Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

09 – Mechanisms
Nuclear Receptors

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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

Various signalling types … now focus on nuclear receptors

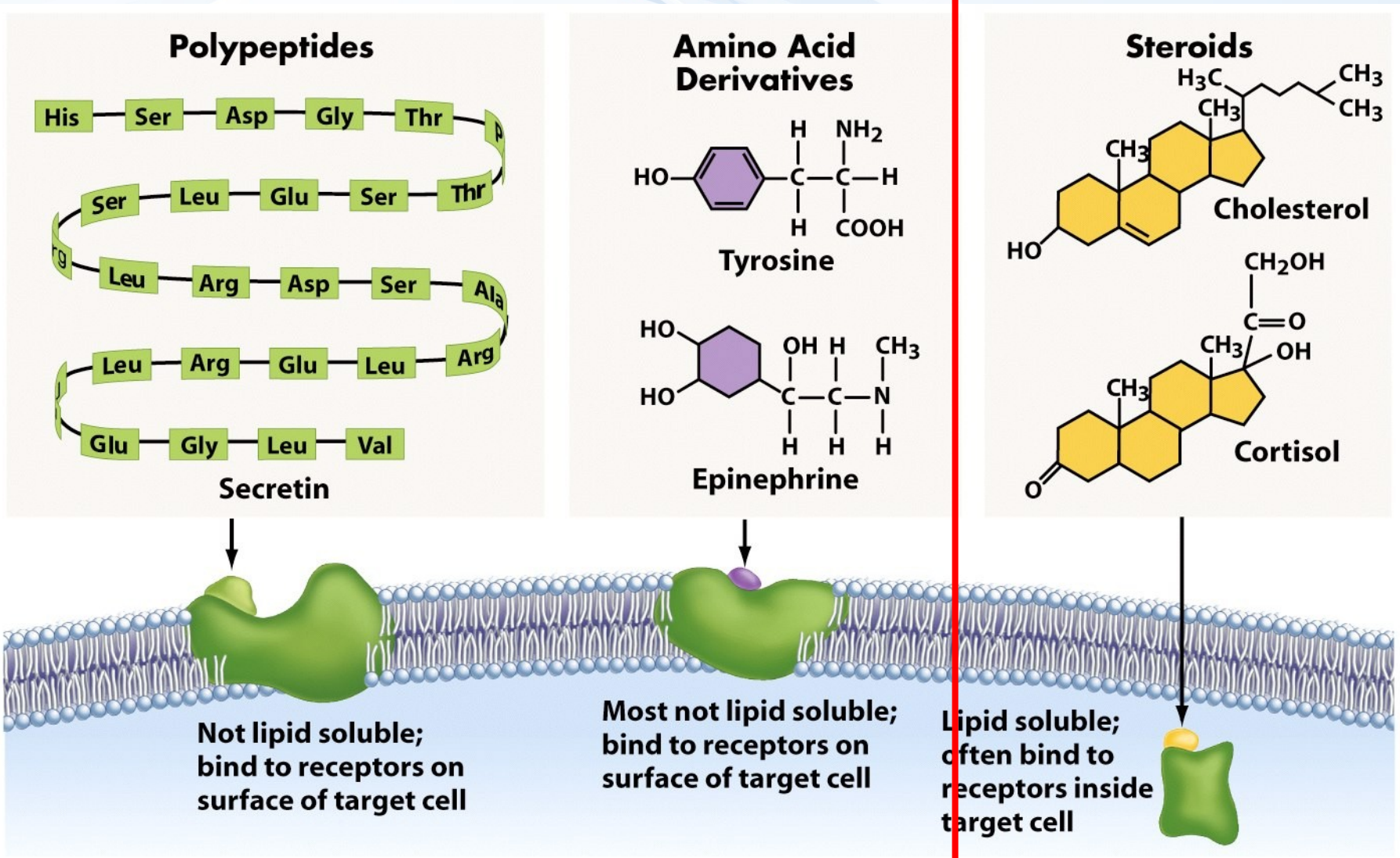


Figure 47-3 Biological Science, 2/e

NUCLEAR (Intracellular) RECEPTORS in summary

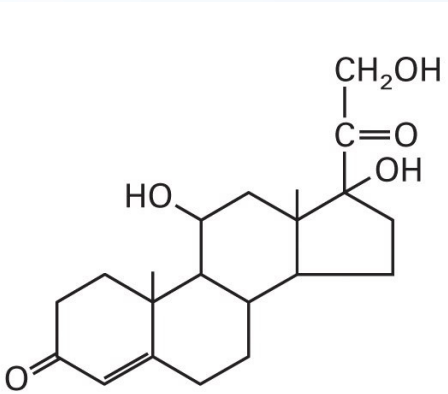
- Important physiological functions, and
- Important roles in pathologies and chemical toxicity
  - Endocrine disruption
  - Dioxin-like toxicity,etc.

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

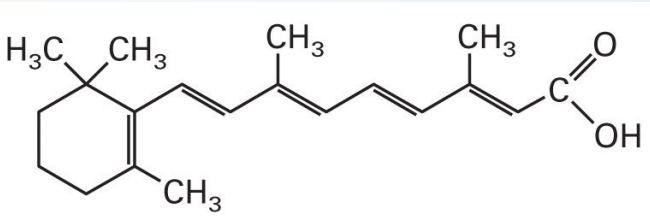
- Natural **ligands are small lipophilic** hormones (steroids, thyroids, retinoids)
  - Role in toxicity – NR are modulated (activated/inhibited) by structurally close xenobiotics

