INTRACELLULAR RECEPTORS

The intracellular (nuclear) receptor superfamily

Steroid hormones, thyroid hormones, retinoids and vitamin D

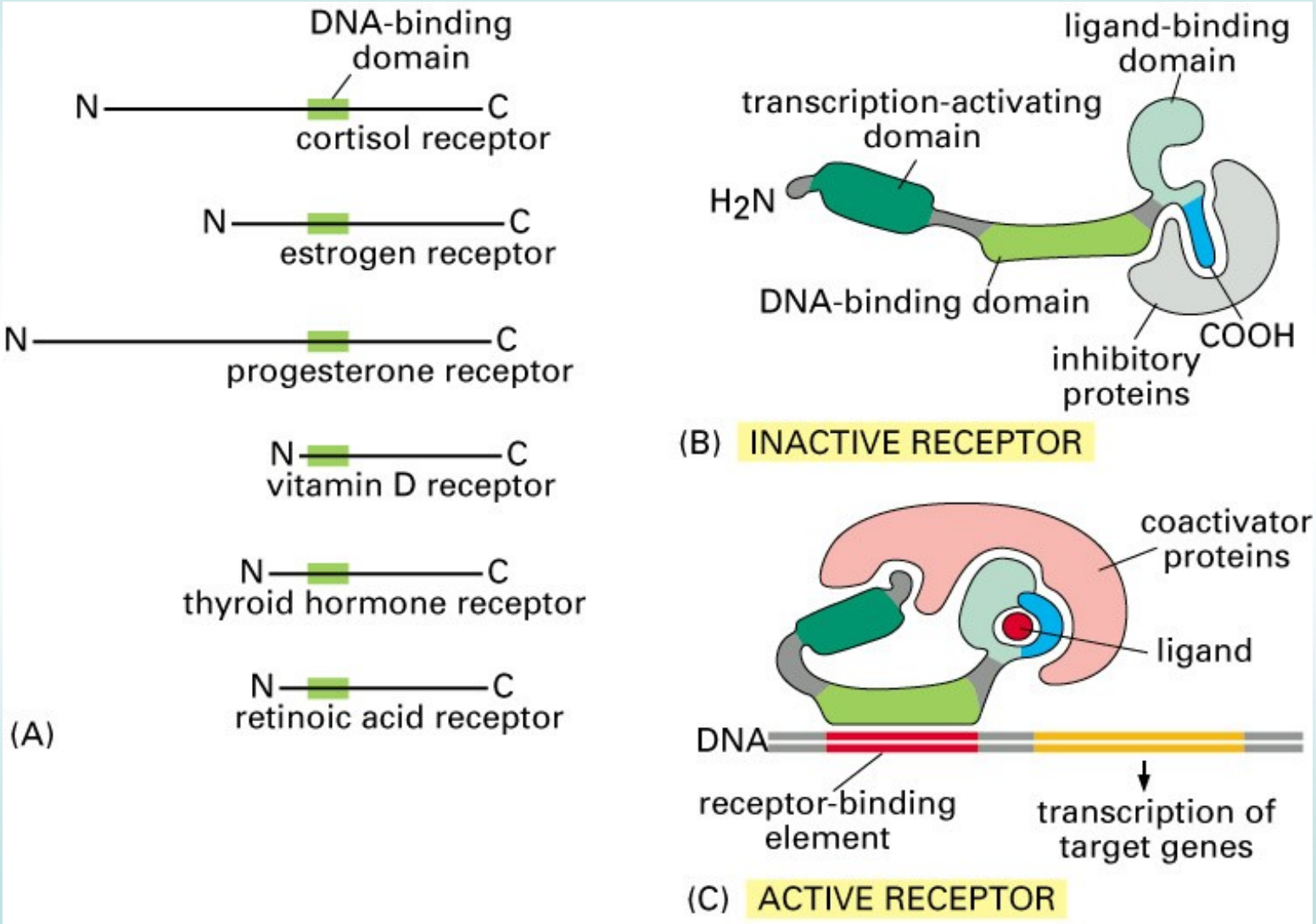
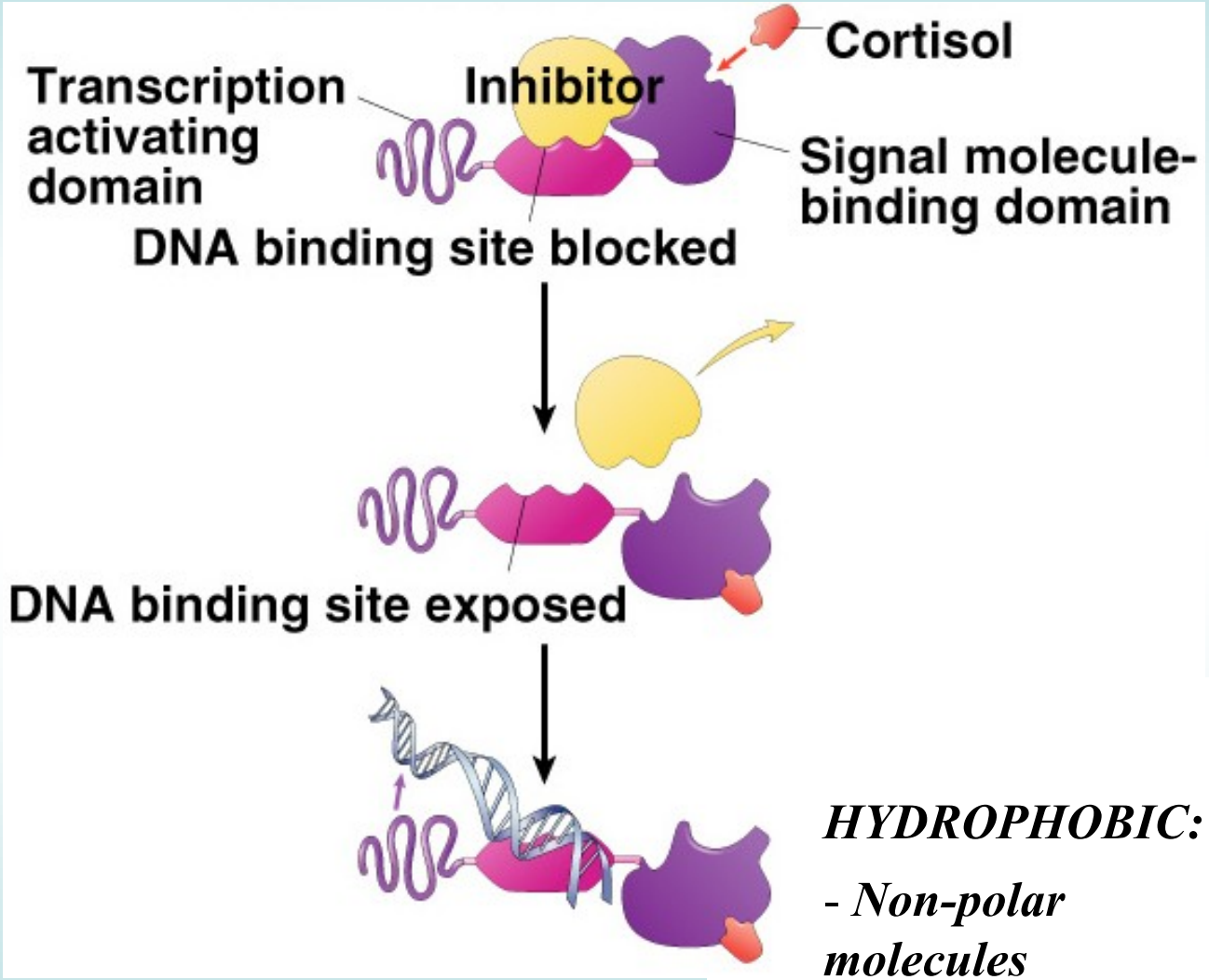


Figure 15–13 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Intracellular receptor

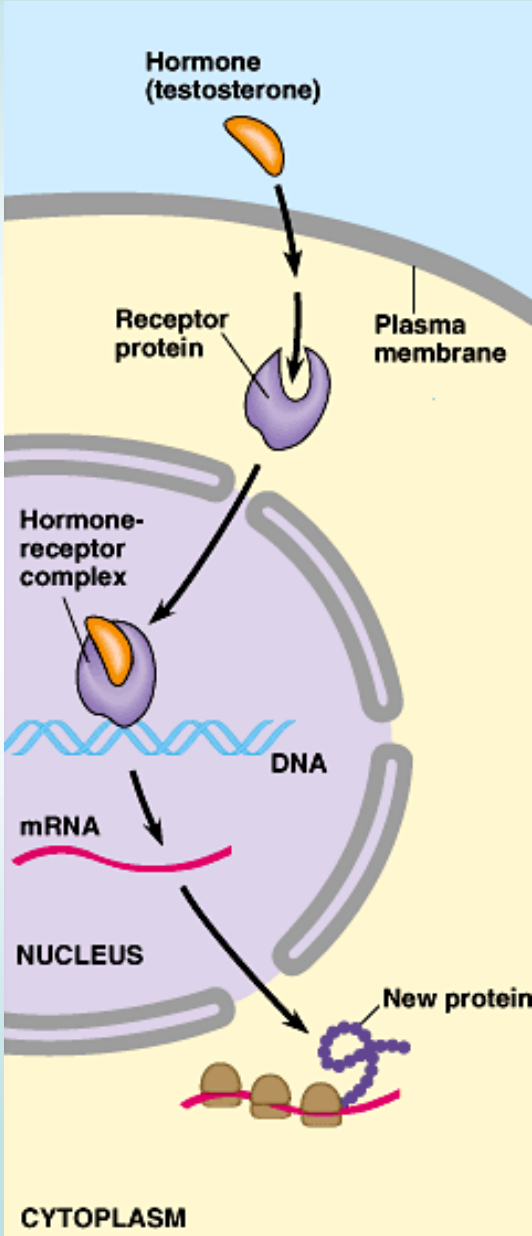


HYDROPHOBIC:

- Non-polar molecules

- Gases

- Steroids



Specificities of some receptors …

- **Steroid hormones** are often required to **dimerize** with a partner to activate gene transcription
- Receptors for vitamin D, retinoic acid and thyroid hormone bind to responsive elements as **heterodimers**
  - Second component of the heterodimer is RXR monomer (i.e, RXR-RAR; RXR-VDR)

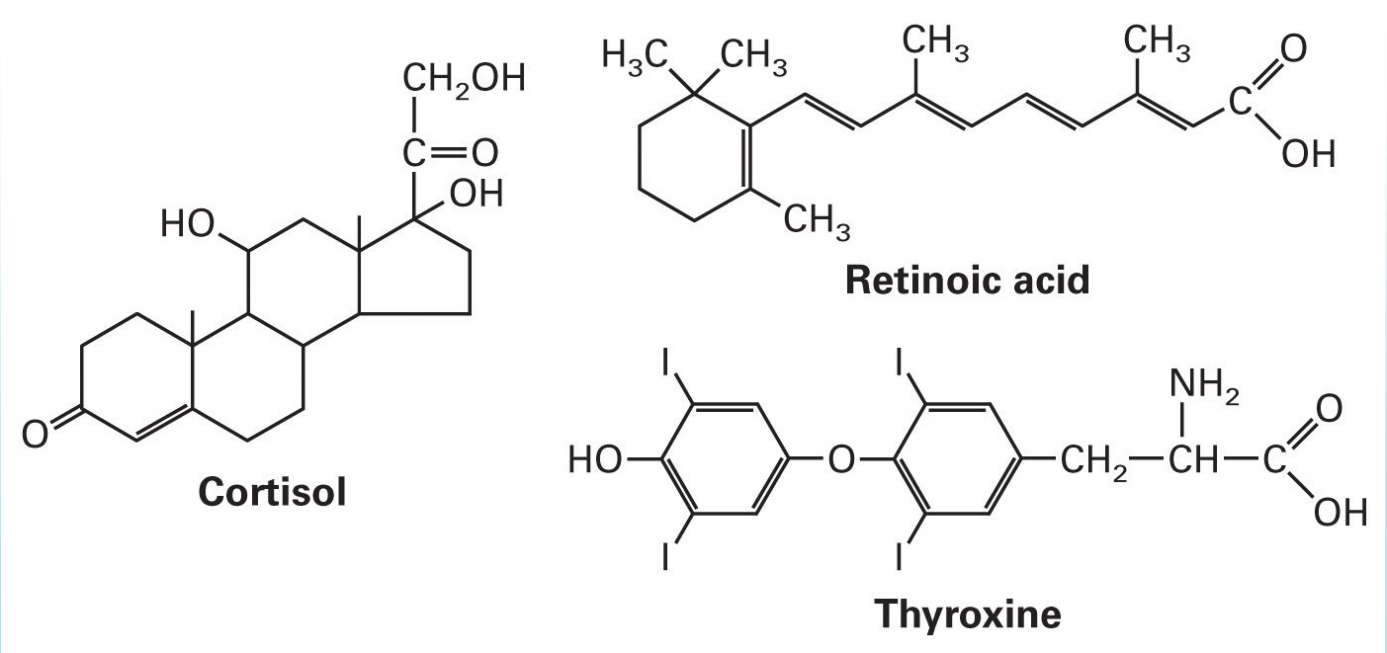
Regulation of transcription activity

- Regulatory mechanisms vary
- Heterodimeric receptors - exclusively nuclear; without ligand, repress transcription by binding to their cognate sites in DNA
- Homodimeric receptors - mostly cytoplasmic (without ligands) & hormone binding leads to nuclear translocation of receptors

- Without ligand - aggregation of receptor with inhibitor proteins (eg Hsp90)

Intracellular signal molecules

- small, lipid-soluble molecules such as steroid hormones, retinoids, thyroid hormones, Vitamin D. (made from cholesterol)
- These molecules diffuse through plasma and nuclear membranes and interact directly with the transcription factors they control.



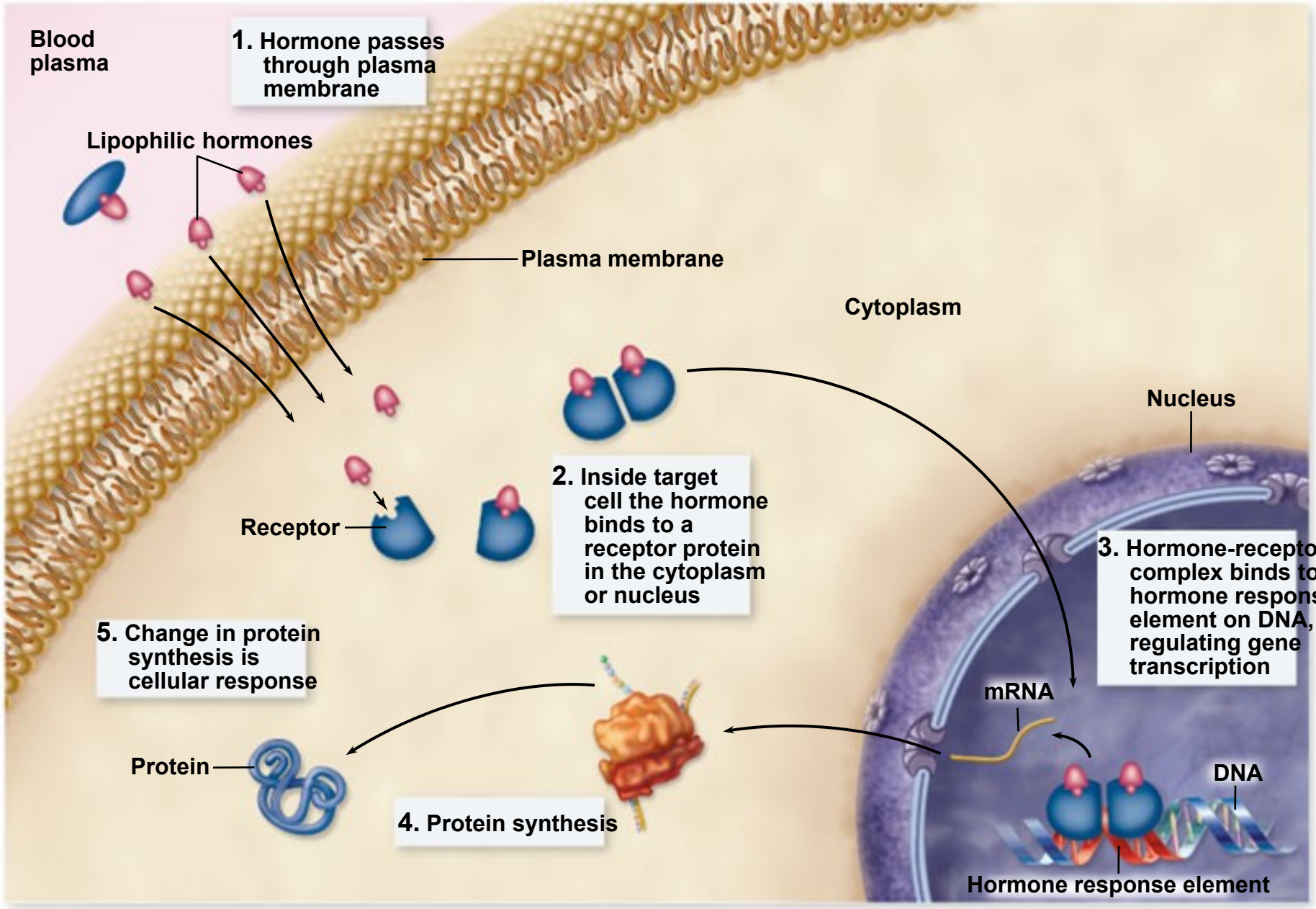
Lipophilic Hormones

Circulation in the blood **bound to transport proteins**

Dissociation from carrier at target cells

Action in the cell

- Pass through the cell membrane and bind to an intracellular receptor, either in the cytoplasm or the nucleus
- Hormone-receptor complex binds to **hormone response elements** in DNA
- Regulate gene expression



Steroid Hormones

STEROID HORMONES:

- sex steroids (estrogen, progesterone, testosterone)
- corticosteroids (glucocorticoids and mineralcorticoids)

OTHER HORMONES

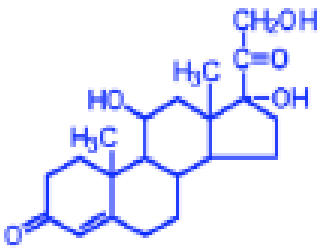
Thyroid hormone, vitamin D3, and retinoic acid have different structure and function but share the same mechanism of action with the other steroids.

Steroid hormones - a review

Steroid hormones are derived from cholesterol metabolism in mitochondria

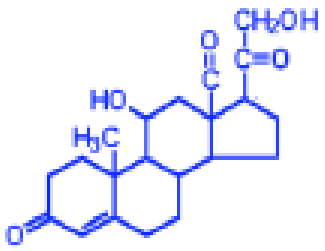
Cortisol

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.



