:** PCBs

- **Effects on retinoid receptors:**
  - RAR, RXR binding and/or transactivation
    - pesticides (chlordane, dieldrin, methoprene, tributyltin…)
    - Effect on ATRA mediated response – TCDD, PAHs

- **Disruption of retinoid metabolism:**
  - **PCDD/Fs,** PAHs, PCBs, pesticides
  - changes of serum concentrations of retinol and RA
  - mobilization of hepatic storage forms

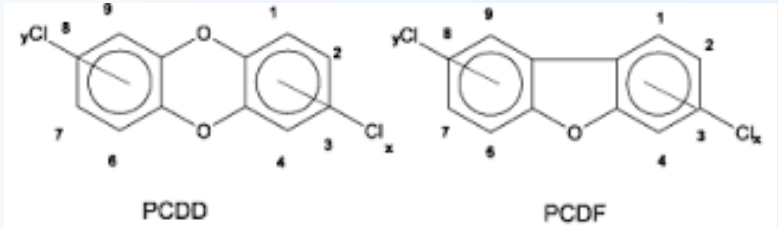
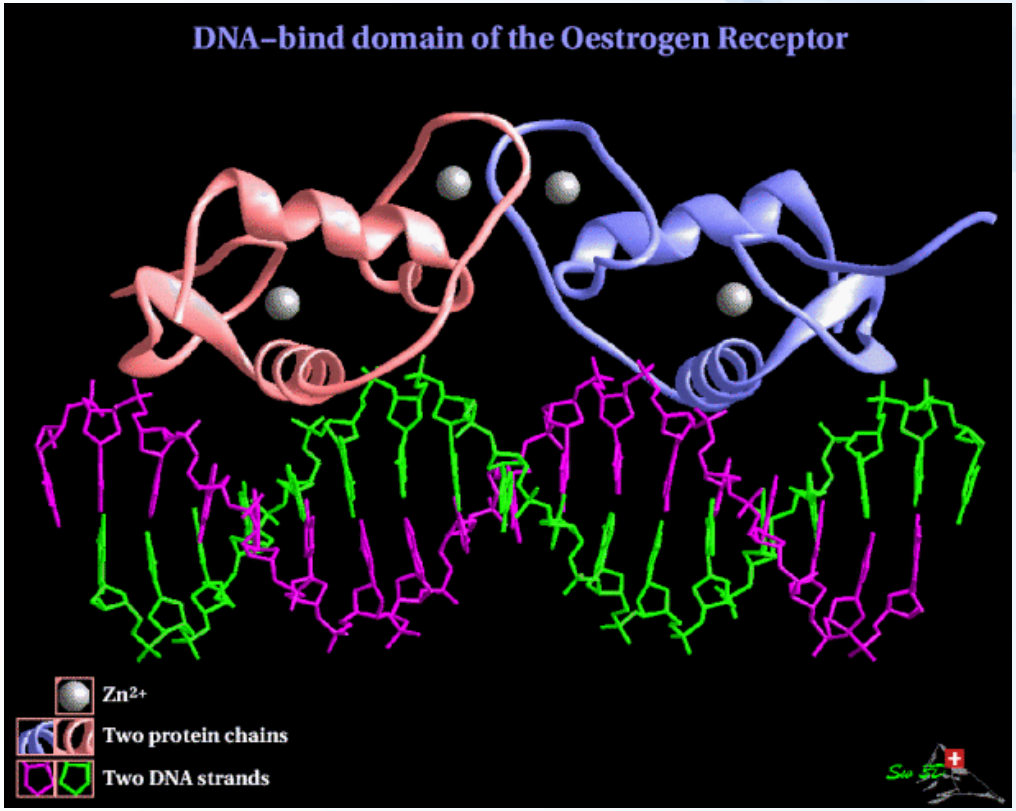


Figure 1. General molecular structure of polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofurans (PCDF)

SECTION 2
OTHER SLIDES (for records only)

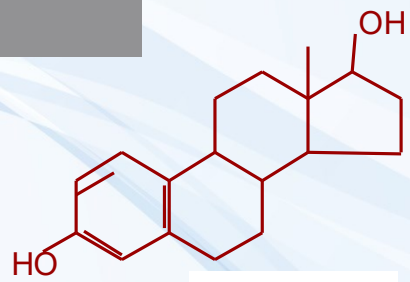
ESTROGEN RECEPTOR – ER

the most studied target of EDCs

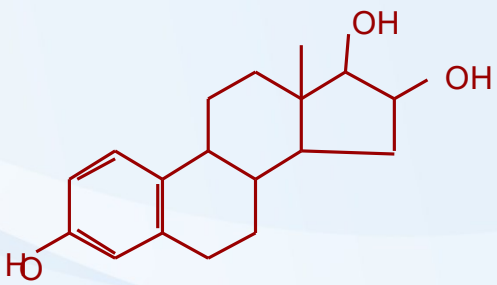


Centrum pro výzkum
toxických látek
v prostředí

Estrogens



17-β-estradiol



estriol

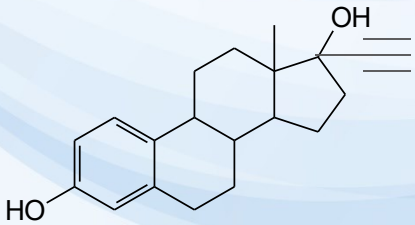
- **Synthesis in ovaries**

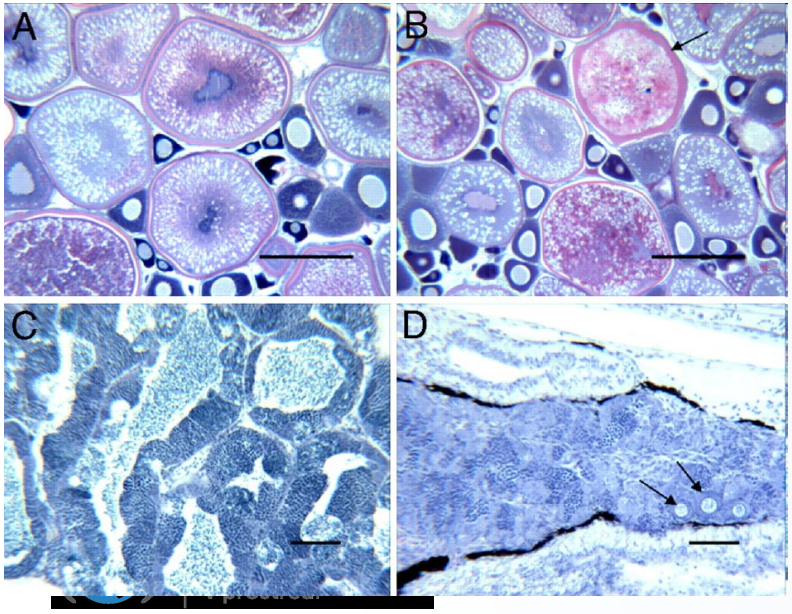
- **Functions**
  - key roles in female hormone regulation and signalling
  - responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
  - involved in the growth, development and homeostasis in a number of tissues
  - control the bone formation, regulation of homeostasis, cardiovascular system and behaviour
  - regulate **production, transport and concentration of testicular liquid and anabolic activity of androgens** in males

- **DISRUPTION OF ESTROGEN SIGNALLING**
  → many documented effects in aquatic biota & laboratory organisms

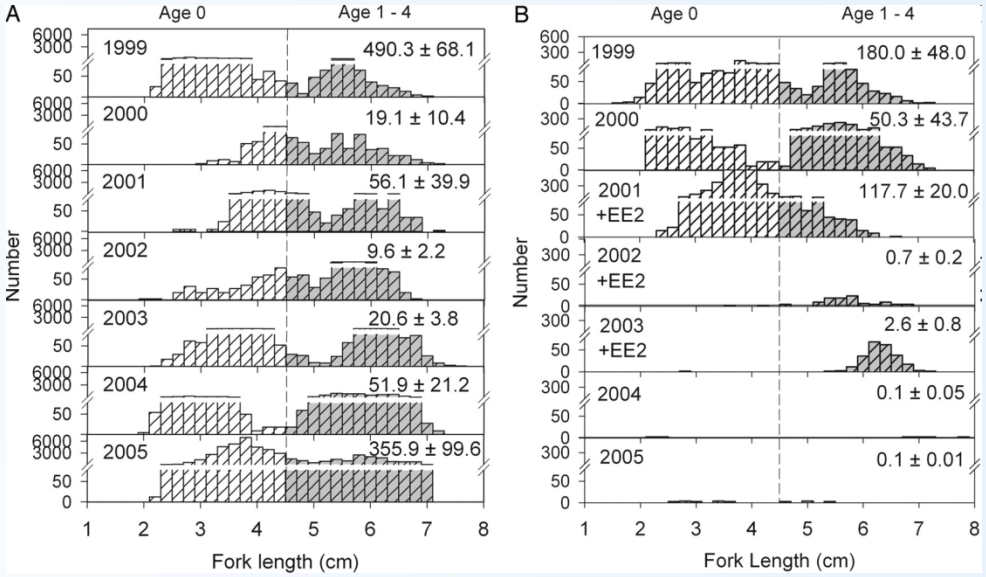
Kidd, K.A. et al. 2007. Collapse of a fish population following exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years