Natural ligands of NR

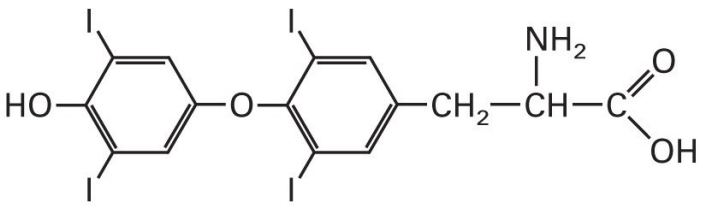
- **Small, lipid-soluble molecules**
  - Diffuse through plasma and nuclear membranes and interact directly with the transcription factors they control.
  - **STEROID HORMONES:**
    - sex steroids (estrogen, progesterone, testosterone)
    - corticosteroids (glucocorticoids and mineralcorticoids)
  - **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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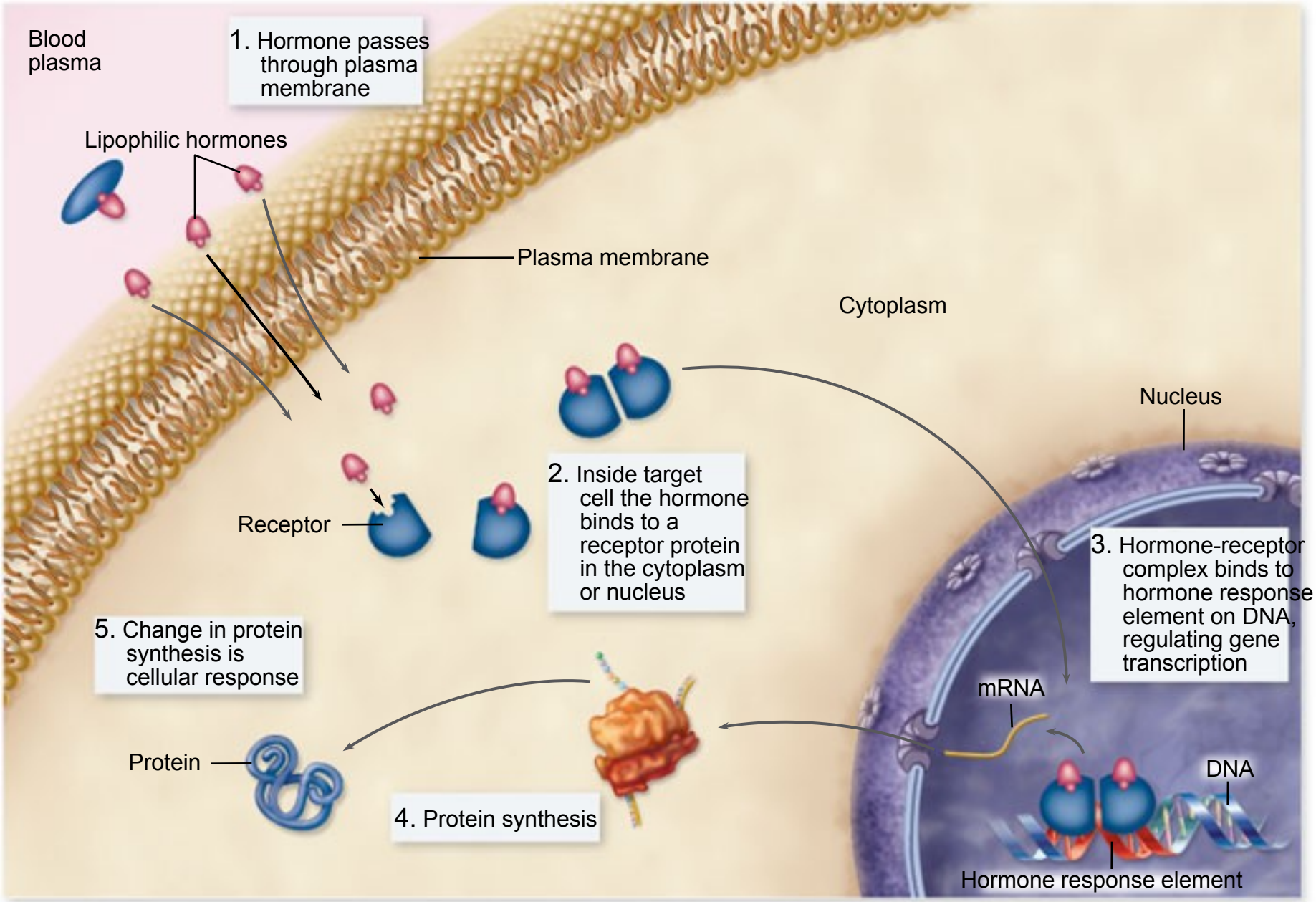
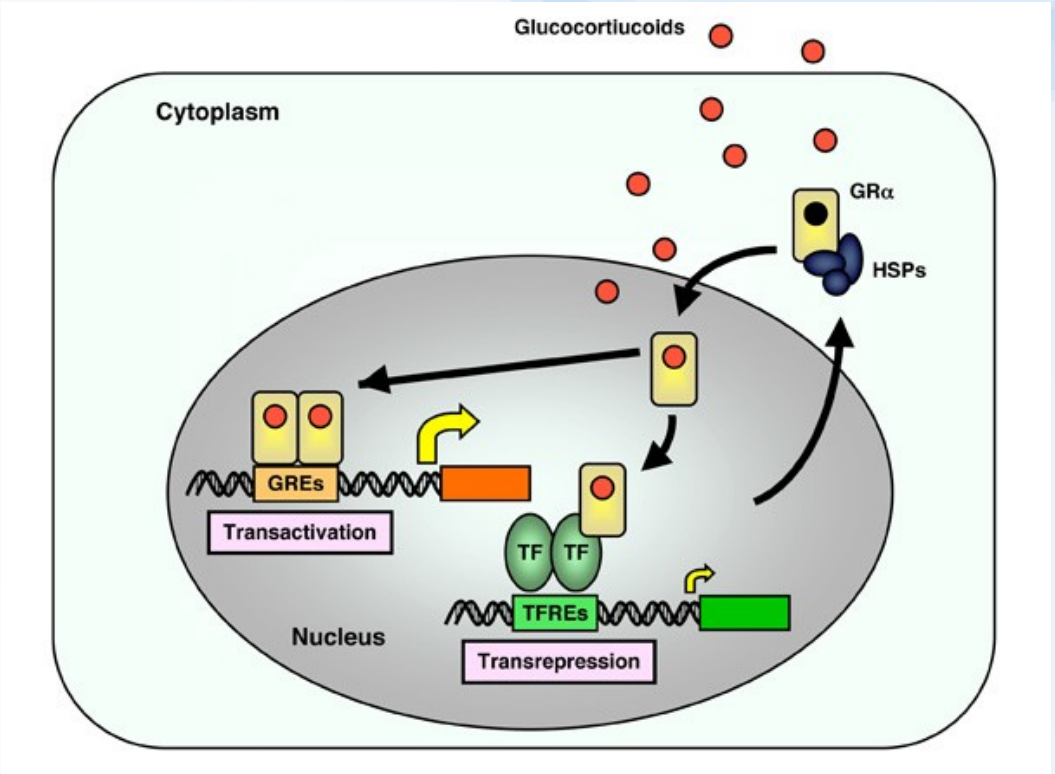


NR signalling is complex … examples of complexity (1)

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 – homodimers (e.g. ER-ER, etc.) AND/OR heterodimers (e.g. AhR-ER, etc.)

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



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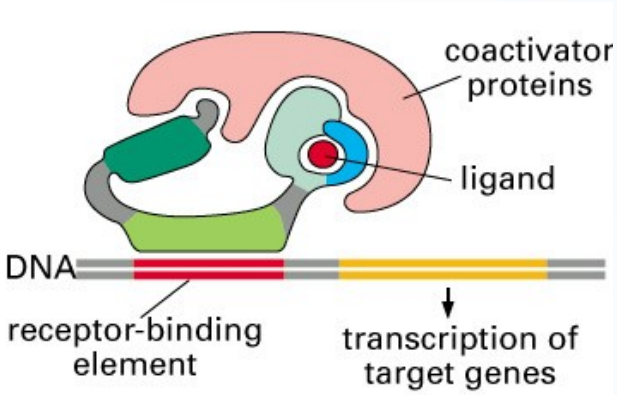
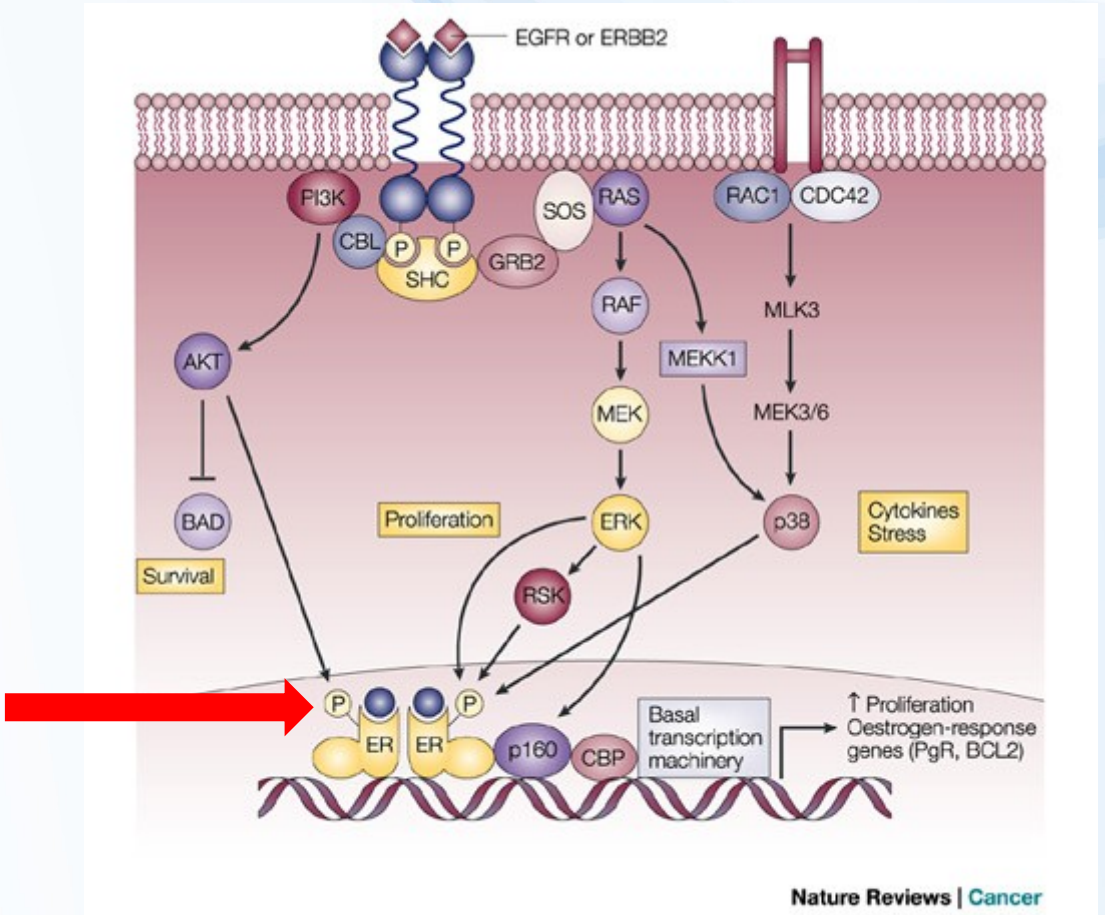
NR signalling is complex … examples of complexity 2