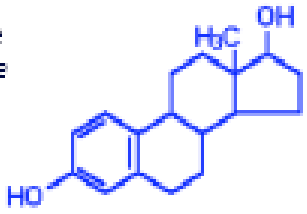
Aldosterone

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



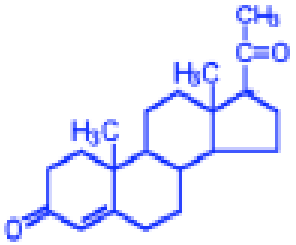
Estradiol

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.



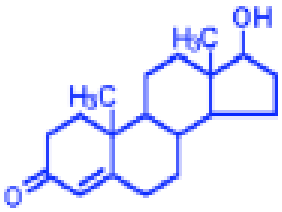
Progesterone

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



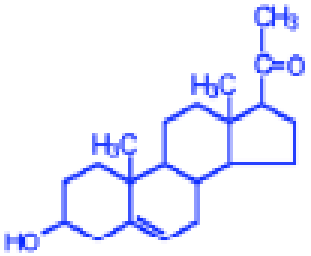
Testosterone

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

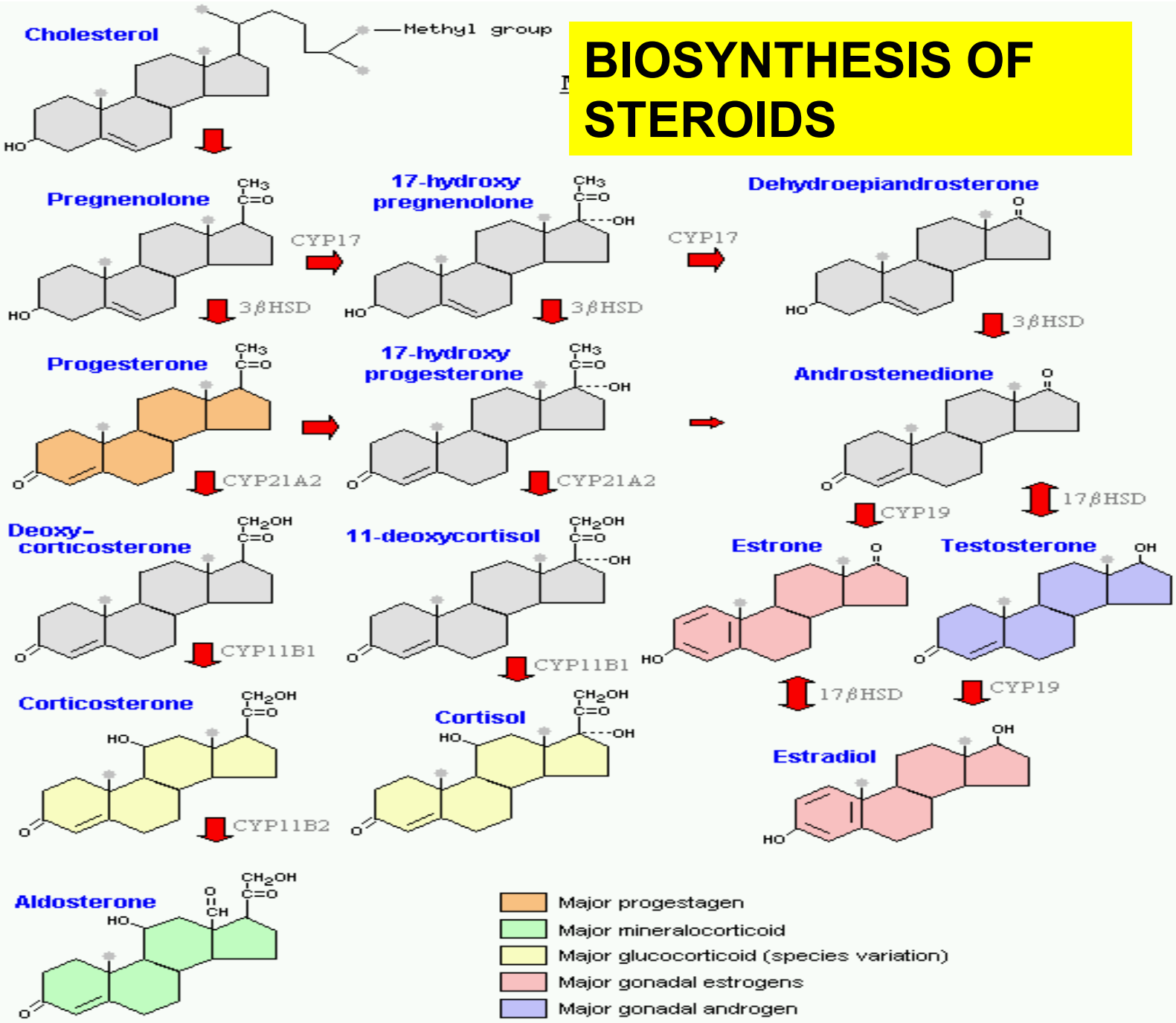


Pregnenolone

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

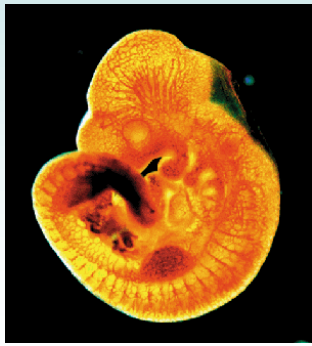


BIOSYNTHESIS OF STEROIDS



Endocrine disruption

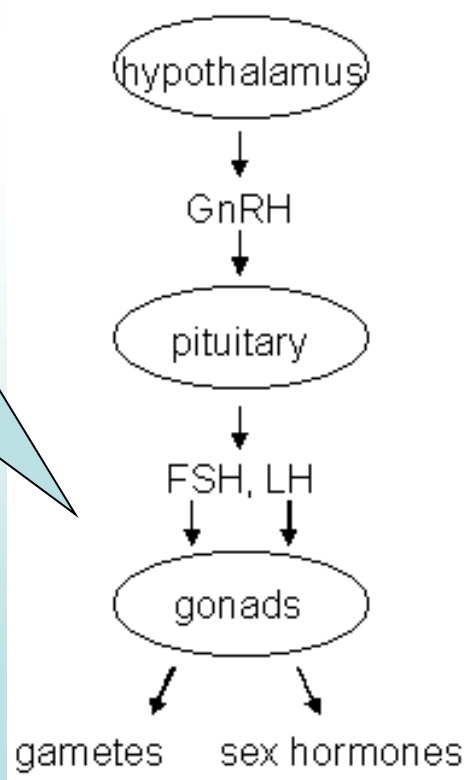
- **Interference of xenobiotics with normal function of hormonal system**



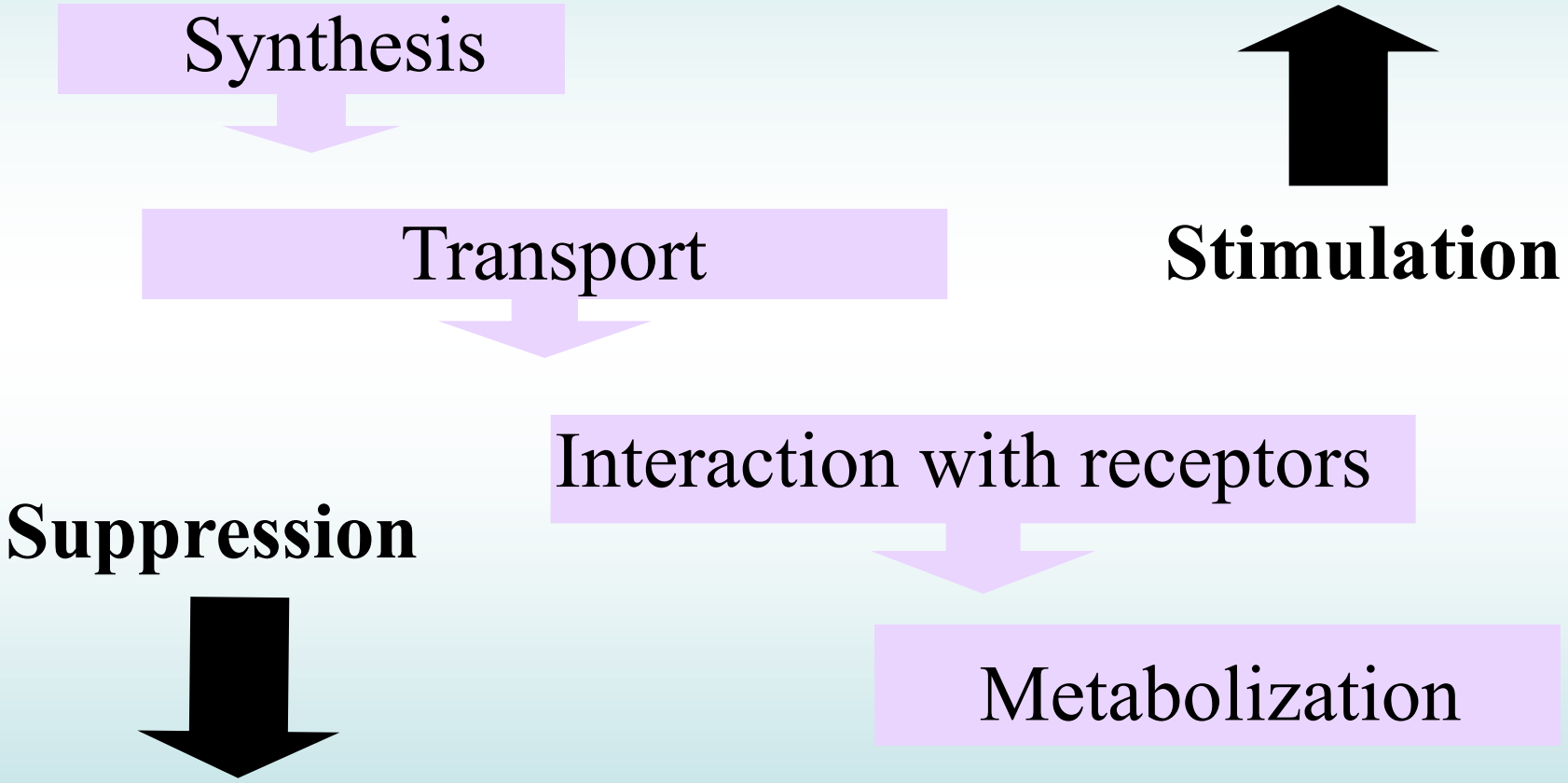
Possible consequences:

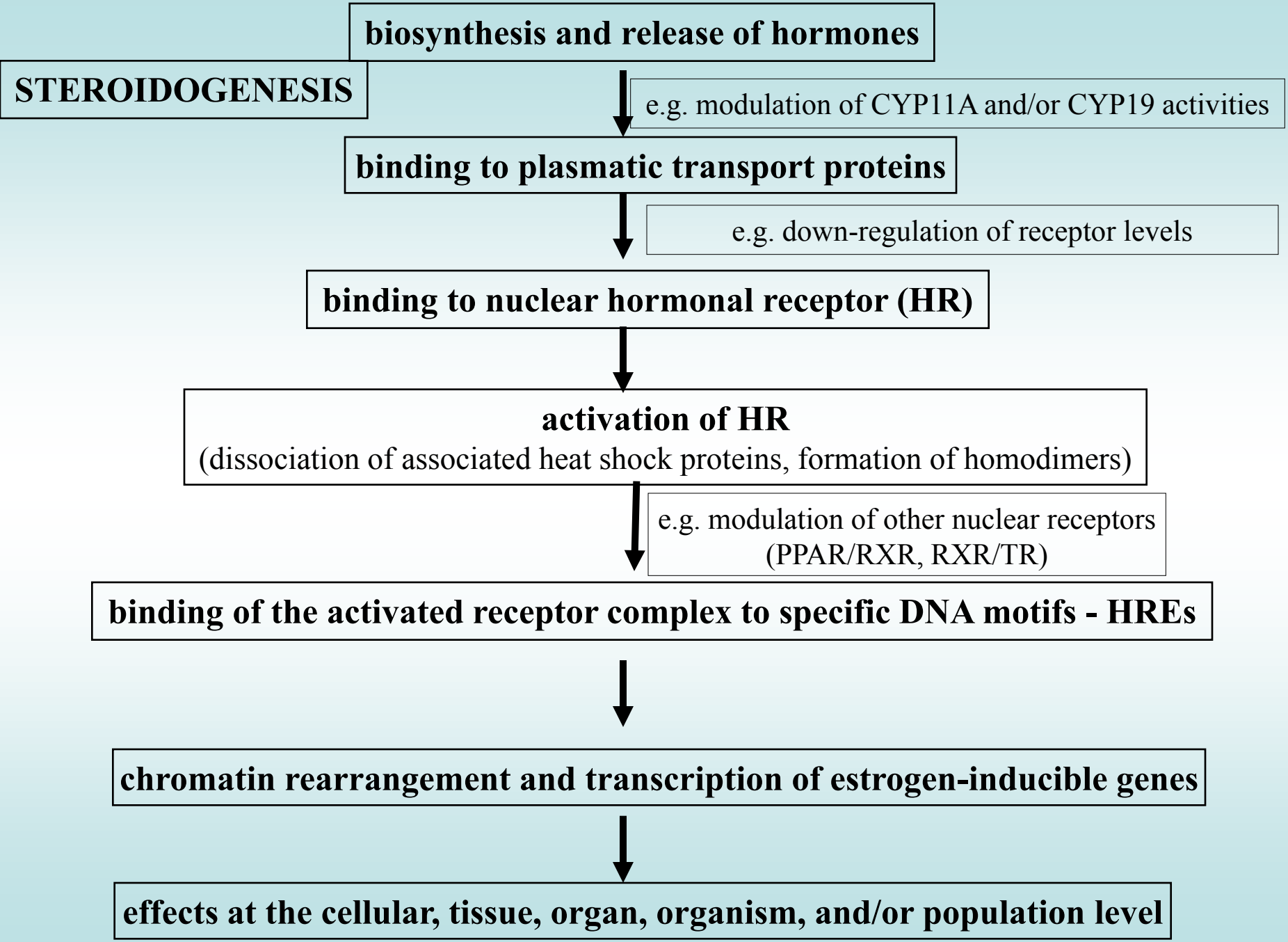
Disruption of homeostasis, reproduction, development, and/or behavior (and oth hormone-controlled processes).

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



Toxicants interact with hormonal system at different levels




biosynthesis and release of hormones
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)
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

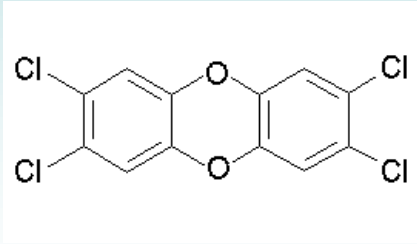
Mechanisms of **steroid hormones signalling disruption**

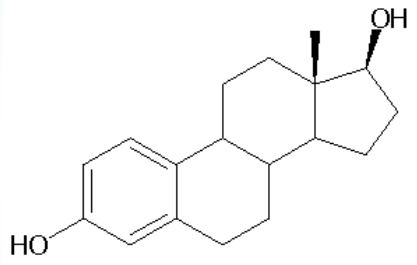
- Nonphysiological activation of hormone receptor (HR)
- Binding to HR without activation
- Decrease of HR cellular levels
- Disruption of the „master“ hormones (FSH/LH)
- Changes in hormone metabolism

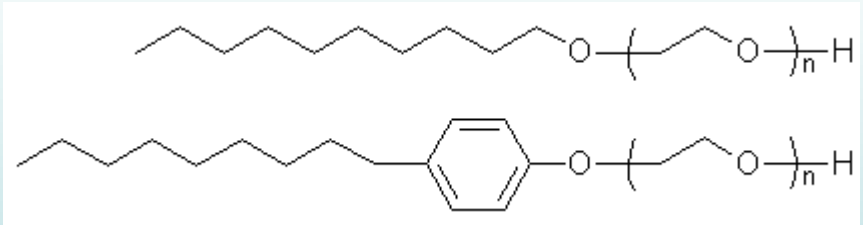
Endocrine disrupters in the environment?

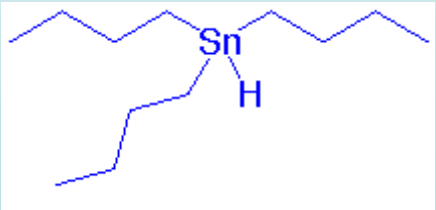
EDCs...

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



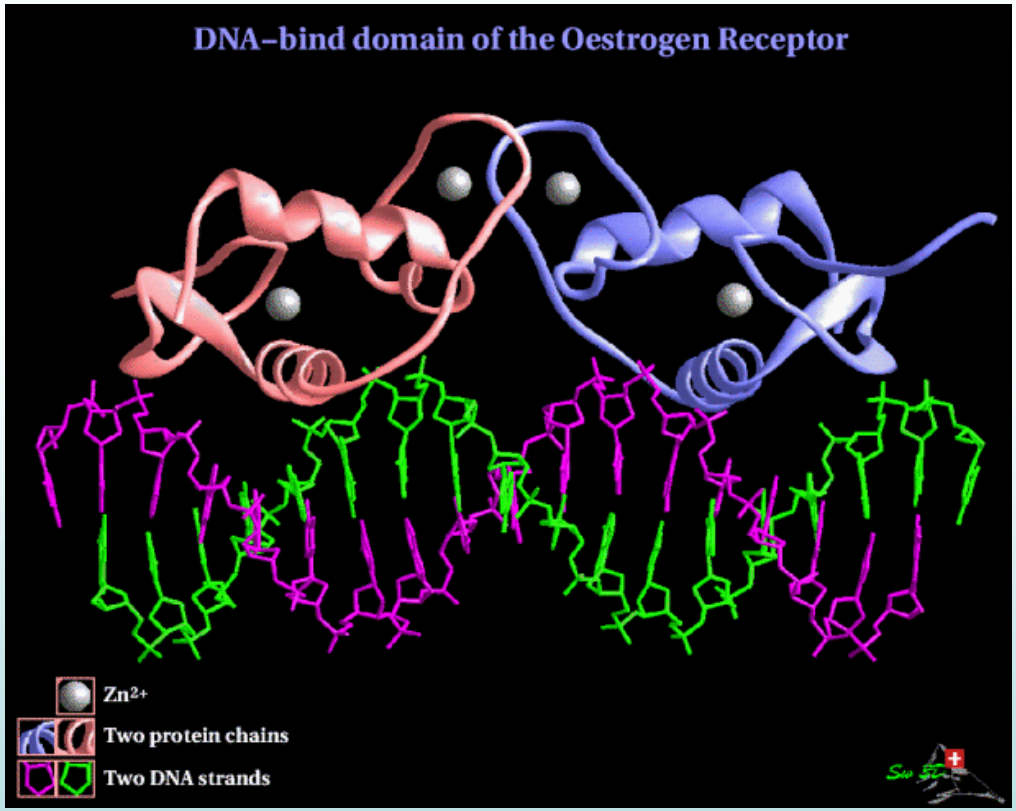




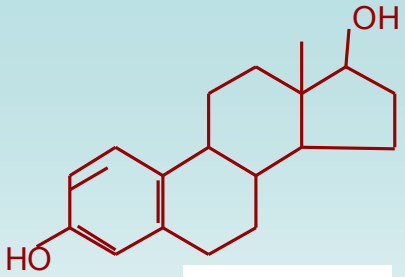


ESTROGEN RECEPTOR – ER

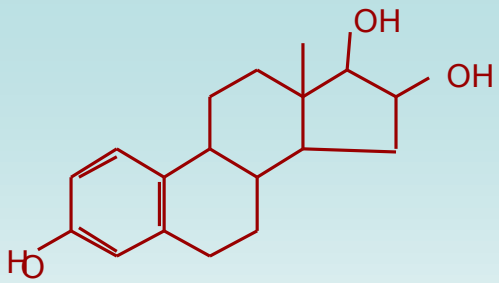
the most studied target of EDCs



Estrogens:



17-β-estradiol



estriol

- play a key role in female hormone regulation and signalling
- are responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
- are involved in the growth, development and homeostasis of 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

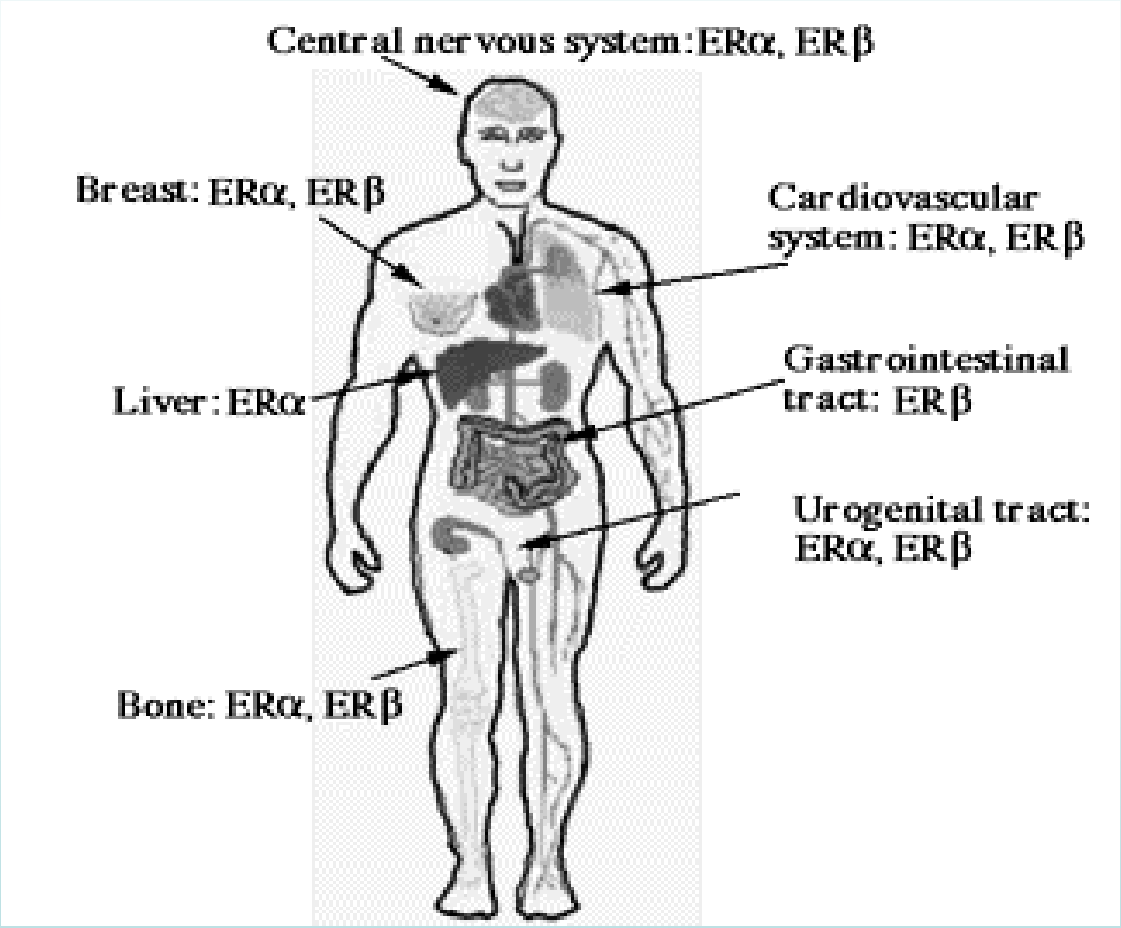
- **<u>Synthesis in ovaries</u>**

- DISRUPTION -> investigated in aquatic biota & laboratory organisms (see notes on EDCs)

ESTROGEN RECEPTORS - ER-α & ER-β:

subtype: 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)

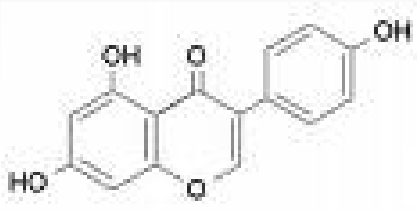


Environmental estrogens (xenoestrogens, exoestrogens)

a diverse group of substances that do not necessarily share structural similarity to the prototypical estrogen (17β-estradiol)
May act as AGONISTS and/or ANTAGONISTS

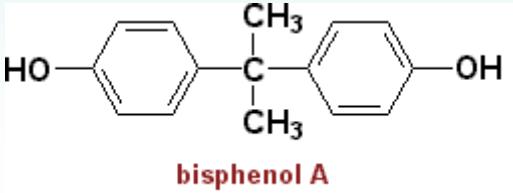
Natural products

genistein
naringenin
coumestrol
zearalenone

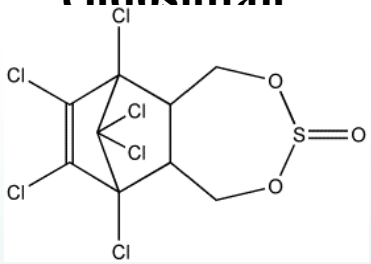


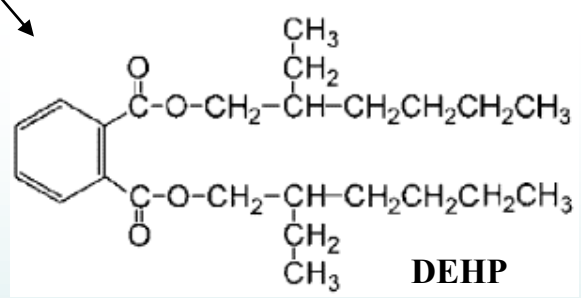
Industrial chemicals

Bisphenol A
Nonionic surfactants
Pthalate esters
endosulfan



bisphenol A





Environmental pollutant

DDT
kepone
PCBs/OH-PCBs
PAHs and dioxins

Pharmaceuticals

Ethinyl estradiol
Diethylstilbestrol
gestodene
norgestrel

Exoestrogens - Relative Potencies to bind to ERα (REPs)

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

Toxicity assessment
number of in vivo and in vitro methods

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.