Controls +Ethinylestradiol

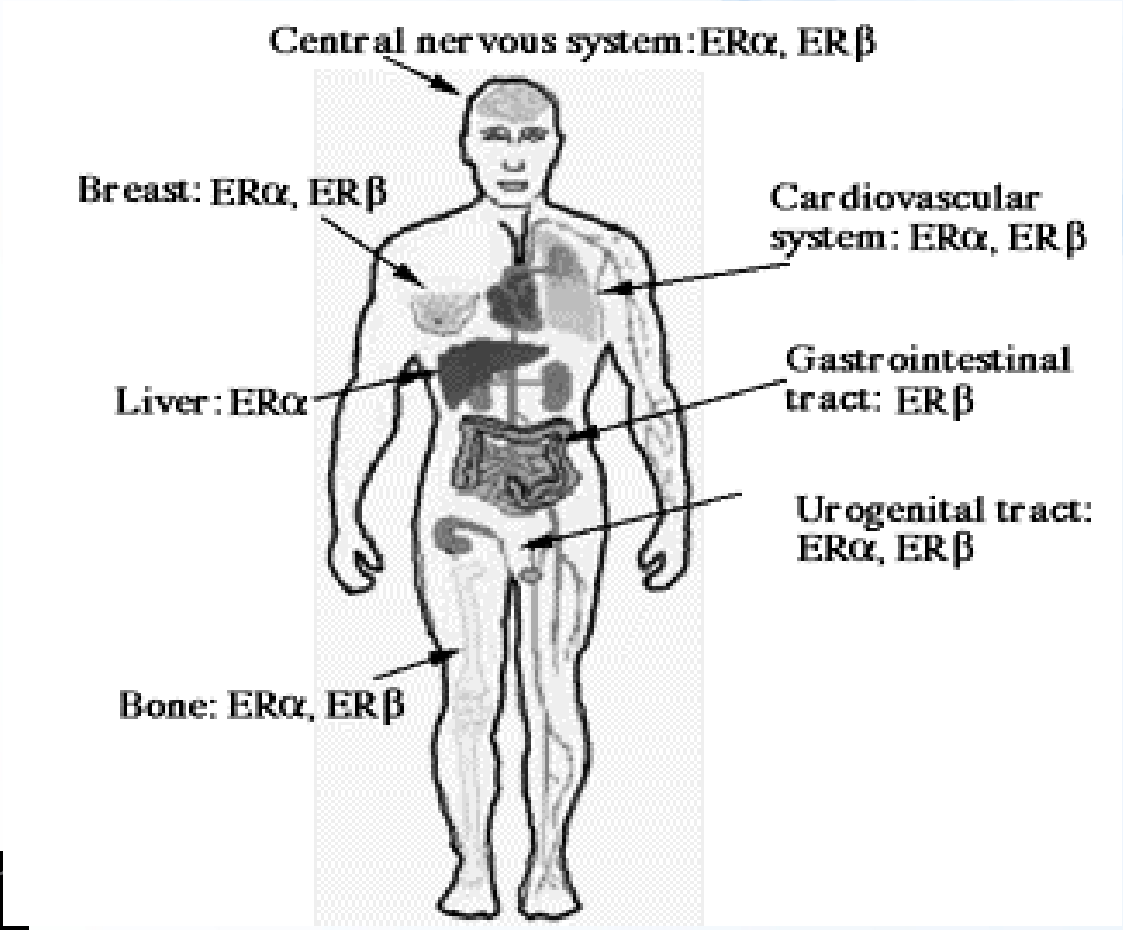


ESTROGEN RECEPTORS - subtypes

ER-α (in breast, ovary, brain, liver, bone and cardiovascular system, adrenals, testis and urogenital tract)

ER-β (in kidneys, prostate and gastrointestinal tract)

(ER-γ in fish)

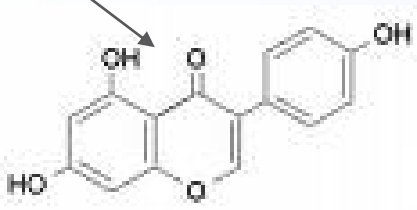


Centrum pro výzku
toxických látek
v prostředí

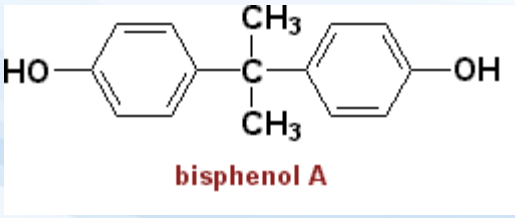
Environmental estrogens (xenoestrogens, exoestrogens)

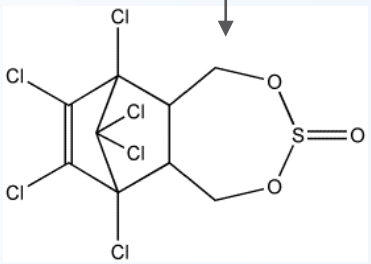
>> Highly diverse group of substances
>> Do not necessarily share structural similarity to the prototypical estrogen 17β-estradiol
>> may act as **AGONISTS** **and/or** **ANTAGONISTS** (depending on situation and concentration!)

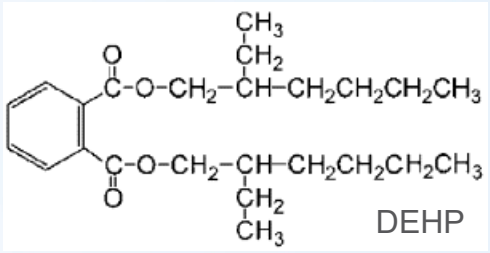
Natural products
genistein
naringenin
coumestrol
zearalenone



Industrial chemicals
Bisphenol A
Nonionic surfactants
Pthalate esters (eg. DEHP)
Endosulfan (pesticide)







Various POPs
DDT
kepone
PCBs/OH-PCBs
PAHs and dioxins

Pharmaceuticals
Ethinyl estradiol
Diethylstilbestrol
gestodene
norgestrel

Centrum pro výzkum toxických látek v prostředí

Exoestrogens - Relative Potencies to bind to ERa (REPs)

REP – a measure of toxic potency of a compound (similar also at other NRs)

Chemical group	Substance	REP
Endogenous hormones	Estradiol	1
	Estriol	$6{,}3.10^{-3}$
	Testosteron	$9{,}6.10^{-6}$
Phytoestrogens	Cuomestrol	$6{,}8.10^{-3}$
	Genistein	$4{,}9.10^{-4}$
Pesticides	o,p´-DDT	$1{,}1.10^{-6}$
PCBs	2,4,6-trichlorbiphenyl-4´-ol	1.10^{-2}
	2,5-dichlorobiphenyl-4´-ol	$6{,}2.10^{-3}$
	3,3´,5,5´tetrachlorobiphenyl-4,4´-diol	$1{,}6.10^{-4}$
alkylphenoles	4-tert-oktylphenol	$3{,}6.10^{-6}$
phthalates	butylbenzylphthalate	4.10^{-6}

REP (RElative Potencies) of selected compounds related to 17-β-estradiol derived from reporter yeast assay

How to assess ESTROGENICITY?

Number of in vivo and in vitro methods available

Assay (ref.)	Exposure type	Detects ER-dependent agents?	Detects non-ER-dependent agents?	Distinguishes agonist versus antagonist?	Pharmacokinetic and metabolism included?
Receptor-based assays					
Receptor binding assay (27)	Cell lysate	Yes	No	No	No
Receptor activation assay (32–34)	Cells in vitro	Yes	No	Yes[a]	No
In vitro estrogen-regulated response assays					
MCF-7 cell proliferation assay (41)	Cells in vitro	Yes	Limited	Yes[a]	No
Induction assays (46,48)	Cells in vitro	Yes	Limited	Yes[a]	No
DNA synthesis assays (47)	Cells in vitro	Yes	Limited	Yes[a]	No
In vivo estrogen-regulated response assays					
Uterotrophic response assay (49)	Whole animal	Yes	Limited	Yes[a]	Yes
Vaginal cornification assay (50)	Whole animal	Yes	Limited	Yes[a]	Yes
Vaginal opening (11)	Whole animal	Yes	Limited	Yes[a]	Yes
Uterine fluid imbibition (11)	Whole animal	Yes	Limited	Yes[a]	Yes
Uterine epithelial hypertrophy (51)	Whole animal	Yes	Limited	Yes[a]	Yes
Inhibition of steroid synthesis assays					
In vitro ovarian steroid assay (55)	Minced tissue	No	Yes	Yes	No
Ex vivo ovarian steroid assay (56)	Whole animal	No	Yes	Yes	Yes

[a]Detection of antagonists requires use of additional groups with test material + estradiol.

Janošek, J., Hilscherová, K., Bláha, L., and Holoubek, I. (2006). Environmental xenobiotics and nuclear receptors-Interactions, effects and in vitro assessment. *Toxicology in Vitro* 20, 18-37.