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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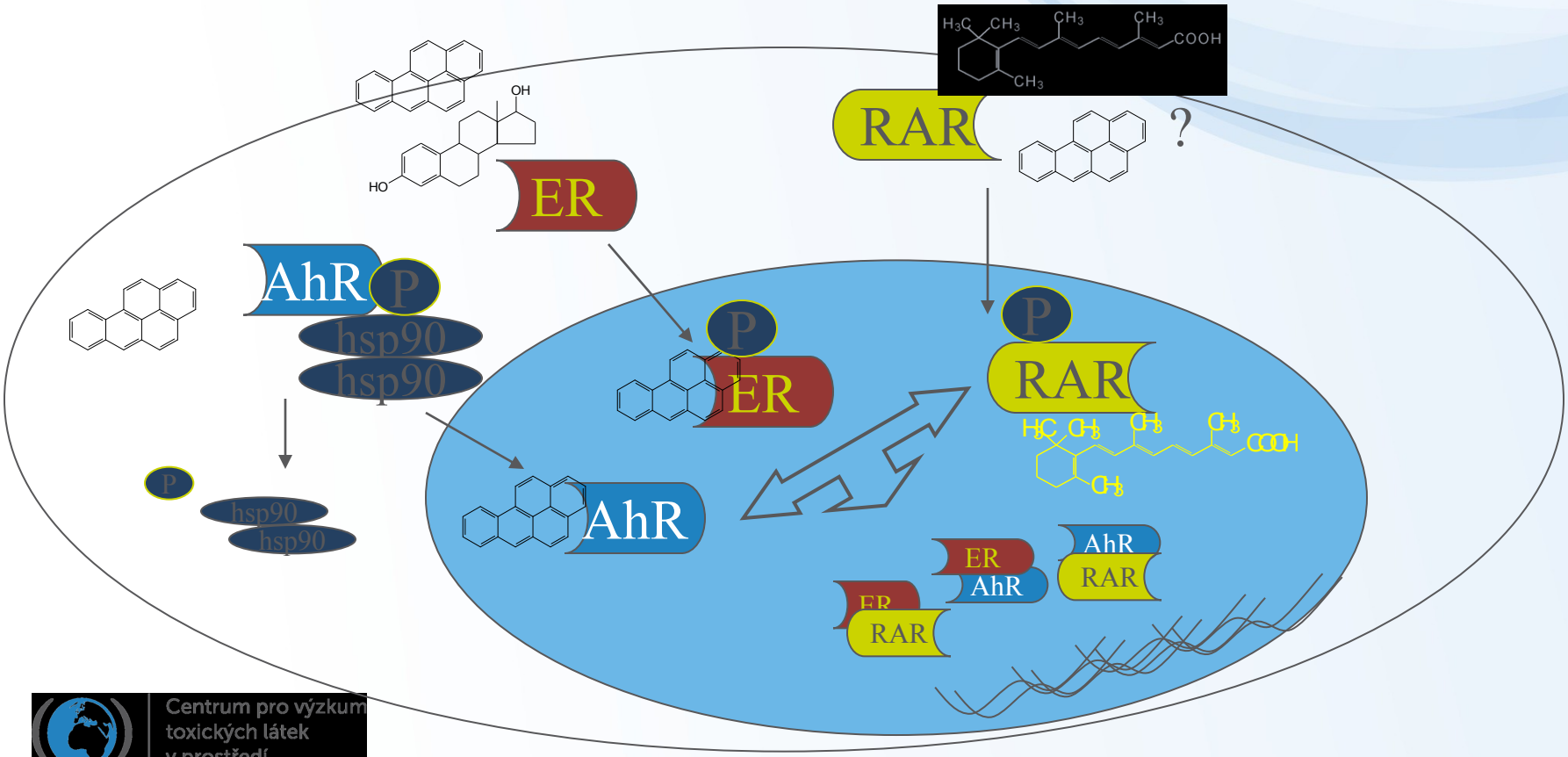
NR signalling is complex … examples of complexity 3

6. Interaction (crosstalk) among various NRs

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



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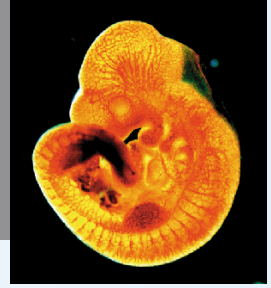
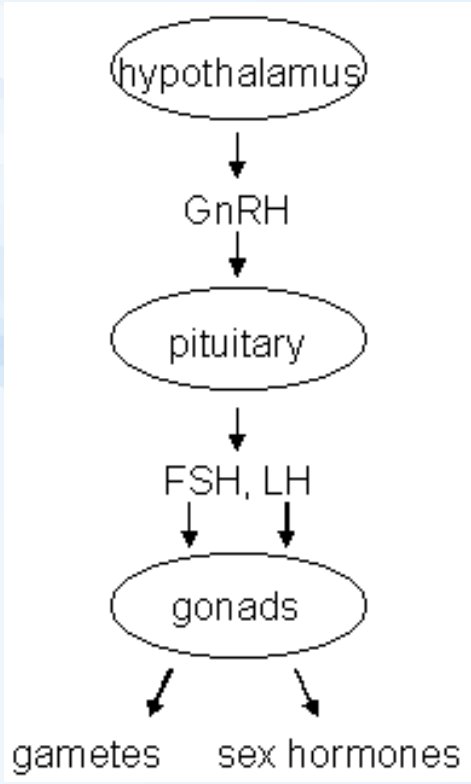
Why are NR important?

→ common mediators of adverse effects due to 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.

ED: toxicants interact with hormone system at all levels

Synthesis

Transport

Interaction with receptors

Metabolization

Consequences of endocrine disruption mechanisms (both are negative!)

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 – Hormone Responsive Elements)

↓

chromatin rearrangement and transcription of hormone-inducible genes

↓

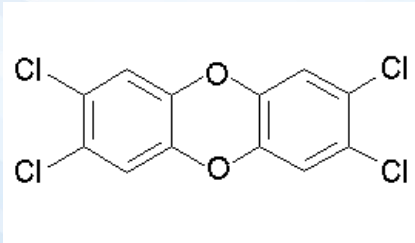
effects at cellular, tissue, organ, organism, and/or population level

Endocrine Disrupting Componds (EDCs) in the environment?

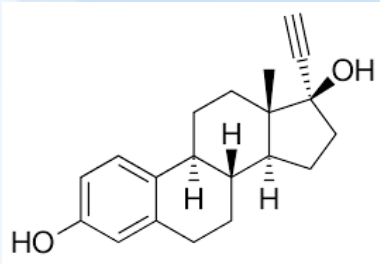
- Persistent Organic Compounds (POPs and their metabolites)
- steroid hormones and their derivatives from contraception pills
- alkylphenols
- organometallics (butyltins)
- pharmaceuticals
- Pesticides

+ many others

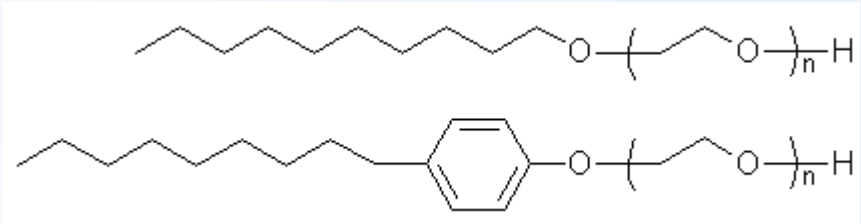
2,3,7,8-TCDD



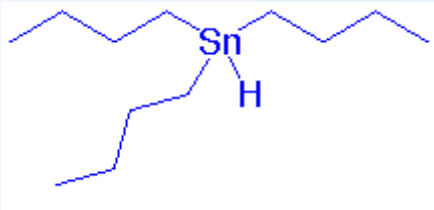
ethinylestradiol



alkylphenols



Tributyl-tin



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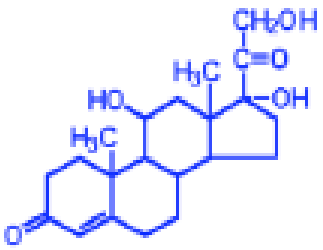
STEROIDs - most studied ligands of nuclear receptors

Steroid hormones - a review

Steroid hormones are derived from cholesterol metabolism in mitochondria

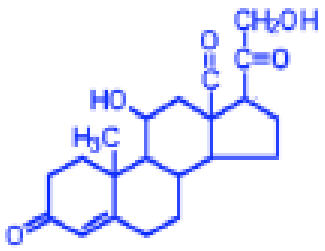
Cortisol

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.



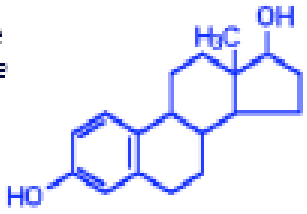
Aldosterone

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



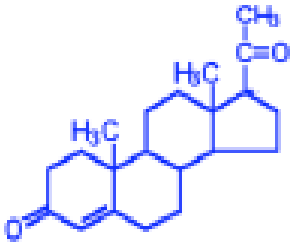
Estradiol

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.