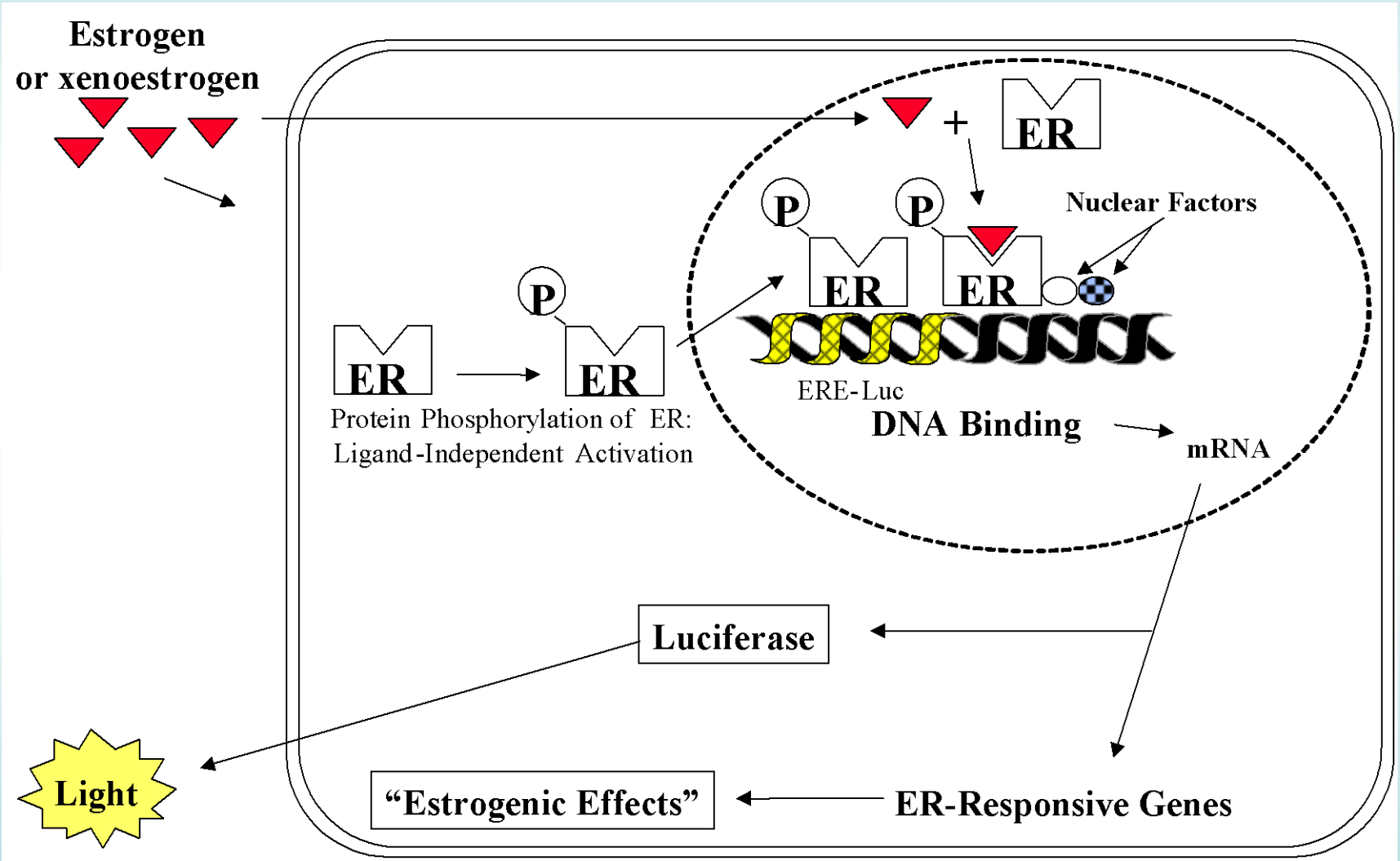
In vitro assays

- **INTERACTION (BINDING) to the receptor**
  - competitive ligand binding assay
  - *Effect unknown (? Activation / suppression / no effect ?)*

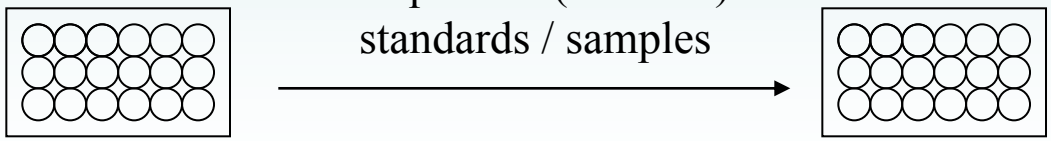
- **Testing the effect at cellular level (interference with receptor biological activity)**
  - cell proliferation assay
  - endogenous protein expression (or enzyme activity) assay
  - reporter gene assay

In vitro ER- mediated effects

luciferase reporter assay



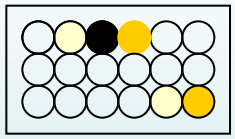
ER- mediated effects
luciferase reporter assay

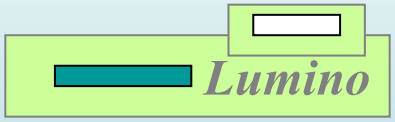


96 microwell plate
cultivation of transgenic cell lines

ER: breast carcinoma MVLN cells

Cell lysis
-> extraction of induced luciferase





Luminescence determination
(microplate luminescence reader)

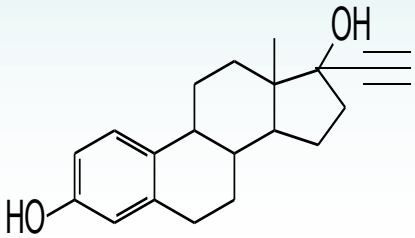
SIMILAR DESIGN FOR OTHER RECEPTORS
(discussed below):
AhR (H4IIE.luc cells)
AR (MDA cells)
RAR/RXR (P19 cells)

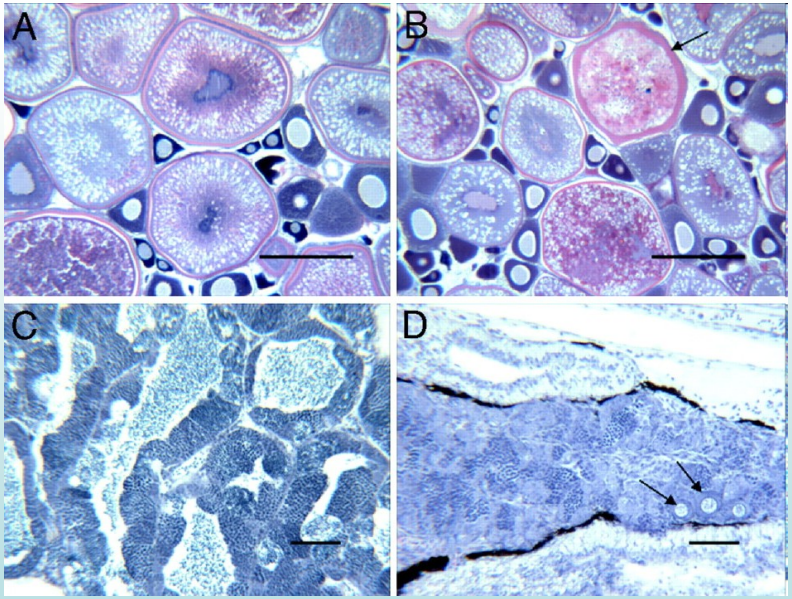
In vivo assays

- uterotropic assay
- vaginal cornification assay
- standard test procedures for reproductive and developmental toxicity (e.g. FETAX)
- production of estrogen-inducible proteins (e.g. vittelogenin and zona radiata protein)

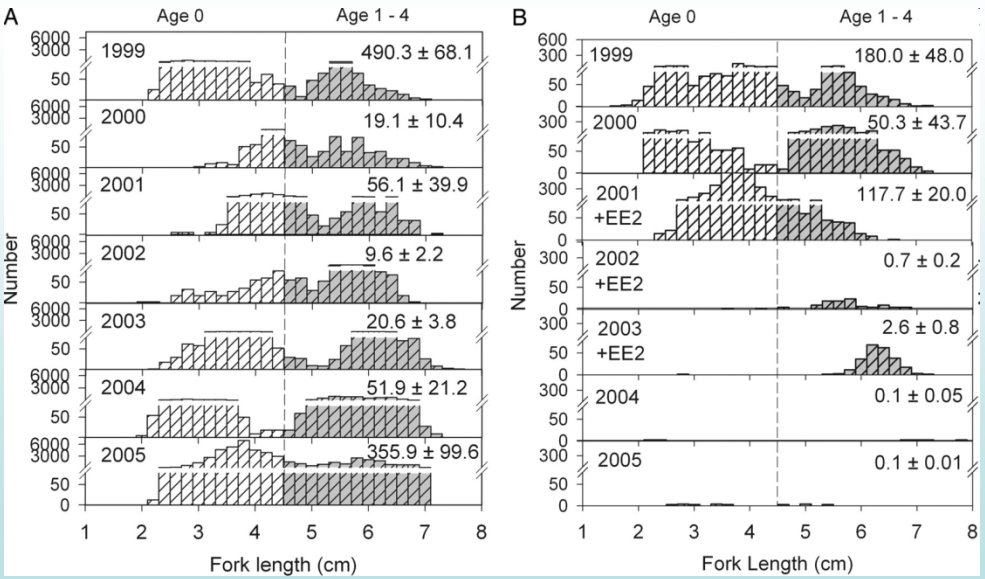
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years





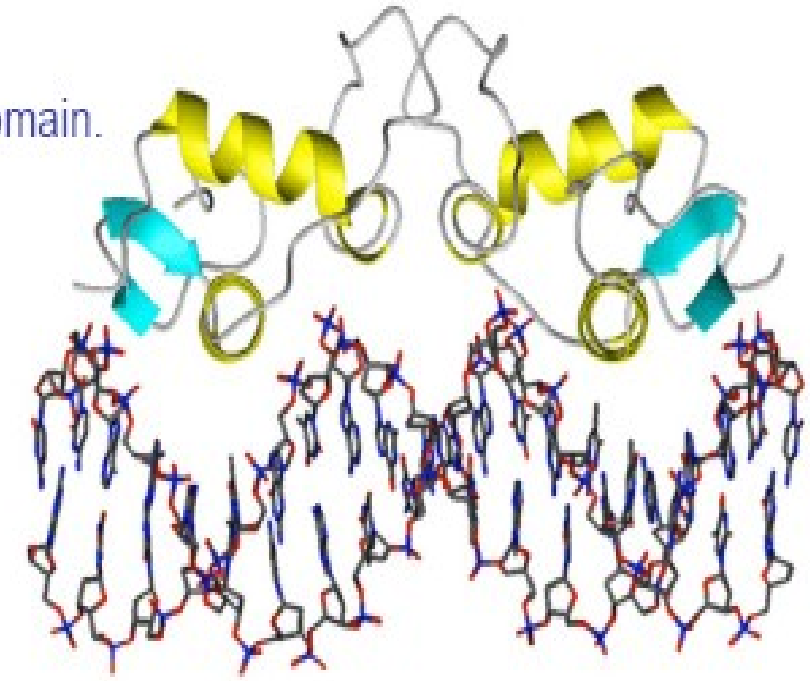
Controls **+Ethinylestradiol**



ANDROGEN RECEPTOR (AR)

effects known but less explored than ER

Androgen receptor DNA binding domain.