IN VIVO ASSAYS FOR ESTROGENICITY

- uterotropic assay
- vaginal cornification assay

- production of estrogen-inducible proteins
  (e.g. **vitellogenin** and zona radiata protein)

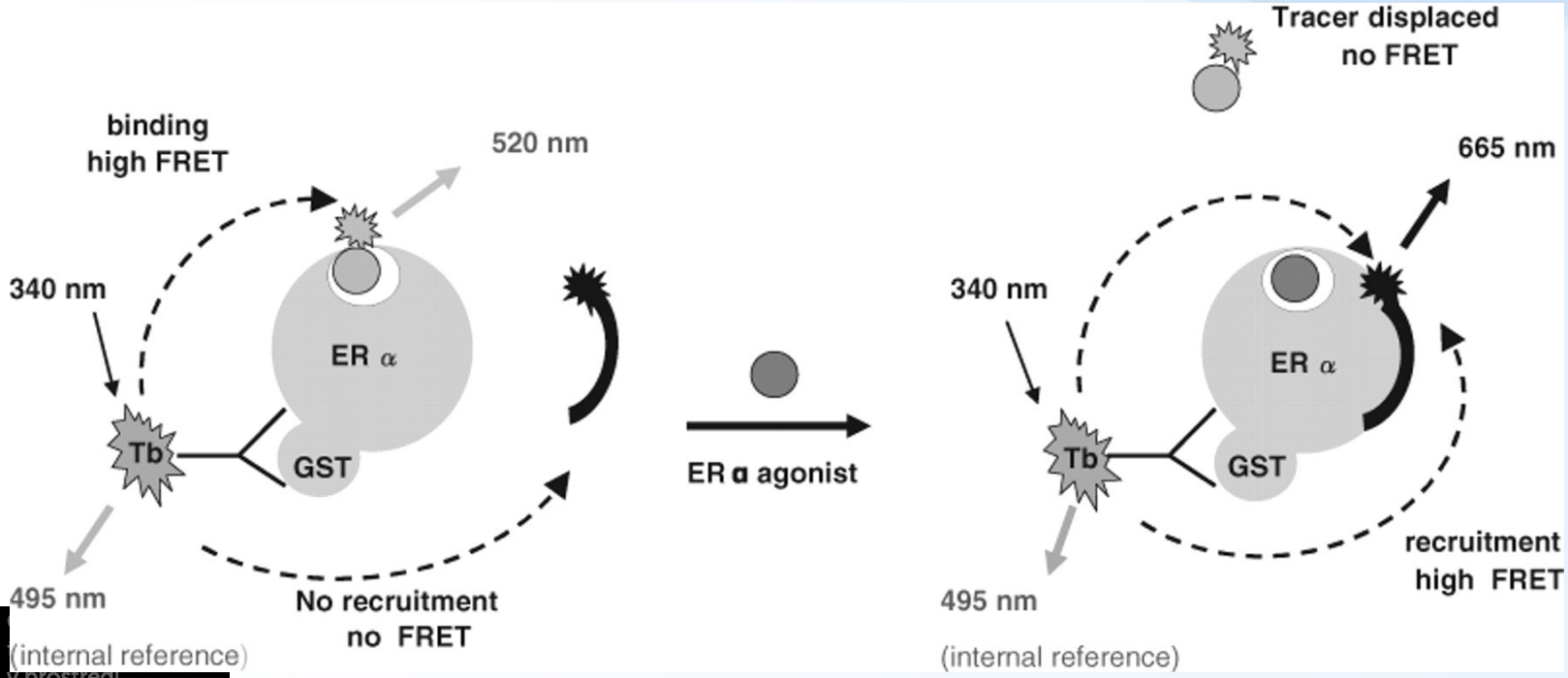
  → also discussed at “biomarkers” part

- standard (in vivo) test procedures for reproductive and developmental toxicity
  - using mice, rats, fish, amphibians etc.

In vitro assays for estrogenicity

- **Level 1 – interaction of toxicant with the protein (receptor)**
  - INTERACTION (BINDING) to the receptor
    - **competitive ligand binding assays**
      - Various variants (e.g. displacement of radioactive substrate, fluorescence resonance energy transfer (*FRET*) techniques etc.

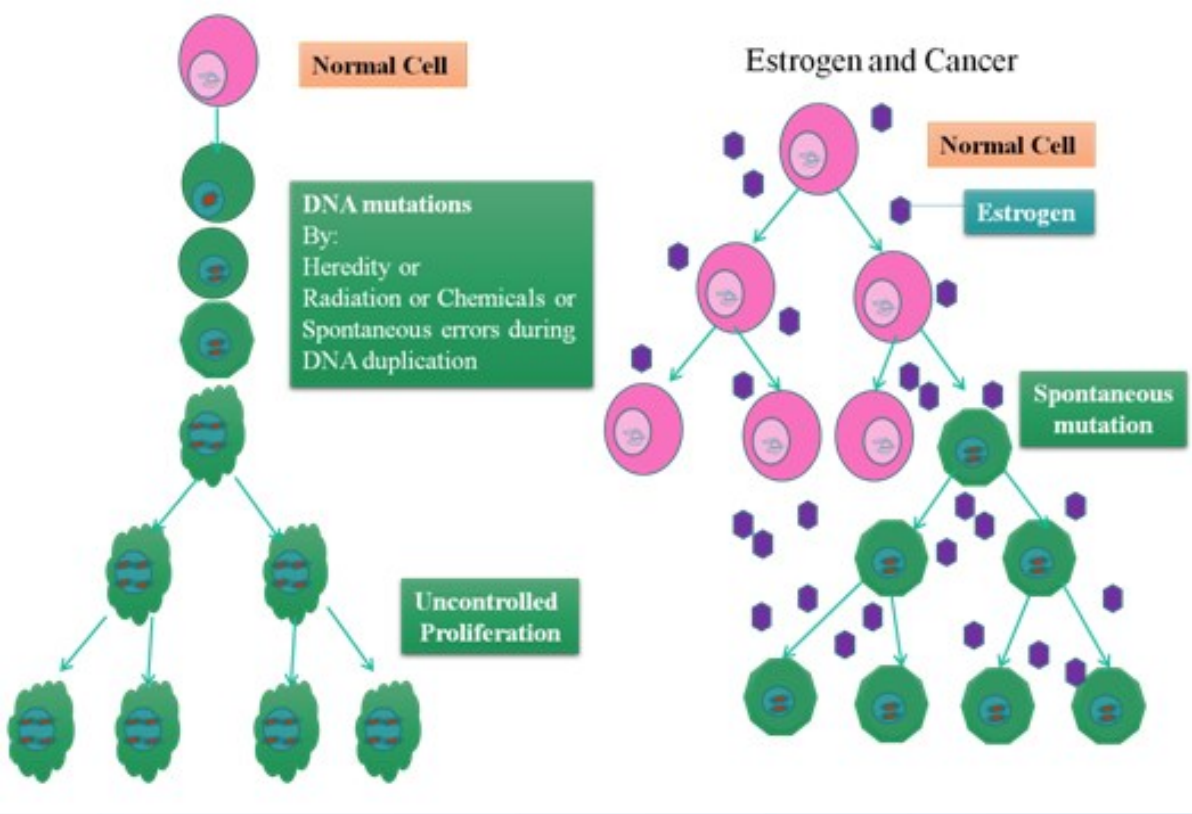
    → information only about "binding potency" but the effect remains unknown (? Activation / suppression / no effect ?)



In vitro assays for estrogenicity

- **Level 2 - effects at cellular level**
  → interference with receptor biological activity

- **Cell proliferation assays**
  – Estrogens induce proliferation



In vitro assays for estrogenicity

- **Level 2 - effects at cellular level**
  → interference with receptor biological activity

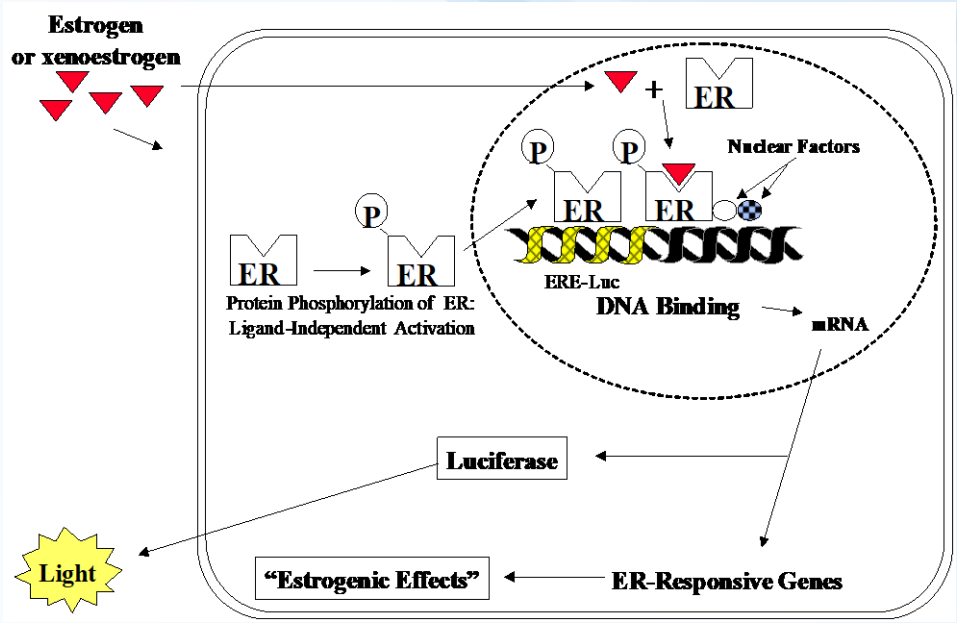
- **Endogenous protein expression** (or enzyme activity) assays
  - **reporter gene assays**

Cell assays *in vitro*

•Cells (e.g. breast carcinoma) naturally carrying functional ER.