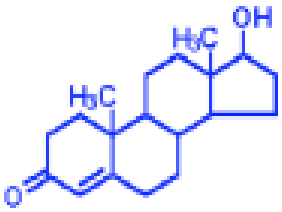
Progesterone

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



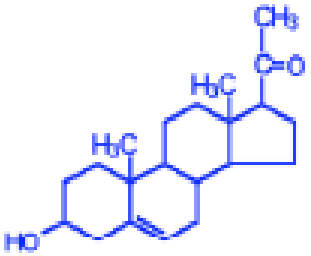
Testosterone

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



Pregnenolone

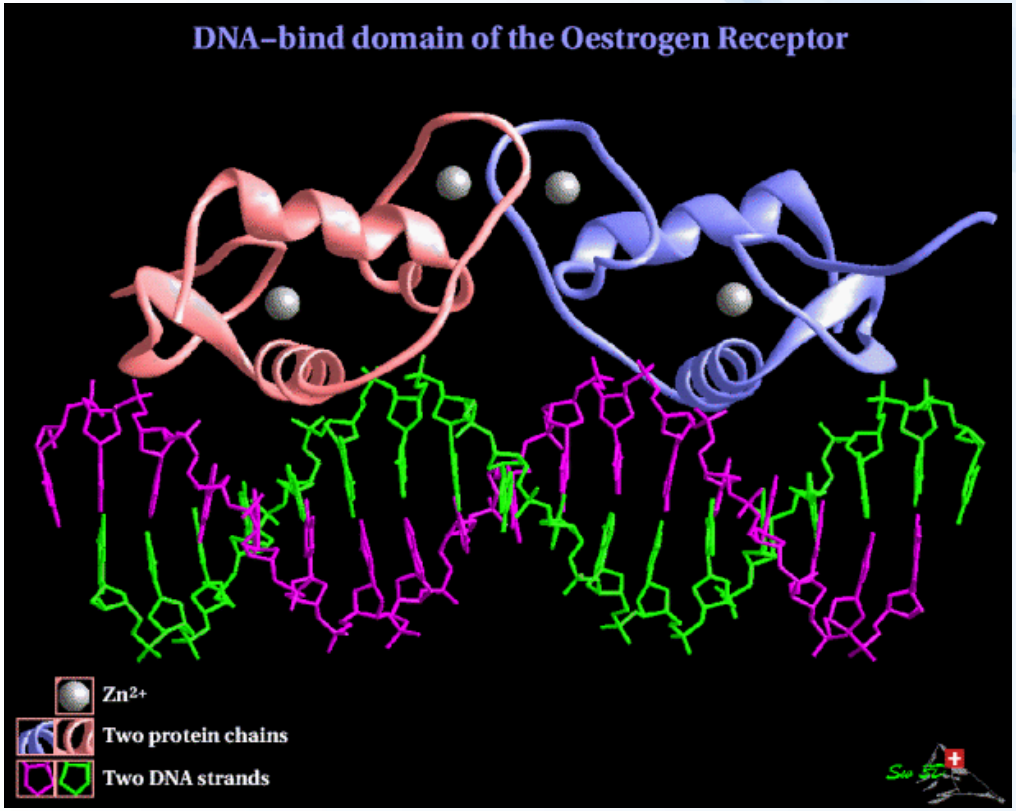
Made directly from cholesterol, the precusor molecule for all C_{18}, C_{19} and C_{21} steroids



STEROID HORMONE biosynthesis

ESTROGEN RECEPTOR – ER

the most studied target of EDCs



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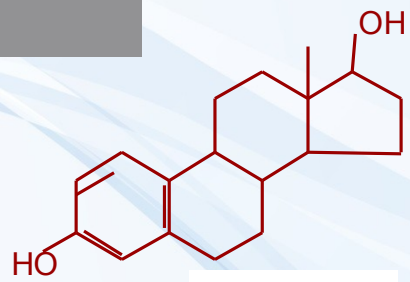
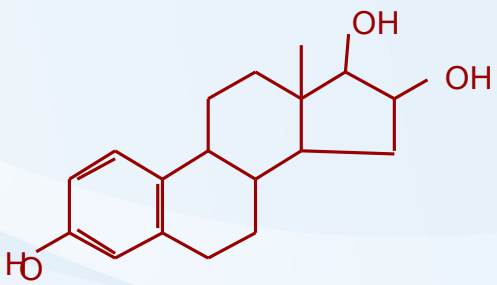
Estrogens



17-β-estradiol



estriol

- **Synthesis in ovaries**

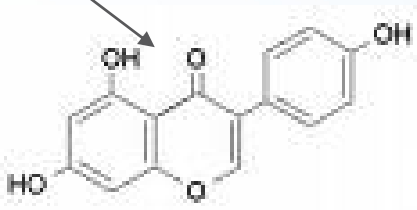
- **Functions**
  - key roles in female hormone regulation and signalling
  - responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
  - involved in the growth, development and homeostasis in a number of tissues
  - control the bone formation, regulation 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

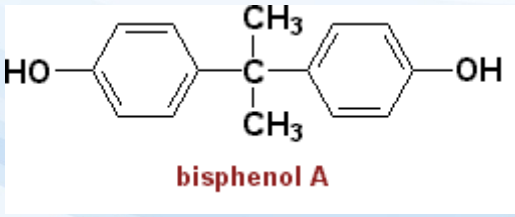
Environmental estrogens (xenoestrogens, exoestrogens)

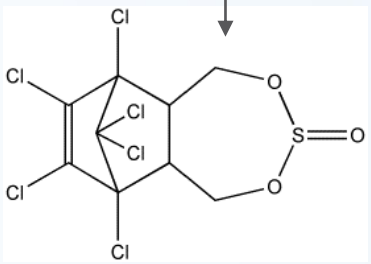
>> Highly diverse group of substances
>> Do not necessarily share structural similarity to the prototypical estrogen 17β-estradiol
>> may act as **AGONISTS** **and/or** **ANTAGONISTS** (depending on situation and concentration!)

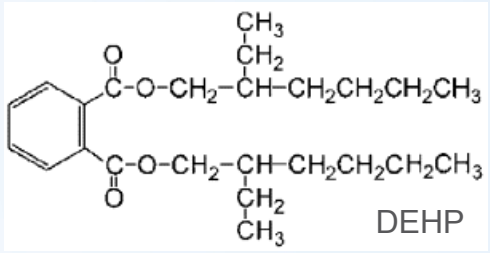
Natural products
genistein
naringenin
coumestrol
zearalenone



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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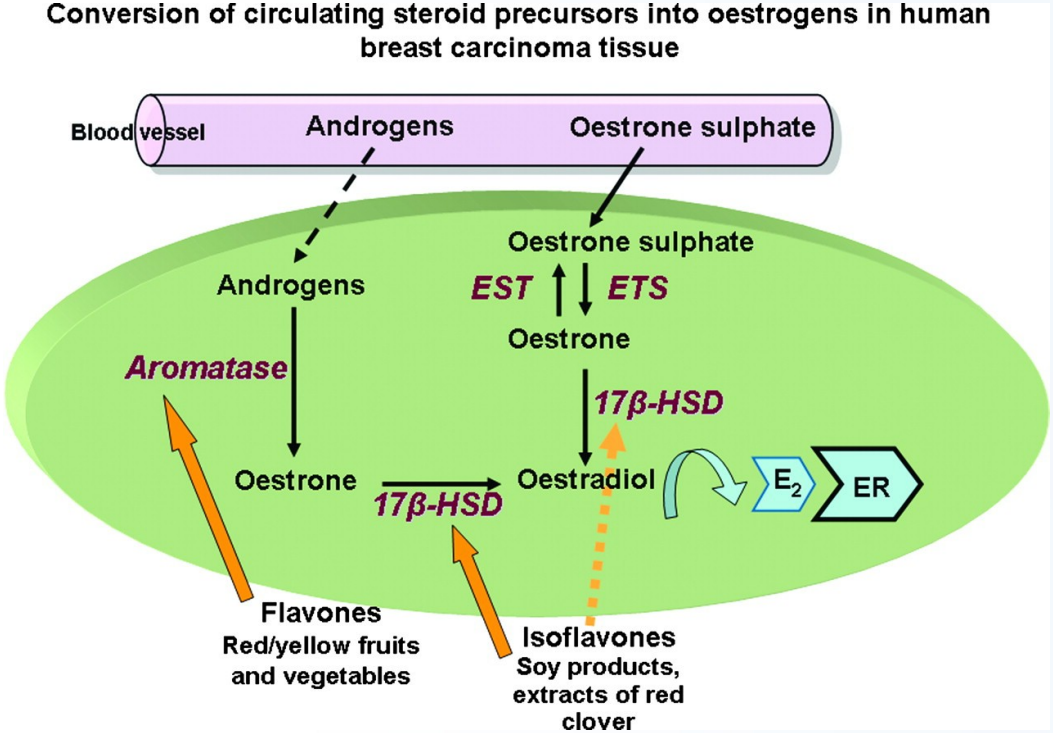
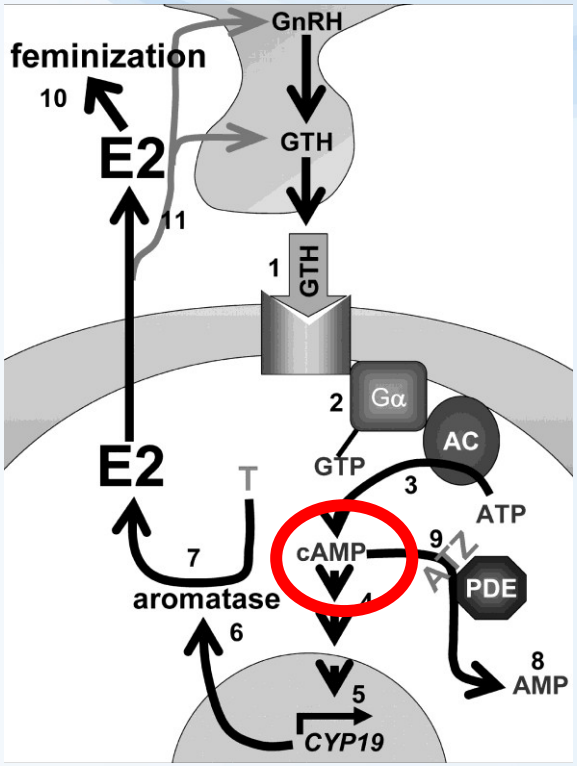
Examples – modulations of synthetic enzyme activities