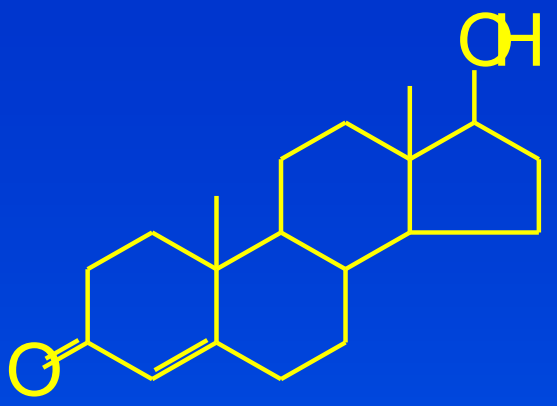


Androgens

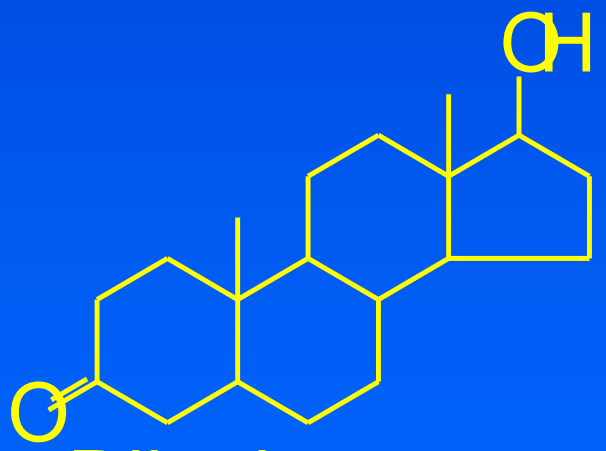
- **Role in males similar to the of estrogens in females**
  - development of male sexual characteristics
  - stimulating protein synthesis, growth of bones
  - cell differenciation, spermatogenesis
  - male type of behaviour

Androgens

- Endogenous ligands – androgen hormones
  - **testosterone (T)**
  - **dihydrotestosterone (DHT)**
  - androstanediol
  - dehydroepiandrosterone
  - androstenedione



Testosterone



Dihydrotestoste

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

Mechanisms of androgen signalling disruption

1) Binding to AR

- Mostly competitive inhibition

  - xenobiotics mostly DO NOT activate AR-dependent transcription

-Only few compounds are able to activate AR in the absence of androgen hormones, and these are also anti-androgenic in the presence of T/DHT (**metabolites of fungicide vinclozoline**, some PAHs)

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

- FSH/LH expression - regulation via negative feedback by testosterone

- Suppression leads to alterations of spermatogenesis

Mechanisms of androgen signalling disruption

3) Alterations of testosterone synthesis

- Inhibition of P450scc needed for side chain cleavage of cholesterol (fungicide **ketoconazol**)

- Inhibition of 17- α-hydroxylase and other CYPs - – enzymes needed for testosterone synthesis (**ketoconazol**)

4) Testosterone metabolic clearance

- Induction of UDP-glucuronosyltransferase or monooxygenases CYP1A, 1B involved in androgen catabolism

- Pesticides **endosulfan, mirex, o-p´-DDT**

Effects of male exposure 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

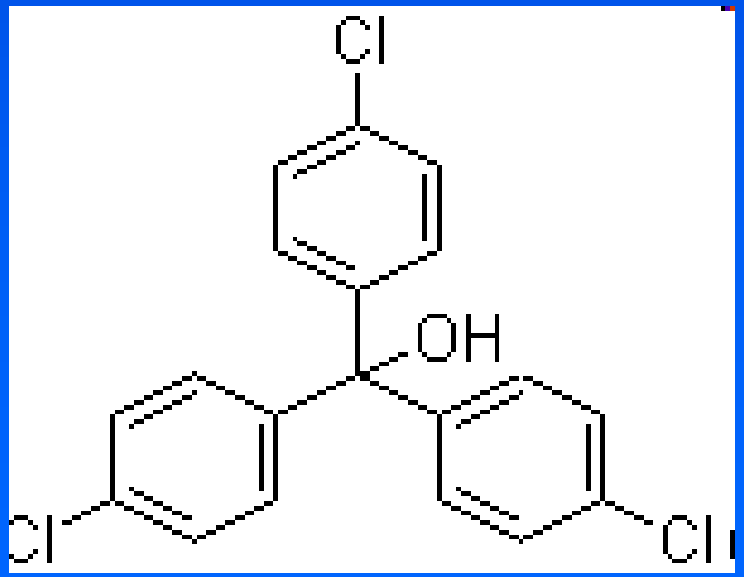
Exposure in **adult** age:

- oligospermia
-azoospermia
-libido diminution

Antiandrogenic compound

tris-(4-chlorophenyl)-methanol

- Ubiquitous contaminant of uncertain origin
- Probable metabolite of DDT-mixture contaminant
- Levels in human blood serum cca. 50nM
- EC50 – cca. 200nM



AR-binding - potencies

(Ref: DHT EC50 ~ 0.1 uM)

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

(*Anti*)androgenicity assessment

In vivo Hershberger assay

- castrated rats treated with examined substance
- Endpoint – after 4-7 days – seminal vesicles and ventral prostate weight

In vivo measurement of testosterone blood levels

In vitro **cell proliferation** assays

– cell lines with androgen-dependent growth
- mammary carcinoma cell lines
- prostatic carcinoma cell lines

¨Receptor-reporter assays

Gene for **luciferase** (or GFP) under control of AR
AR-CALUX (human breast carcinoma T47D)
PALM (human prostatic carcinoma PC-3)
CHO515 (Chinese hamster ovary CHO)

Yeast transfected cells
beta-galactosidase reporter

Treatment:
tested chemical only
-> androgenicity
Cotreatment with DHT
->
antiandrogenicity

Thyroid hormones

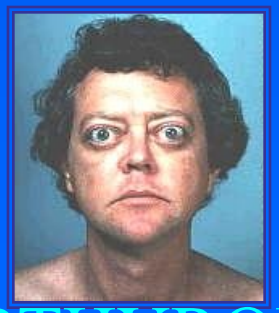
Thyroid hormones

Play crucial roles in stimulating 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

HYPOTHYROIDISM



HYPERTHYROIDISM

Thyroid hormones

Thyroxine (T4)

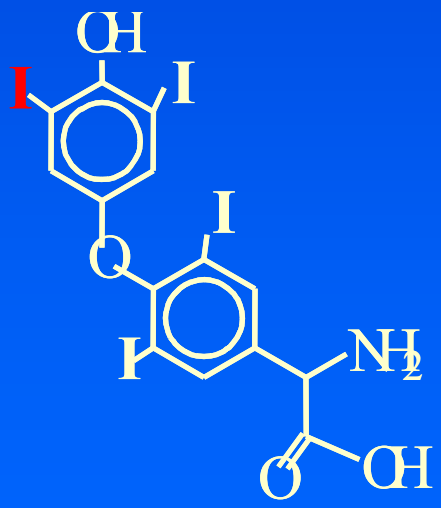
Also called tetraiodothyronine
Contains 4 iodide ions

Triiodothyronine (T3)

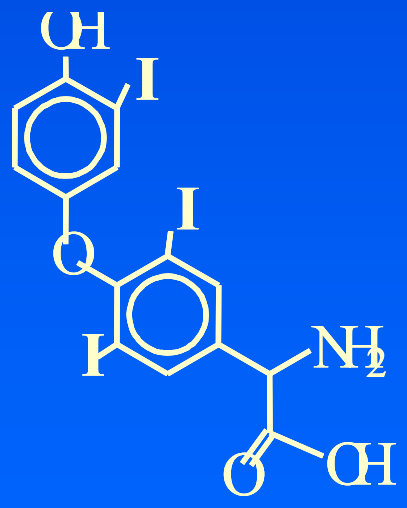
Contains 3 iodide ions

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

- T4 – prohormone
- 5′-deiodination leads to active form, T3

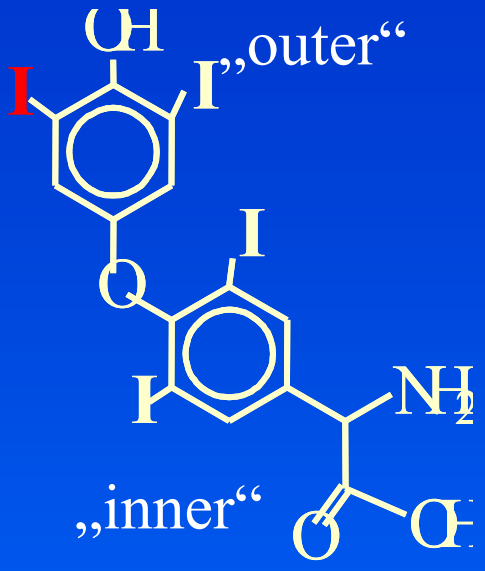


Thyroxine (T4)



3,5,3′-Triiodothyronine (T3)

Enzymes involved in thyroid metabolism



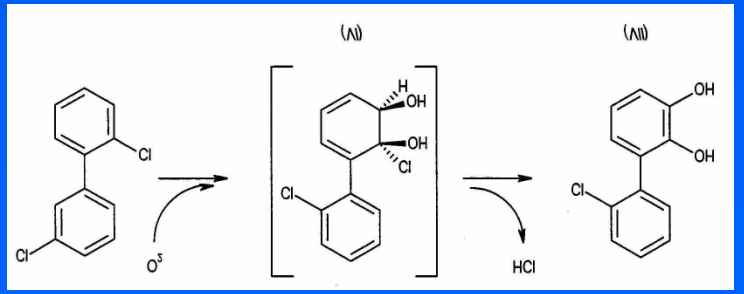
- **Thyroid peroxidases**
  - iodination of tyrosyl residues
  - coupling of iodinated tyrosyl residues
- **Thyroid deiodinases**
  - D1, D2 - activation of T4 into T3 via deiodination on „outer“ ring
  - D3 - deactivation into rT3 via deiodination on „inner“ ring

EDCs -> may affect metabolism of these key enzymes

Thyroid hormones are transported in the blood by **thyroid binding proteins**

- Regulating free T4 and T3 levels in blood
- 3 types :
  - -Thyroid-binding prealbunin (transthyretin) (20-25%)
  - -Albumin (5-10%)
  - -Thyroid binding globulin (75%)

- **NUMBER OF ENVIRONMENTAL TOXICANTS act at transport proteins**
  - -OH-PCBs, brominated and chlorinated flame retardants, DDT, dieldrin
  - -OH-PCBs – equal affinity to TBP as T4 and T3 (!!!)