•Genetic modification - stable transfection with firefly **luciferase gene**: under the control of ER

•Estrogens in media → light induction

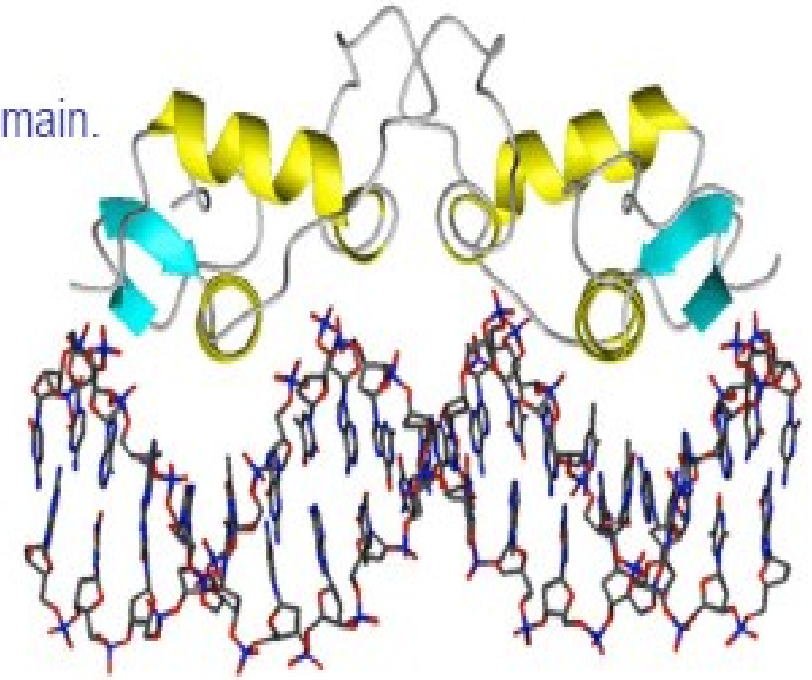


Centrum pro výzkum toxických látek v prostředí

ANDROGEN RECEPTOR (AR)

role in toxicity confirmed ... but less explored than ER

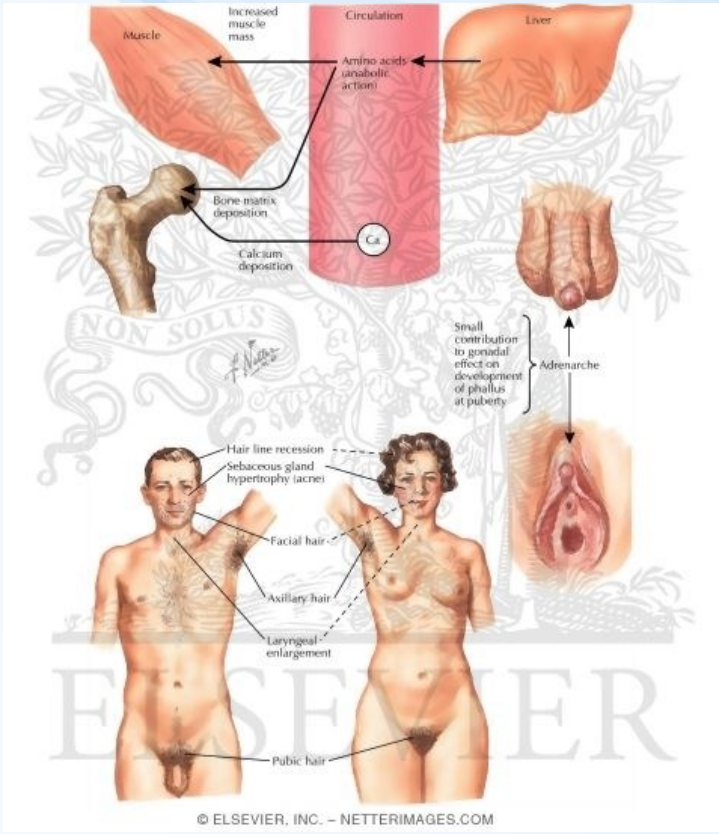
Androgen receptor DNA binding domain.



Centrum pro výzkum toxických látek v prostředí

Androgens

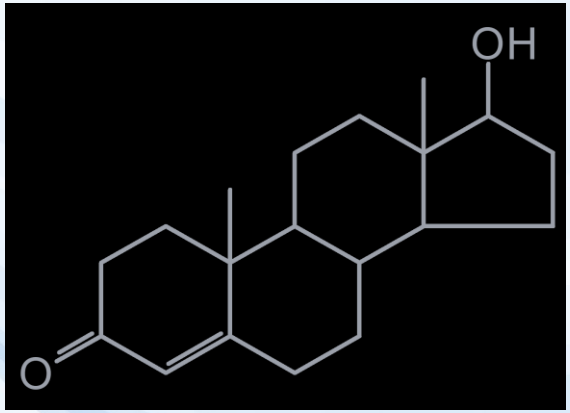
- **Role of androgens in males is similar to that of estrogens in females**
  - development of male sexual characteristics
  - stimulating protein synthesis, growth of bones
  - cell differenciation, spermatogenesis
  - male type of behaviour



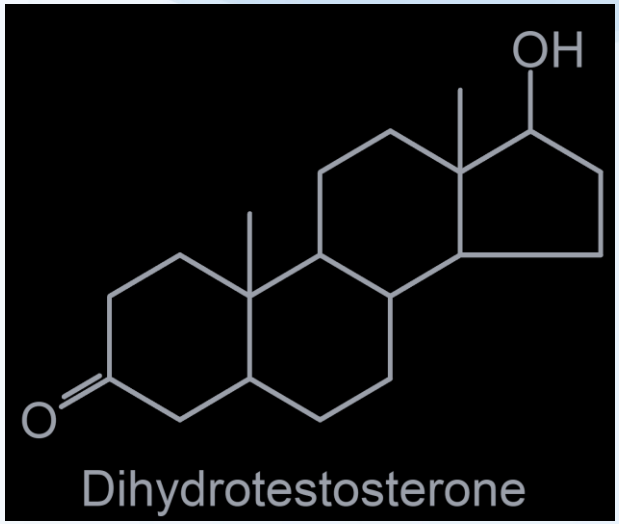
Centrum pro výzkum toxických látek v prostředí

Androgens – endogenous ligands

- **Endogenous ligands – androgen hormones**
  - **Two key androgens**
    - **testosterone (T)**
    - **dihydrotestosterone (DHT)**
  - Other androgens – androstanediol, dehydroepiandrosterone, androstenedione



Testosterone



Dihydrotestosterone

- **T: synthesis in testis (Leydig cells)**
  - in lesser extent in adrenals
- **DHT**: Formed **extratesticulary** from T
  - In several tissues (seminal vesicles, prostate, skin) higher affinity to androgen receptor than T
  - Daily production 5-10% of testosterone

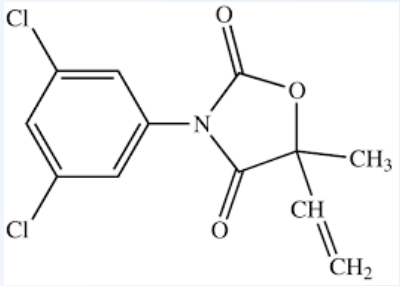
Several mechanisms how „xenoandrogens“ disrupt natural androgen signalling and action

1) Binding to AR

- Mostly competitive **inhibition** (xenobiotics mostly do not activate AR-dependent transcription)

- Only few compounds able to activate AR in the absence of androgen hormones but they are **anti-androgenic in the presence of strong androgens like T or DHT**
  - metabolites of **fungicide vinclozoline**
  - some PAHs

vinclozoline



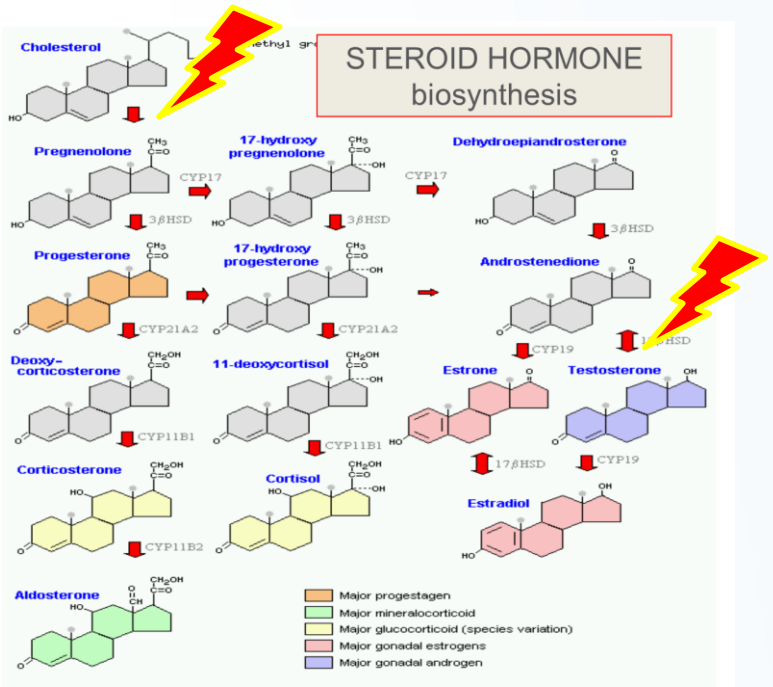
2) FSH/LH (gonadotropins) signalling disruption – less explored

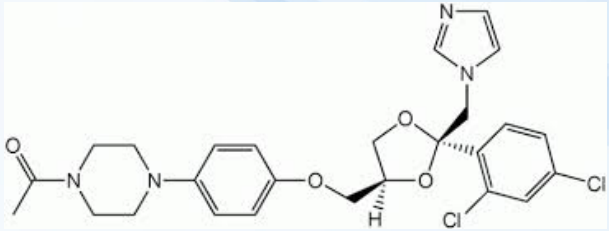
- *FSH/LH expression - regulation via negative feedback by testosterone*
- *Suppression → alterations of spermatogenesis*

Mechanisms of androgen signalling disruption

3) Alterations of *de novo* testosterone synthesis

- Inhibition of P450scc needed for side chain cleavage of cholesterol or inhibitions of 17-beta-hydroxylase and other CYPs
  - **fungicide ketoconazol**