Phytoestrogens induce genes involved in estradiol (E2) synthesis (**e.g. CYP19 Aromatase; 17b-HSD**) inducing thus elevante E2 concentrations (→ feminization)

Modulation of E2 synthesis via a crosstalk with other signalling pathways (such as **cAMP**), which can be target to toxicants (*see slides on signalling*)





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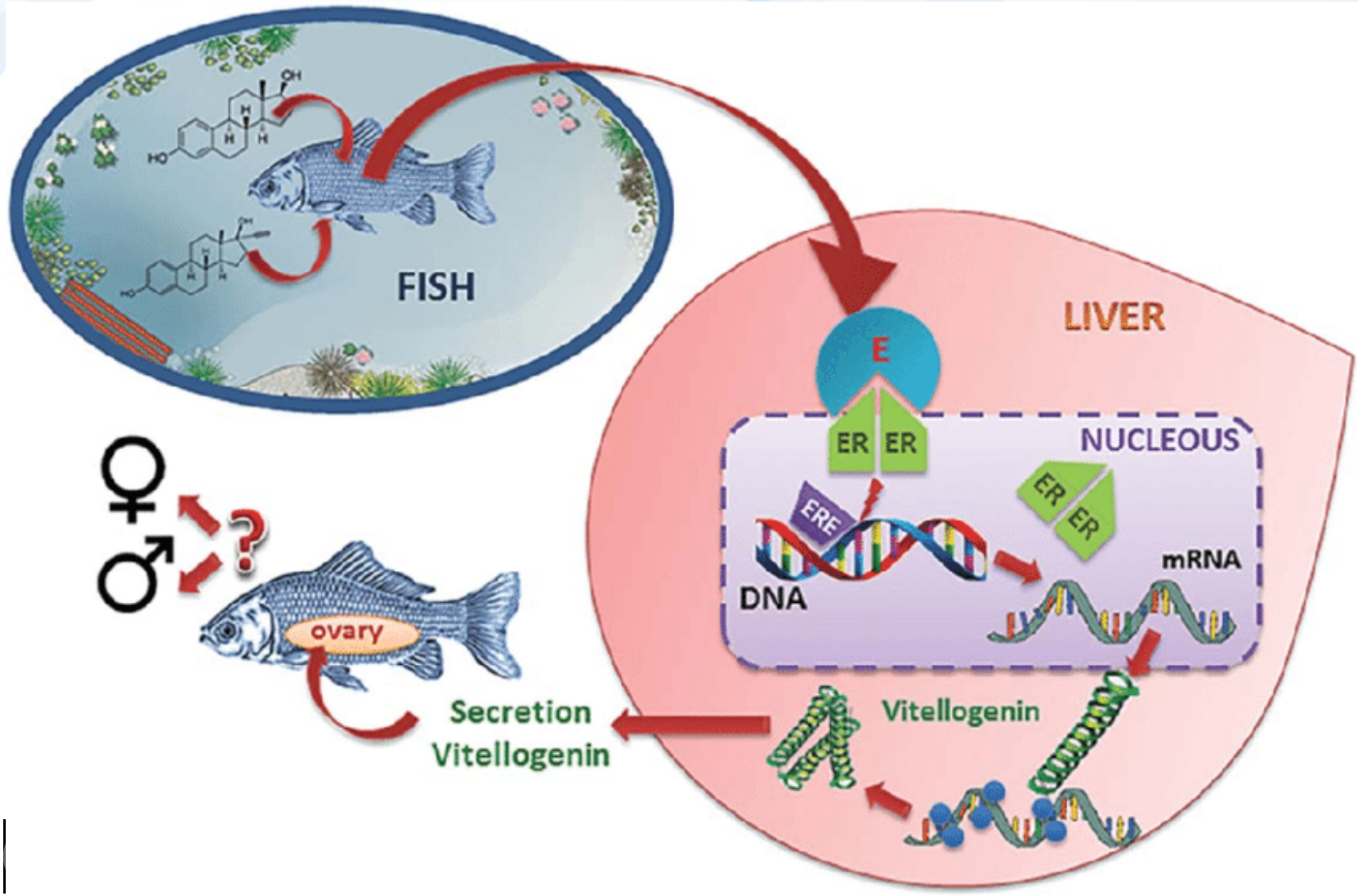
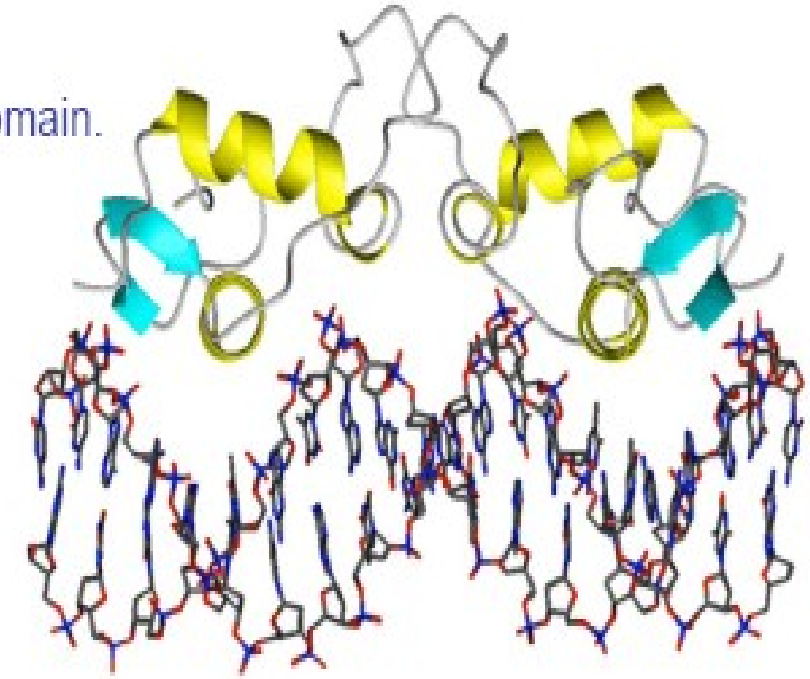
Activation of ER by xenoestrogens (e.g. synthetic hormones) in fish leading to disruption of reproduction



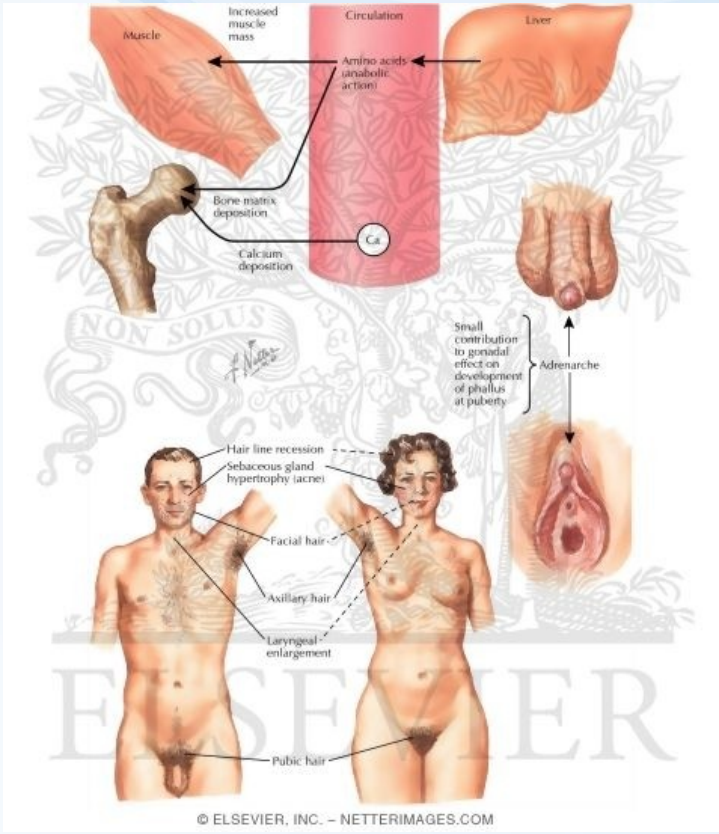
ANDROGEN RECEPTOR (AR)

Androgen receptor DNA binding domain.