Mechanisms of androgen signalling disruption

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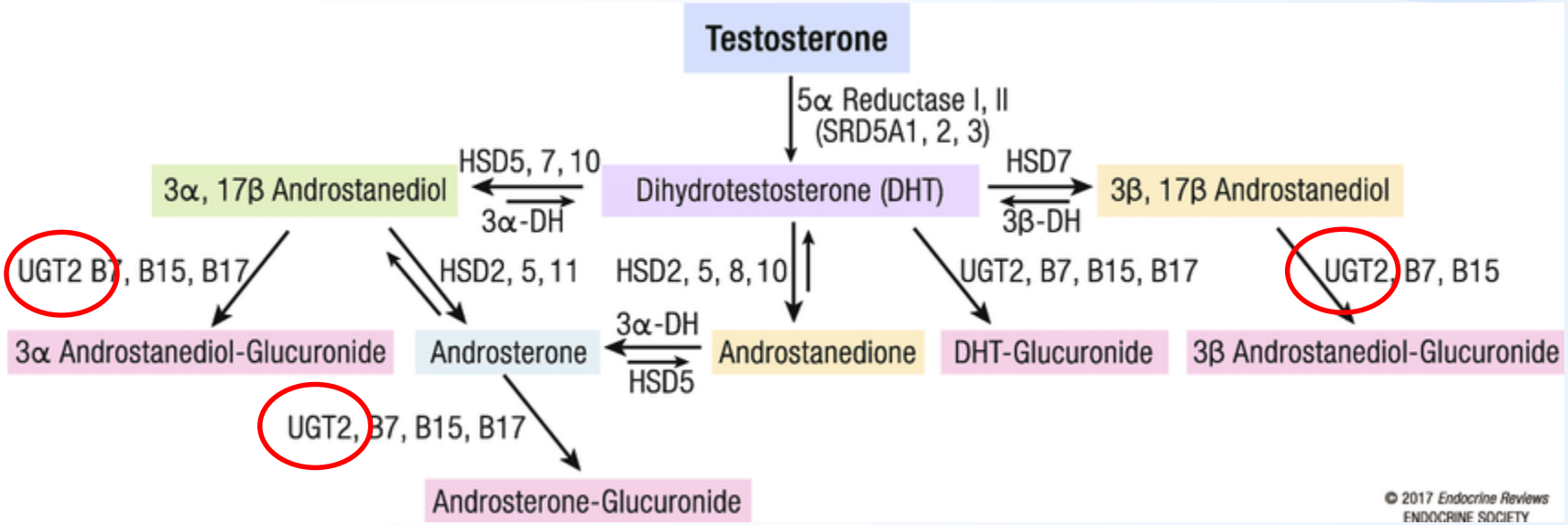
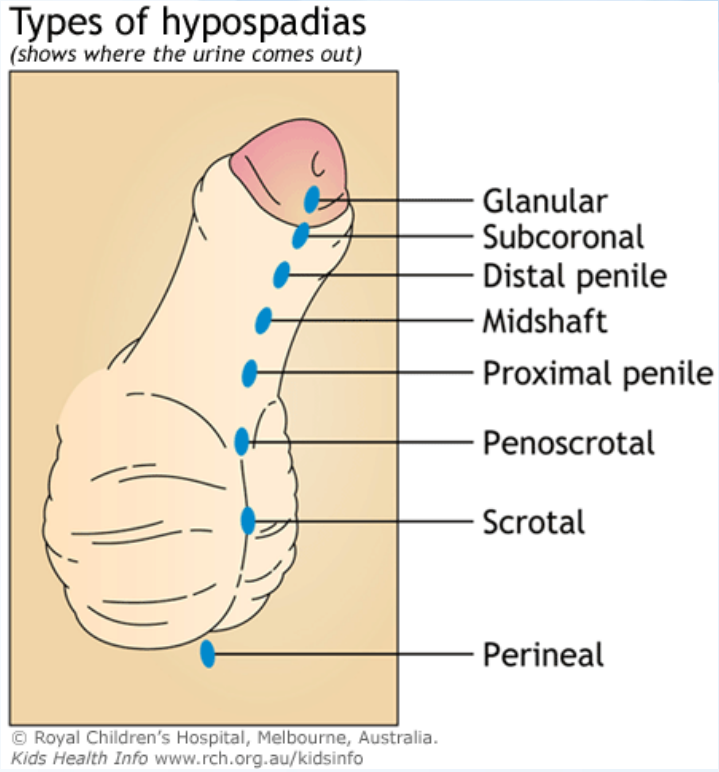


Effects of male exposures to **antiandrogens**

- **Exposure during prenatal development:**
  - malformations of the reproductive tract
    - reduced anogenital distance
    - **hypospadias** (abnormal position of the urethral opening on the penis)
    - vagina development
    - undescendent ectopic testes
    - atrophy of seminal vesicles and prostate gland

- **Exposure in prepubertal age:**
  - delayed puberty
  - reduced seminal vesicles
  - reduced prostate

- **Exposure in adult age:**
  - oligospermia
  - azoospermia
  - loss of sexual libido



Centrum pro výzkum toxických látek v prostředí

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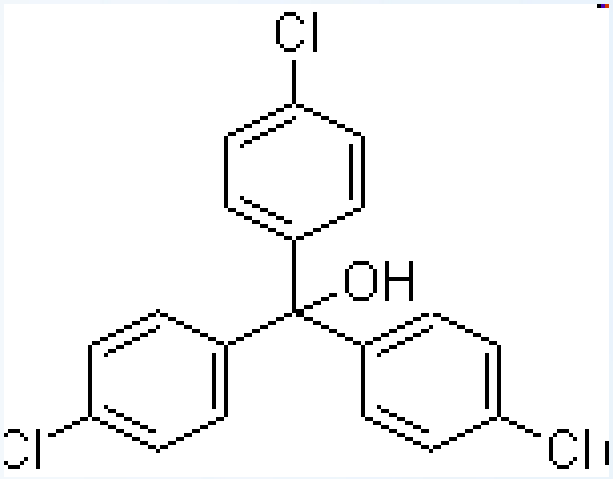
hydroxyflutamide	5
Aroclor typical values	0.25-1.11
Individual PCBs typical values	64 - 87
***tris*-(4-chlorophenyl)-methanol**	**0.2**

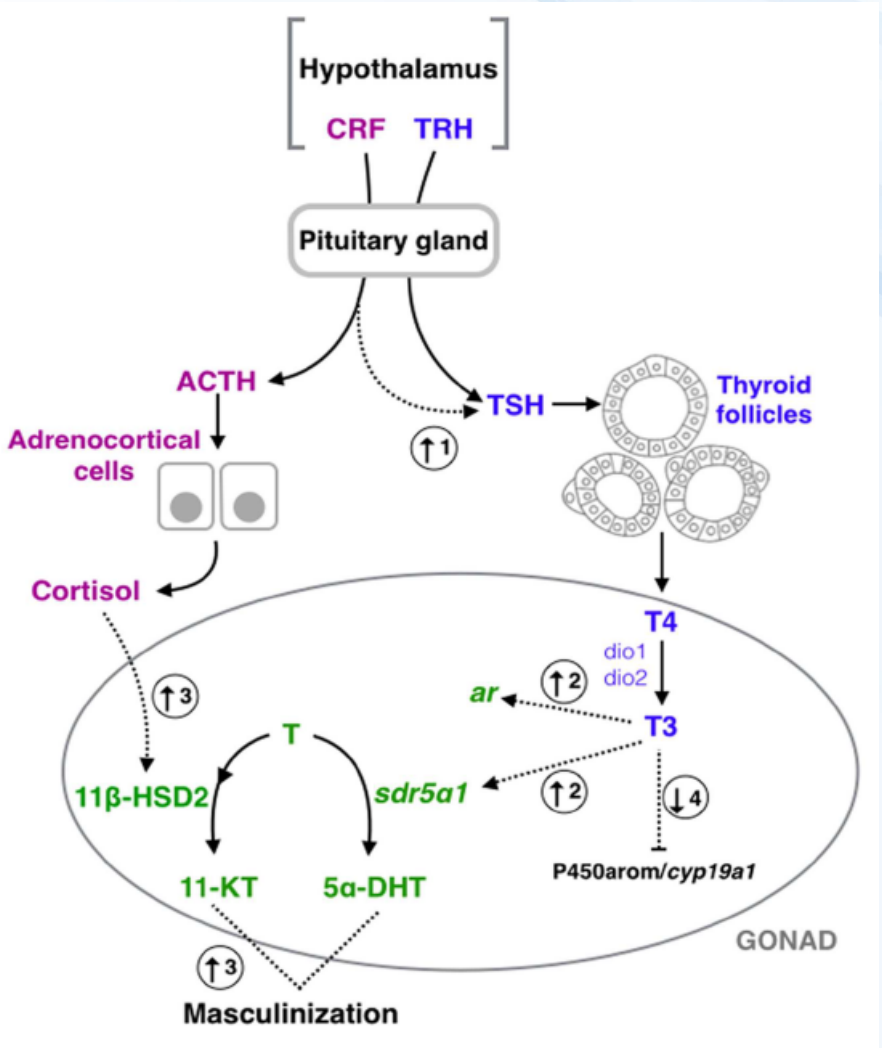
Antiandrogenic compound

- **tris-(4-chlorophenyl)-methanol**
  - Ubiquitous contaminant of uncertain origin
  - Probable metabolite of DDT-mixtures
  - Levels in human blood serum cca. 50nM
    (antiAR effective EC50 – cca. 200nM)



Centrum pro výzkum toxických látek v prostředí

THYROID SIGNALLING



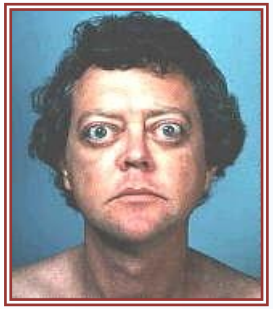
Centrum pro výzkum toxických látek v prostředí

Thyroid hormones

- Crucial roles in metabolism, development and maturation
  - Regulation of metabolism
    - increasing oxygen consumption
    - modulating levels of other hormones (insulin, glucagon, somatotropin, adrenalin)
  - Important in cell differenciation
  - Crucial role in development of CNS, gonads and bones