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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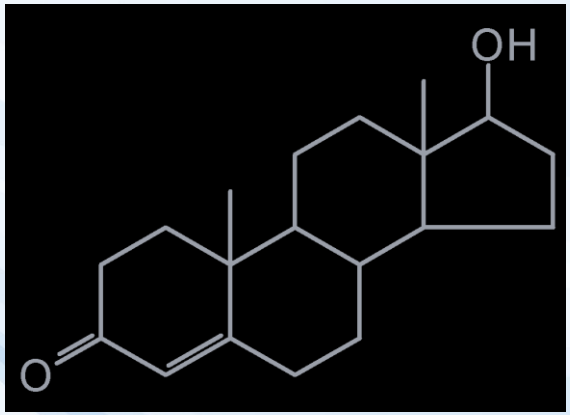
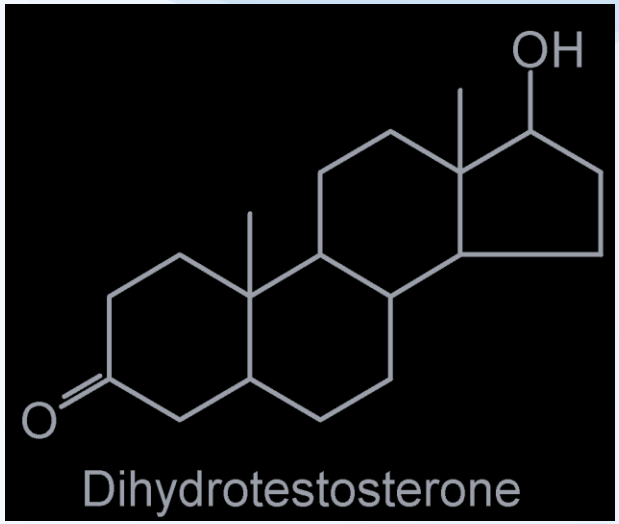
Androgens – endogenous ligands

- **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

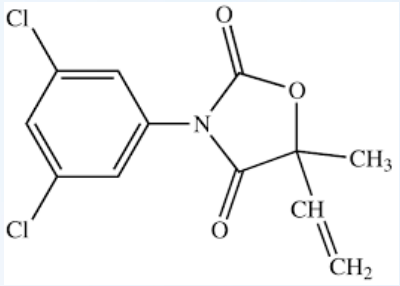
Several mechanisms how „xenoandrogens“ disrupt natural androgen signalling and action

1) Binding to AR

- Mostly competitive **inhibition** (xenobiotics mostly do not activate AR-dependent transcription) → outcome is the feminization

- Few compounds activate AR in the absence of androgen hormones … but they are still **anti-androgenic** in the presence of natural androgens like T or DHT
  - metabolites of **fungicide vinclozoline**
  - some PAHs

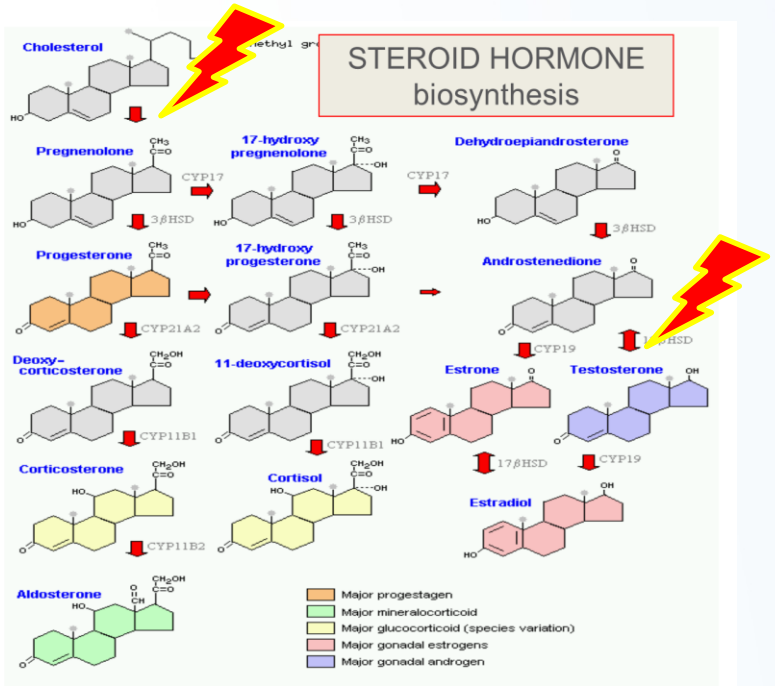
vinclozoline

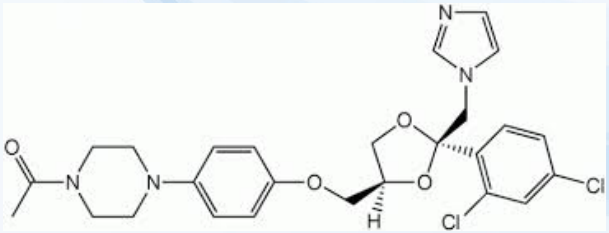


Mechanisms of androgen signalling disruption

2) 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**



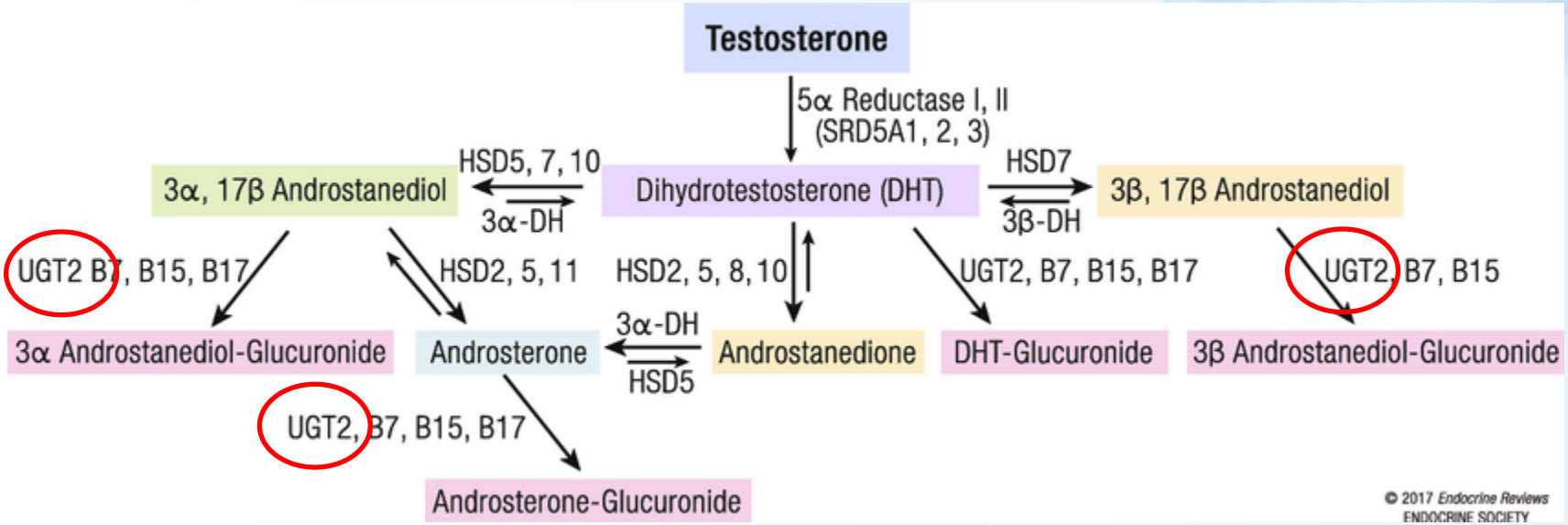


The toxic outcome = feminization

Mechanisms of androgen signalling disruption

3) 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: feminization)*

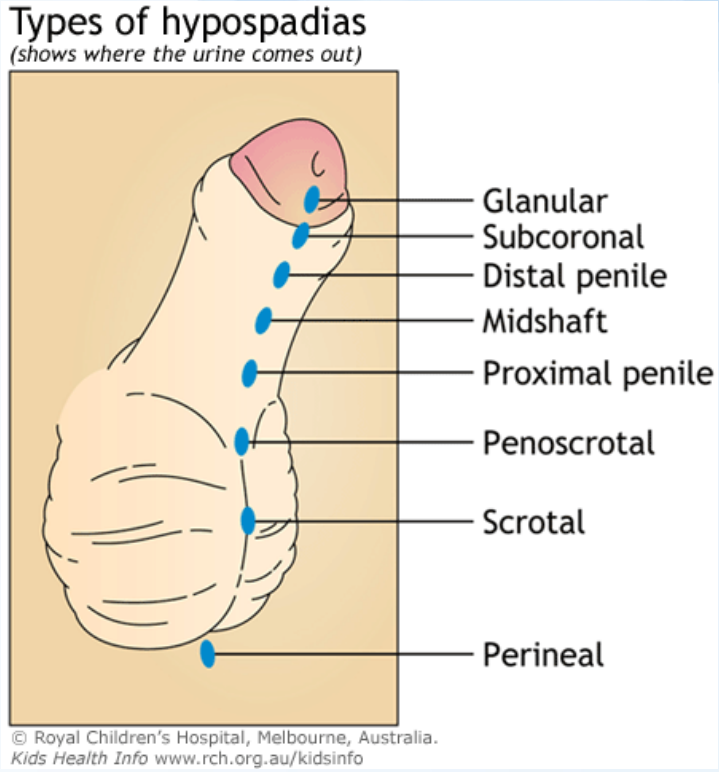


Effects of exposures to **antiandrogens** in males

- **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



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Other selected nuclear receptors – examples of mechanisms beyond the toxic effects

Receptors for **THYROID** hormones

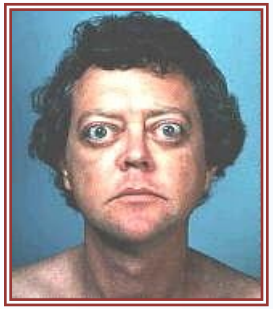
Receptors for **RETINOID-like** compounds

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

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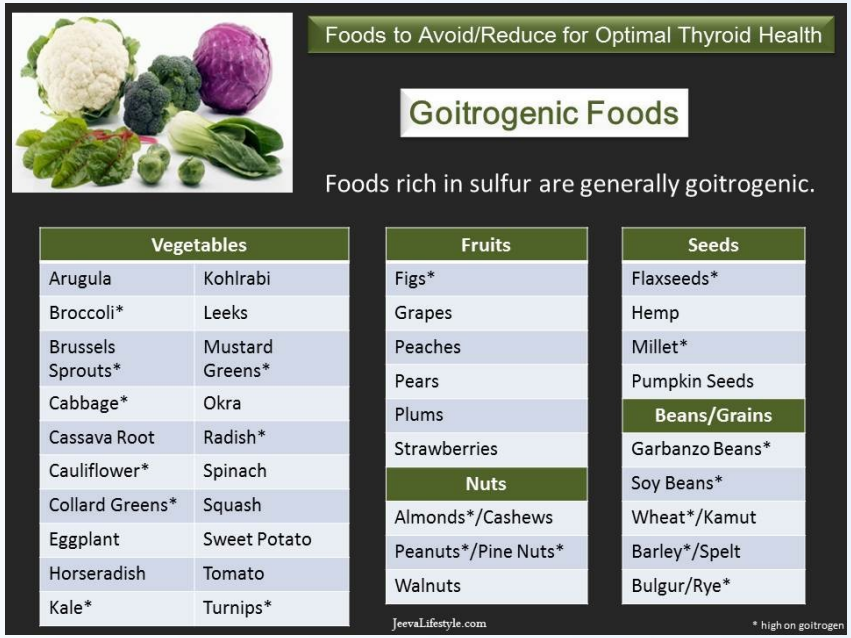


Thyroxine (T4)

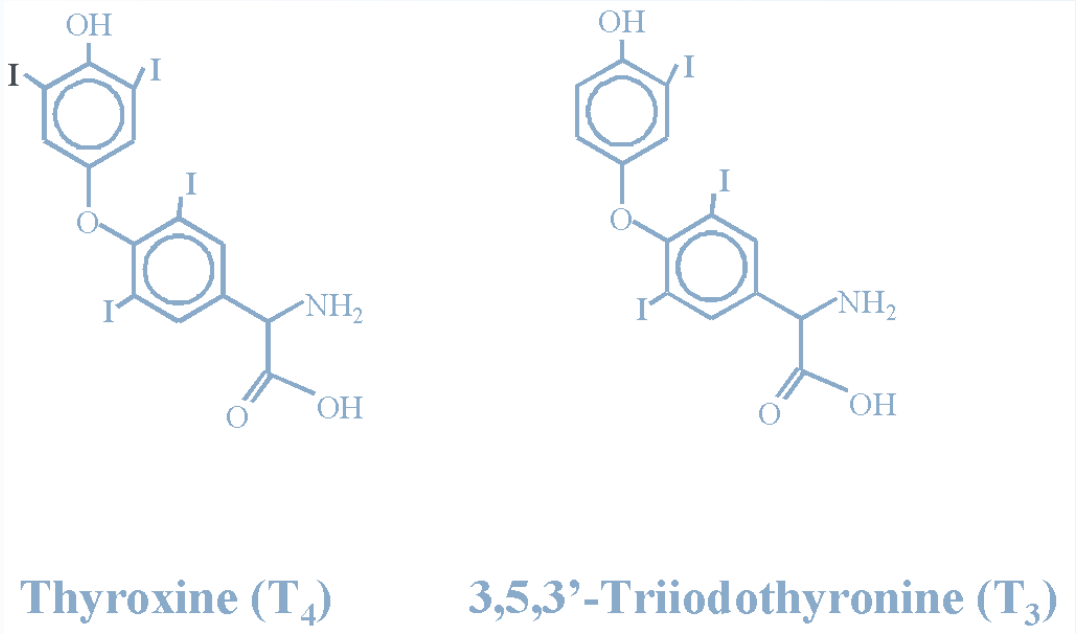
Also known as tetraiodothyronine
Contains 4 iodide (I) ions

T4 – pro-hormone
5 -deiodination → **active form: T3**

Triiodothyronine (T3)

Contains 3 iodide ions

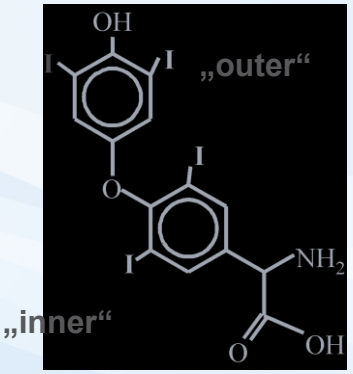
-Most T3 is produced by deiodination (deiodinases) in target tissues



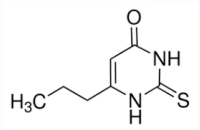
Thyroxine (T_4) 3,5,3'-Triiodothyronine (T_3)

Disruption of enzymes involved in Thyroid metabolism

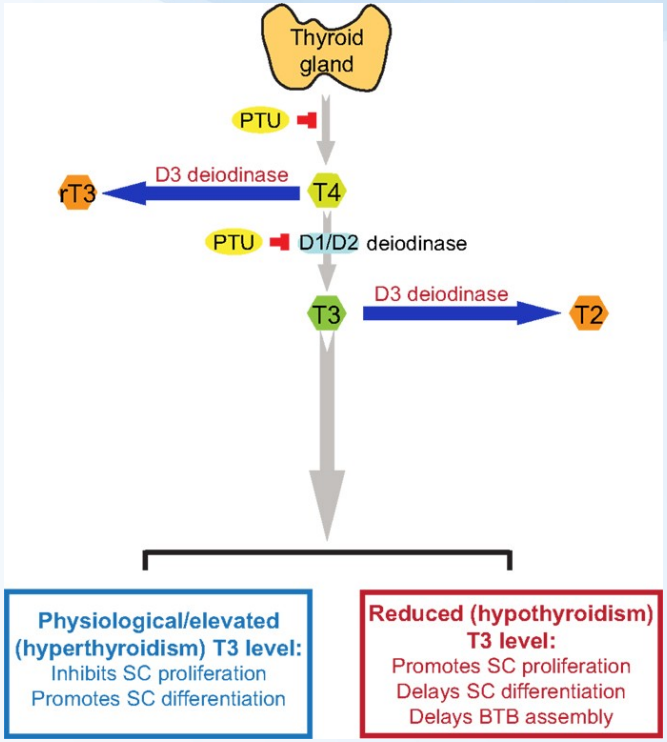
- **Thyroid peroxidases**
  - iodination of tyrosyl residues
  - coupling of iodinated tyrosyl residues
- **Thyroid deiodinases**
  - D1, D2 - activation of T4 into T3 via deiodination on „outer“ ring
  - D3 - deactivation into rT3 via deiodination on „inner“ ring



- **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



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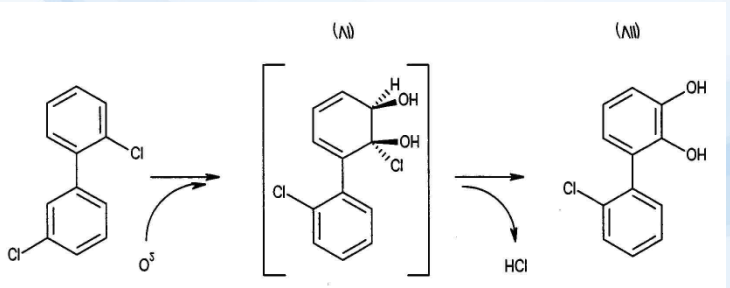
Disruption of transport of thyroid hormones in blood

- 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%)**

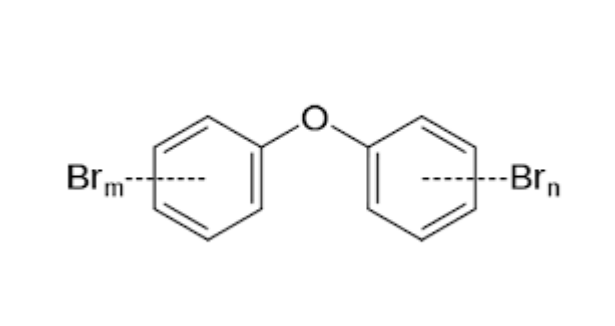
- **NUMBER OF EDCs → act on transport proteins**
  - **Brominated and chlorinated flame retardants**, DDT, dieldrin
  - **OH-PCBs** – equal affinity to **TBP** as T4 and T3 (!!!)