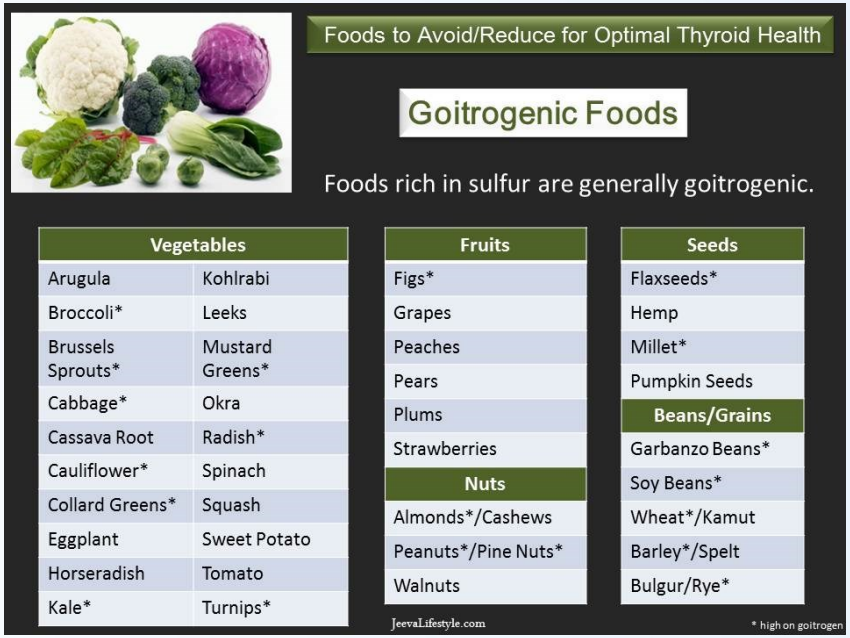
- EDC compounds interfering with thyroid signalling **"GOITROGENS"**
- Many food (vegetables) contain goitrogens

HYPOTHYROIDISM



HYPERTHYROIDISM

Centrum pro výzkum toxických látek v prostředí



Thyroid hormones

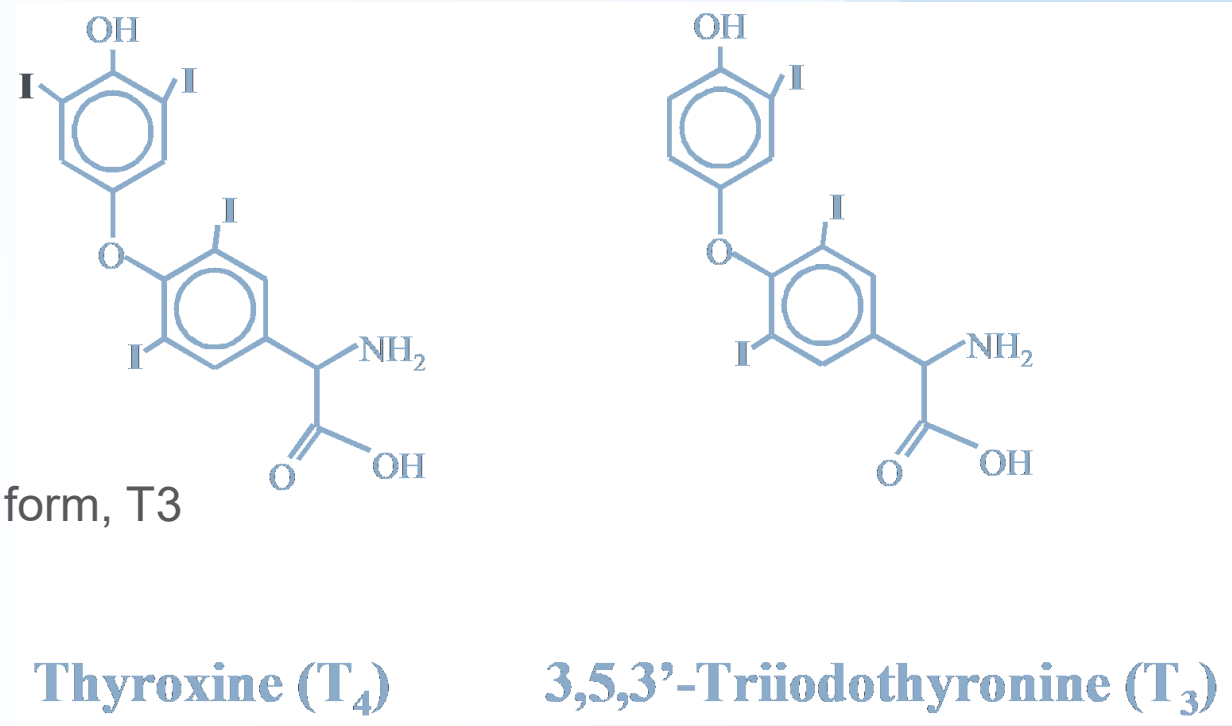
Thyroxine (T4)

Also called tetraiodothyronine
Contains 4 iodide ions

Triiodothyronine (T3)

Contains 3 iodide ions

-Most T3 produced
by deiodination
in target tissues (deiodinases)

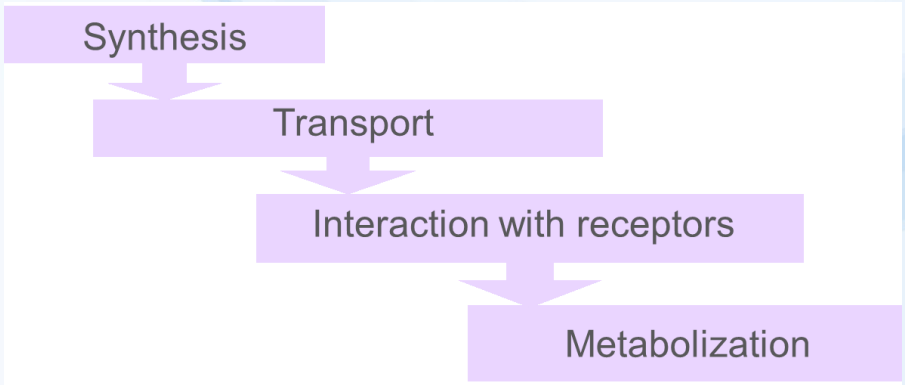


T4 – prohormone
5´-deiodination → active form, T3

Centrum pro výzkum toxických látek v prostředí

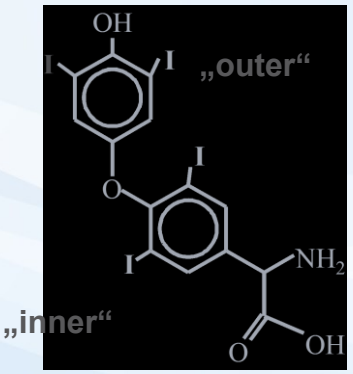
Multiple mechanisms of thyroid signalling disruption

- Xenobiotics are known to affect
  - Synthesis & activation (deiodinases)
  - Transport in blood
  - *(Direct effects on nuclear receptors - ThR – less important)*

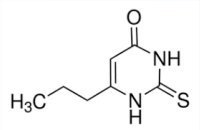


Disruption of enzymes involved in Thyroid metabolism

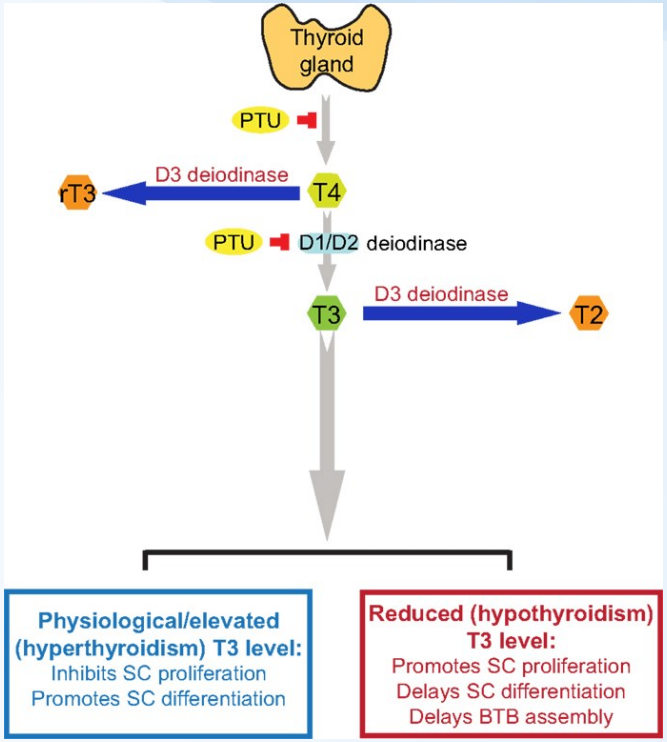
- **Thyroid peroxidases**
  - iodination of tyrosyl residues
  - coupling of iodinated tyrosyl residues
- **Thyroid deiodinases**
  - D1, D2 - activation of T4 into T3 via deiodination on „outer“ ring
  - D3 - deactivation into rT3 via deiodination on „inner“ ring



- **Many goitrogens** affect expression, activities and outcomes of these key enzymes
  - **PTU – propylthiouracil**
    →effect deiodinases



  - **Thiocyanate ([SCN]⁻) or perchlorate ($NaClO_4$)**
    →effect on iodine uptake



Centrum pro výzkum toxických látek v prostředí

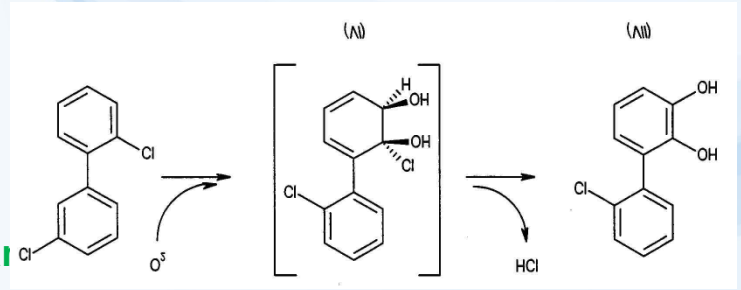
Disruption of transport of thyroid hormones in blood

- SPECIFIC TRANSPORTERS in blood
  - regulating free T4 and T3 levels
  - 3 types :
    - Thyroid-binding prealbunin (transthyretin) (20-25%)
    - Albumin (5-10%)
    - **Thyroid binding globulin (TBP, 75%)**

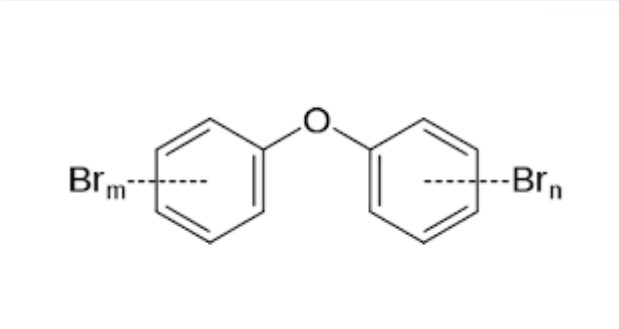
- **NUMBER OF EDCs → act on transport proteins**
  - OH-PCBs, **brominated and chlorinated flame retardan**
    DDT, dieldrin
  - **OH-PCBs** – equal affinity to **TBP** as T4 and T3 (!!!)