- 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

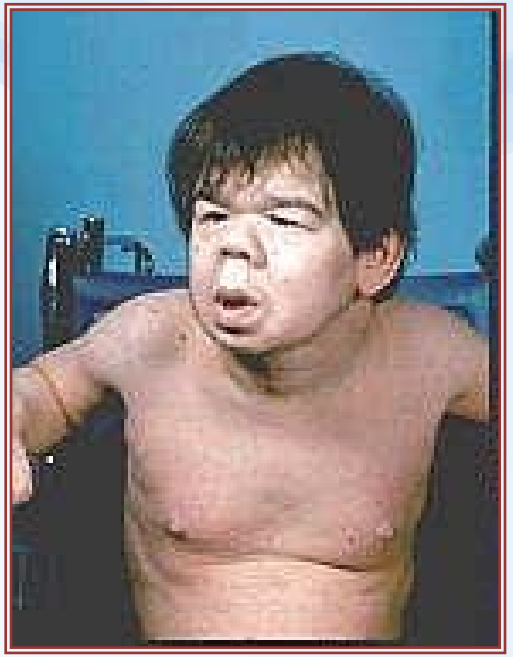


Polybrominated diphenyl ethers (PBDEs) – flame retardants



Effects of thyroid disruption

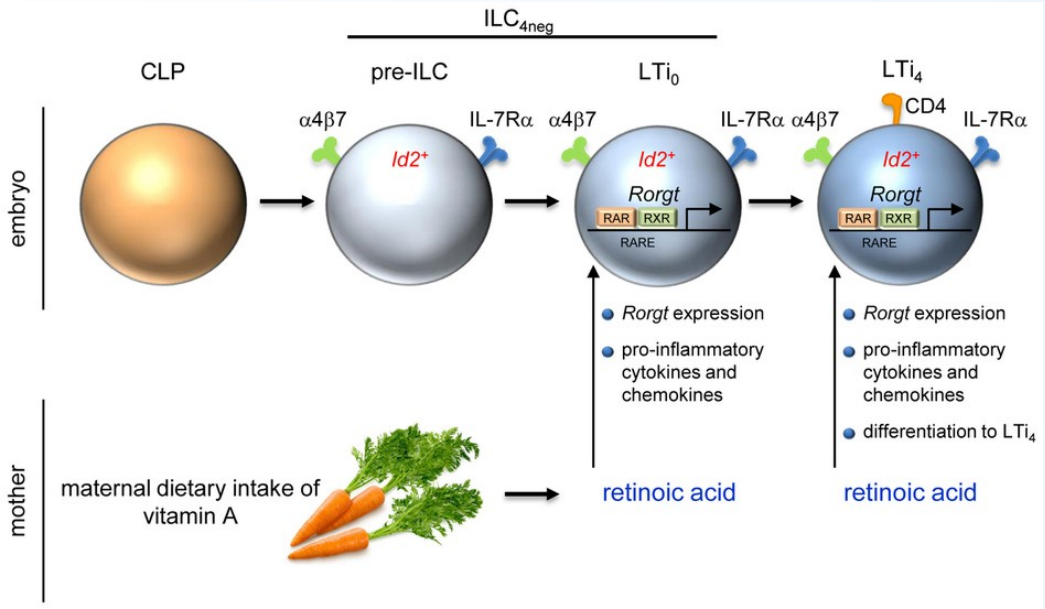
- **Exposures to goitrogens during prenatal stages**
  - severe damage of CNS (cretenism, delayed eye opening, cognition)
  - Megalotestis
  - Histological changes in thyroid gland (goitre)



- **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



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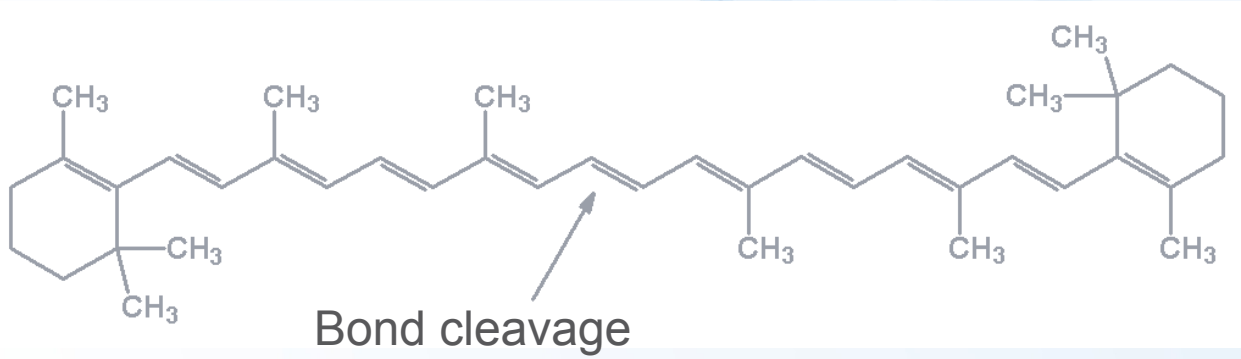
RETINOIDS

Sources: from diet - dietary hormones

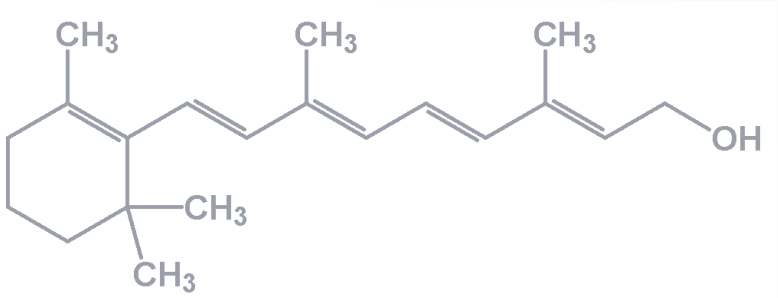
Retinyl esters – animal sources

Plant carotenoids

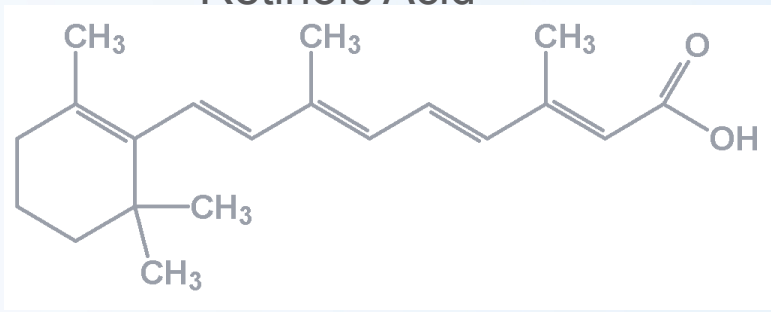
β-karoten



Retinol (vitamin A)



atRA – all trans - Retinoic Acid



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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

Effects caused by retinoid (RAR/RXR) disruptors – see next slide

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

- **Modes of action – disruption of retinoid signalling:**
  - **Metabolization** of retinoids by detoxication enzymes (**PCDD/Fs,** PAHs, PCBs, pesticides
  - Disruption of binding retinoids **to transport proteins** (PCBs and their metabolites)
  - Retinoids as antioxidants may be **consumed by oxidative stress** induced by xenobiotics (general oxidative stress)
  - Interference during **binding to RAR/RXR**
    - pesticides (chlordane, dieldrin, methoprene, tributyltin…)
    - Effect on ATRA mediated response – TCDD, PAHs

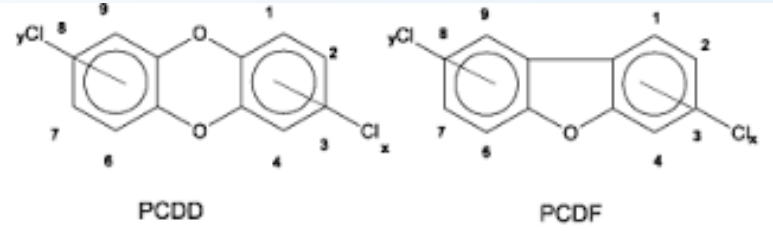

Figure 1. General molecular structure of polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofurans (PCDF)

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