- Increased levels of "free T4" in blood
  - negative feedback to TSH release
    → increased depletion
    → increased weight, changes in thyroid gland
  - Documented after exposures to POPs in vertebrates

Hydroxylated PCB formation



Polybrominated diphenyl ethers (PBDEs) – flame retardants



Centrum pro výzkum toxických látek v prostředí

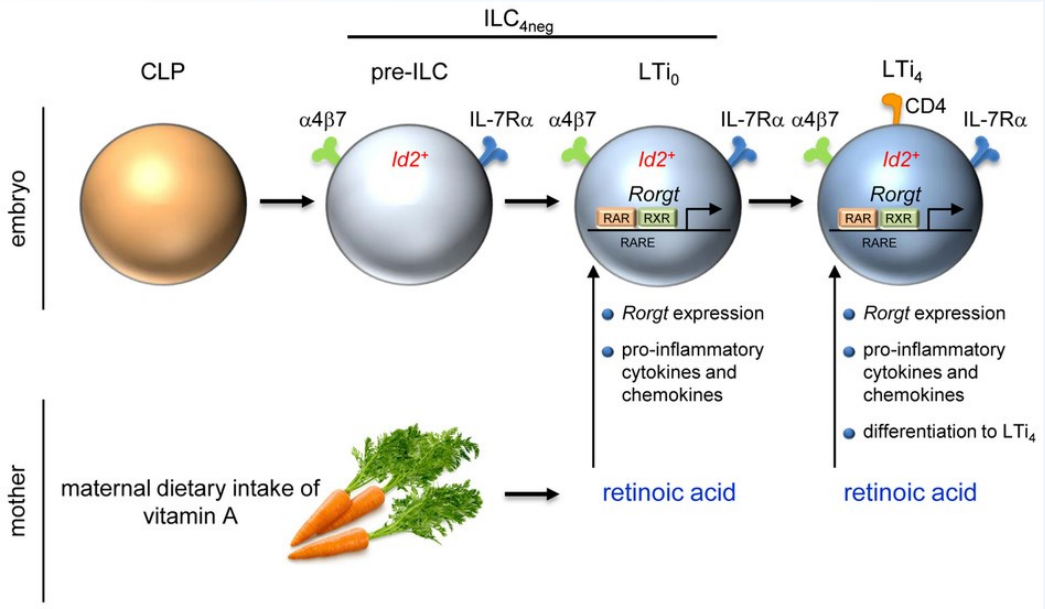
Effects of thyroid disruption

- **Exposures to goitrogens during prenatal stages**
  - severe damage of CNS (cretenism, delayed eye opening, cognition)
  - Megalotestis
  - Histological changes in thyroid gland (goitre)

- **Exposures during development**
  - nervous system fails to develop normally
  - mental retardation
  - skeletal development

RAR/RXR receptors
- vitamin A and its derivatives: RETINOIDS -

& their role in toxicity



Centrum pro výz
toxických látek
v prostředí

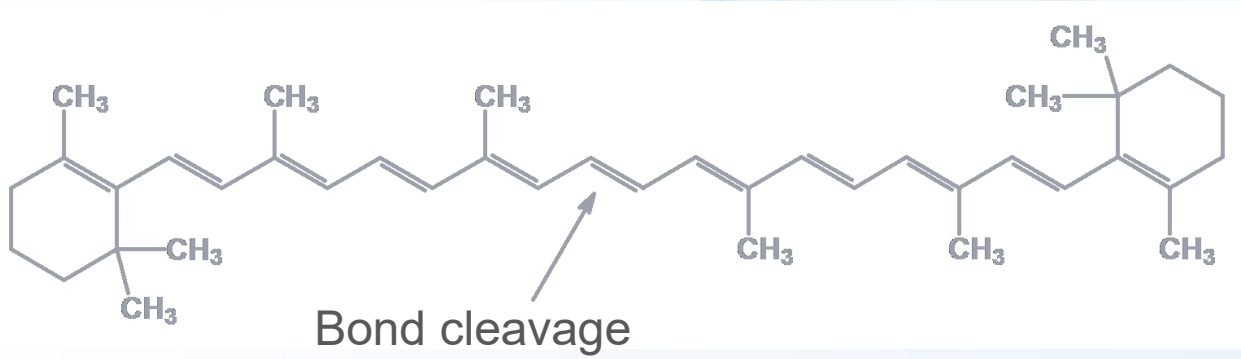
RETINOIDS

Sources: from diet - dietary hormones

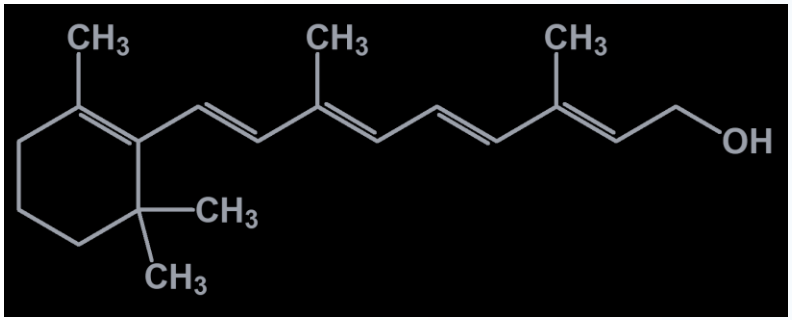
Retinyl esters – animal sources

Plant carotenoids

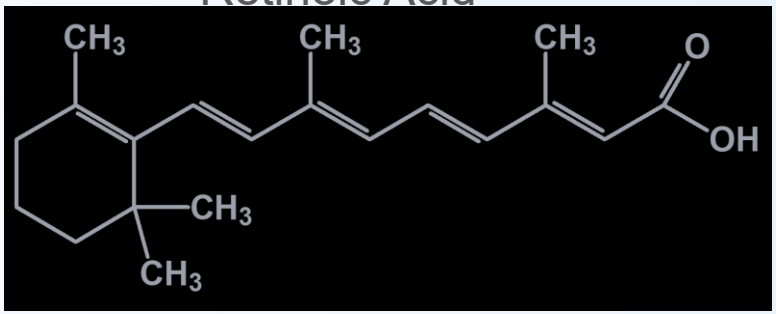
β-karoten



Retinol (vitamin A)



atRA – all trans - Retinoic Acid



Centrum pro výzkum toxických látek v prostředí

Retinoids and their functions

- Regulation of development and homeostasis in tissues of vertebrates and invertebrates
- Development of embryonic, epithelial cells (gastrointestinal tract, skin, bones)
- Necessary for vision
- Suppressive effects in cancer development
- Important for cell growth, apoptosis and differenciation
- Antioxidative agent
- Affect nervous and immune function

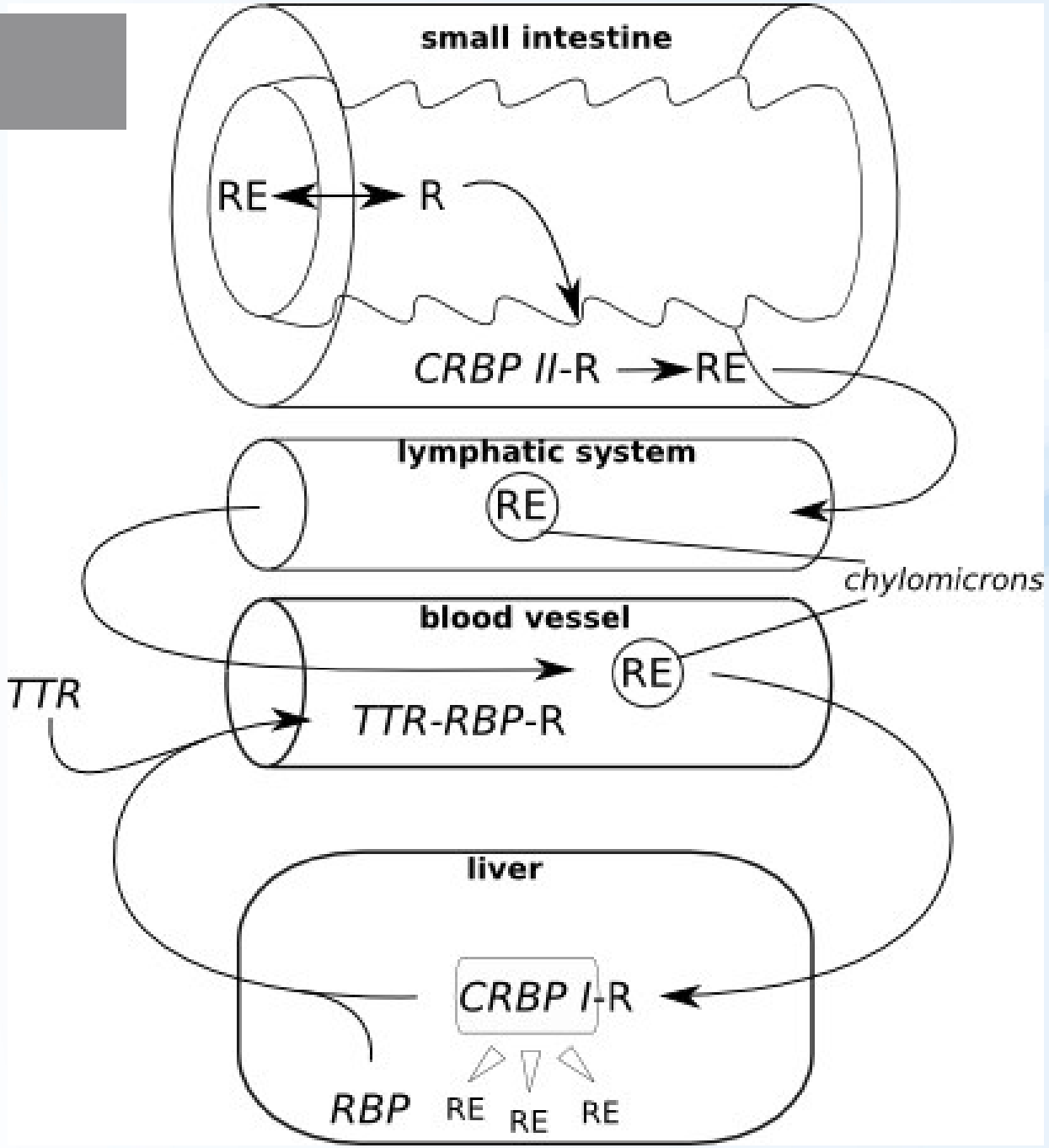
Retinoid transport

RE: Retinol-Ester

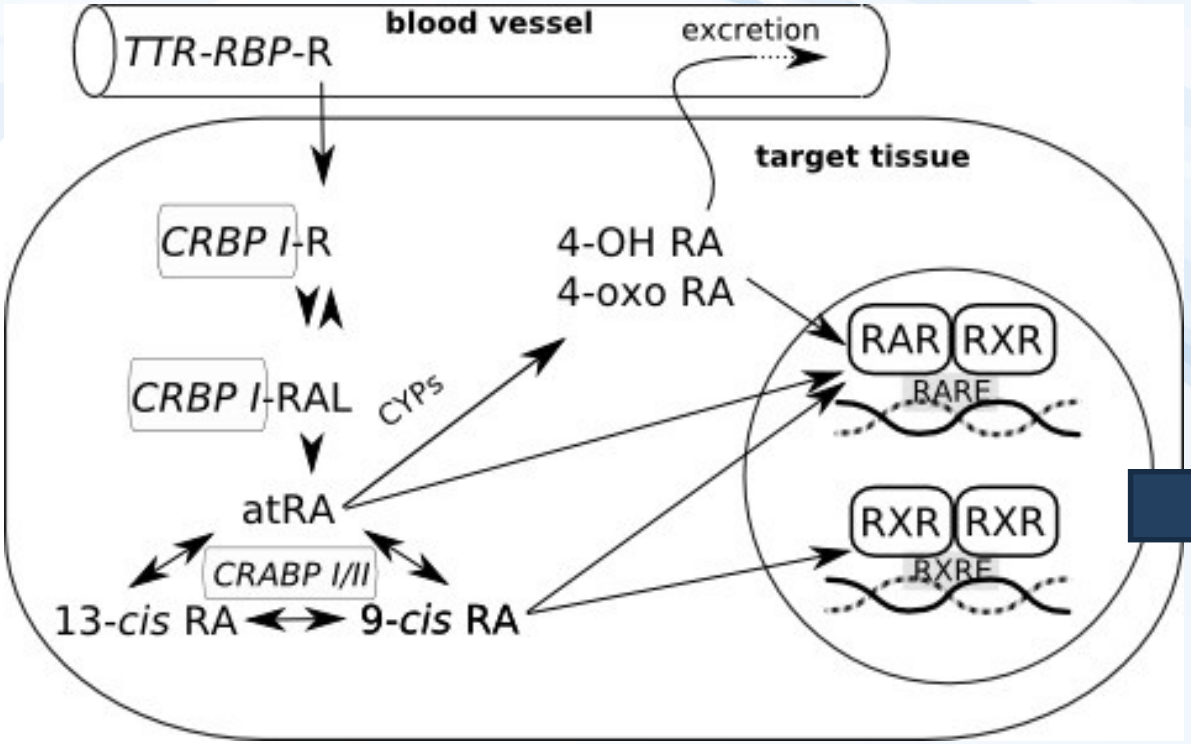
R: Retinol

RBP: Retinol Binding Protein (*LMW*)

TTR: Transthyrethin (*HMW*)



Retinoid fate in the cells



Gene expression

Retinoid binding proteins

CRBP – cellular retinol binding protein
- binding of retinol, immediate decrease of retinol concentration

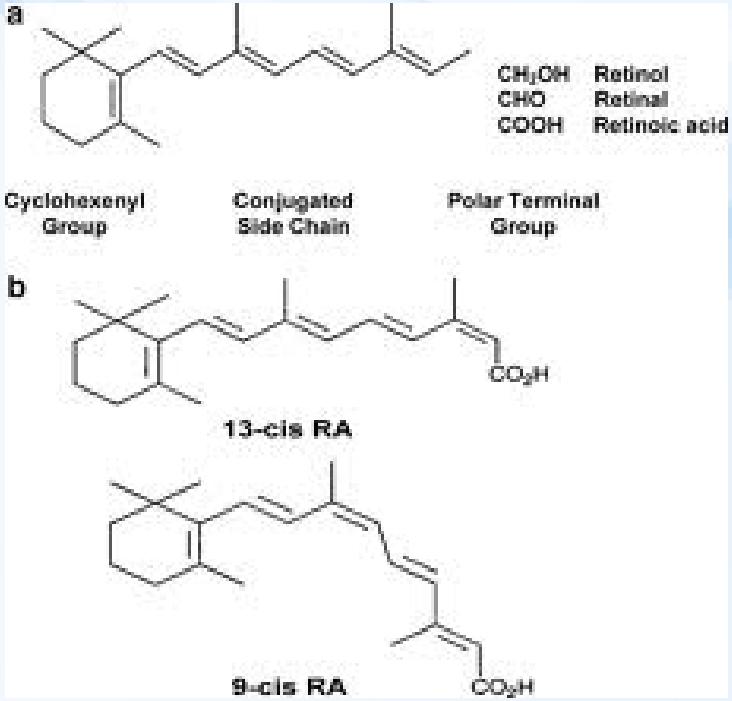
CRBAP – cellular retinoic acid binding protein
- Controlling the ratio free retinol/free retinoic acid

RAR/RXR and RA

- Isoforms of RAR a RXR
  - Formation of homo- and heterodimers
  - 48 possible RAR-RXR heterodimers

  → sensitive regulation of gene expression
- RXR – heterodimers with other receptors
  - VDR, TR, PPAR ... → see crosstalk

- **RETINOIC ACID (RA)**
- 3 basic subtypes
  - all-trans- (ATRA)
  - 9-cis- and 13-cis-retinoic acid
- All-trans RA (ATRA) binds selectively to RAR
- Cis RA bind to both receptor types



Centrum pro výzkum toxických látek v prostředí

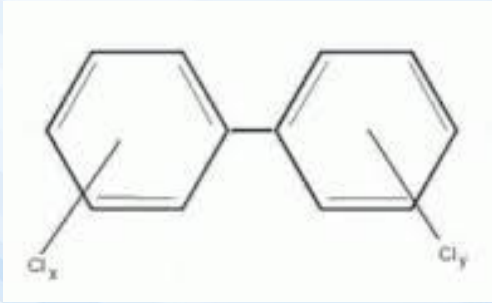
Disruption of retinoid signalling by xenobiotics

- **Possible modes of action – disruption of retinoid signalling:**
  - **Metabolization** of retinoids by detoxication enzymes
  - Disruption of binding retinoids **to transport proteins**
  - Retinoids as antioxidants may be **consumed by oxidative stress** induced by xenobiotics
  - Interference during **binding to RAR/RXR**

- **Effects**
  - **Decreased retinoid levels in organisms**
    - Downregulation of growth factors
    - Xerophtalmia, night blindness
    - Embryotoxicity, developmental abnormalities
  - **Increased ATRA concentration**
    - teratogenic effects

Disruption of retinoid signalling by xenobiotics

- **Polluted areas**
  - **mostly decrease of retinoid levels**
    - Documented in aquatic birds, mammals and fish



- **Disruption of retinoid transport:** PCBs

- **Effects on retinoid receptors:**
  - RAR, RXR binding and/or transactivation
    - pesticides (chlordane, dieldrin, methoprene, tributyltin…)
    - Effect on ATRA mediated response – TCDD, PAHs

- **Disruption of retinoid metabolism:**
  - **PCDD/Fs,** PAHs, PCBs, pesticides
  - changes of serum concentrations of retinol and RA
  - mobilization of hepatic storage forms

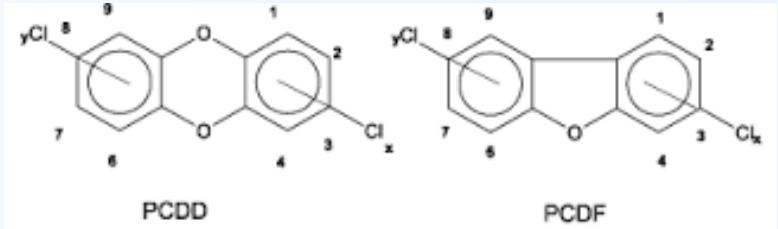


Figure 1. General molecular structure of polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofurans (PCDF)