- More of free T4 in blood

⇒negative feedback to TSH release

=> increased depletion

=> increased weight, histological changes in thyroid gland

Observed after exposure to POPs in mammals, birds, fish

Other possible effects of EDCs on Thyroid signalling

Competitive binding to TR

- **Probably less important than binding to TBP**
- Chemicals that affect thyroid signalling in vivo mostly don´t bind to TR (DDT, PCBs) or bind with much lesser affinity than T3 (OH-PCBs – 10000x)

Accelerated depletion of TH

- UDP-glucuronosyltransferase – detoxication enzyme (II.biotransformation phase)
  - Induced by PCBs, dioxins
  - Key enzyme in thyroid catabolism
- Increased by disruption of TBP binding

Effects of thyroid disruption

- nervous system fails to develop normally
- mental retardation
- skeletal development

<u>Disruption during prenatal development</u>

- severe damage of CNS (cretenism, delayed eye opening, cognition)
- Megalotestis
- Histological changes in thyroid gland (goitre)

Assessment of effects

- **In vivo approaches**
- **TH serum levels** – simple, nondestructive x variation within time of day, age, sensitive to other than biochemical stresses
- Thyroid gland weight and folicular cells number
- Developmental toxicity assays - delayed eye opening, abnormalities in brain development and cognition, increased testis weight and sperm counts
- Perchlorate discharge test (TH synthesis)
- Hepatic UDP-glucuronosyltransferase activity (marker of enhanced TH clearance from serum)

- **In vitro**
- Enzyme inhibition assays (thyroid peroxidase, deiodinases) – assessment of thyroid metabolism
- Competitive binding assays with TBP
- TH- dependent proliferation assay (pituitary tumor GH3, thyroid tumors like FRTL-5 cell line) or TSH-dependent proliferation assay (thyroid tumors)
- Receptor-reporter gene assays with luciferase (monkey kidney CV-1, chinese hamster ovary CHO or insect Sf9 cell lines)

Retinoids

Vitamin A and its derivatives

Toxicants affect retinoid action but effects are much less explored

Retinoids

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

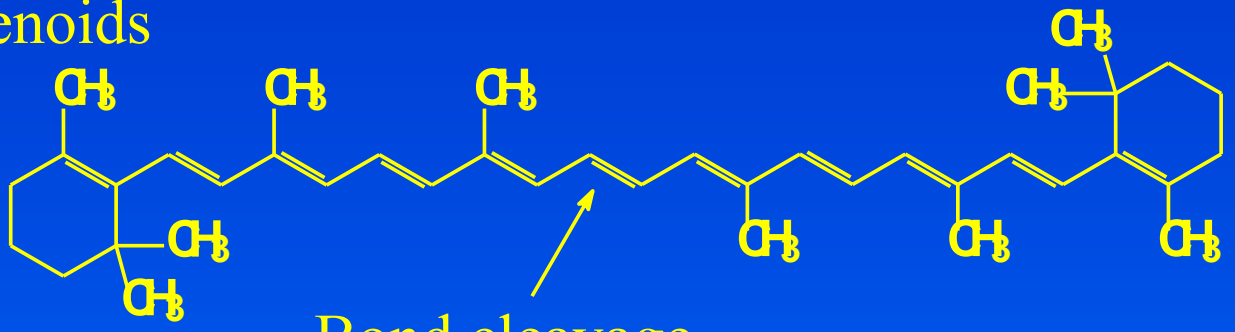
Antioxidative agent

Affect nervous and immune function

Retinoids

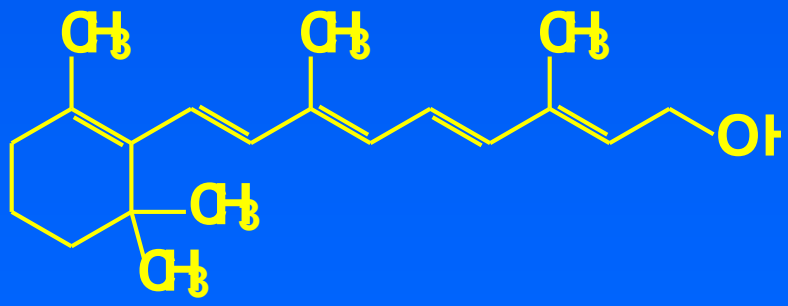
Sources: from diet (dietary hormones)
Retinyl esters – animal sources
Plant carotenoids

β-karoten

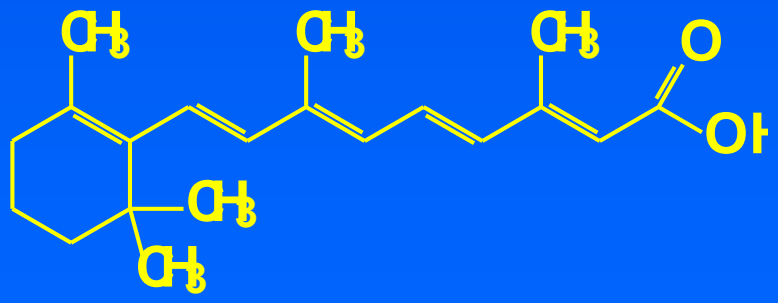


Bond cleavage

Retinol (vitamin A)



Retinoic Acid



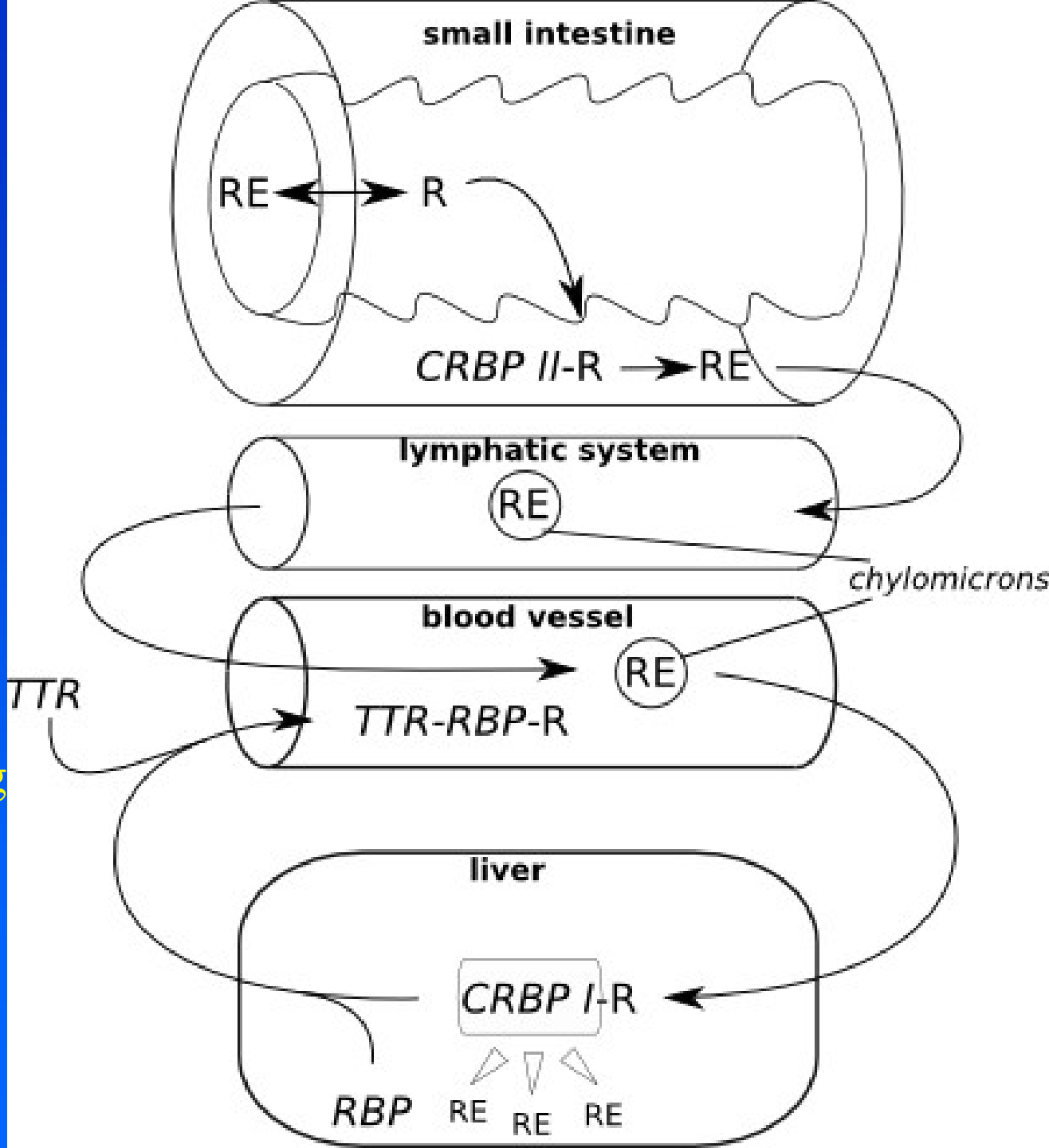
TRANSPORT OF RETINOIDS

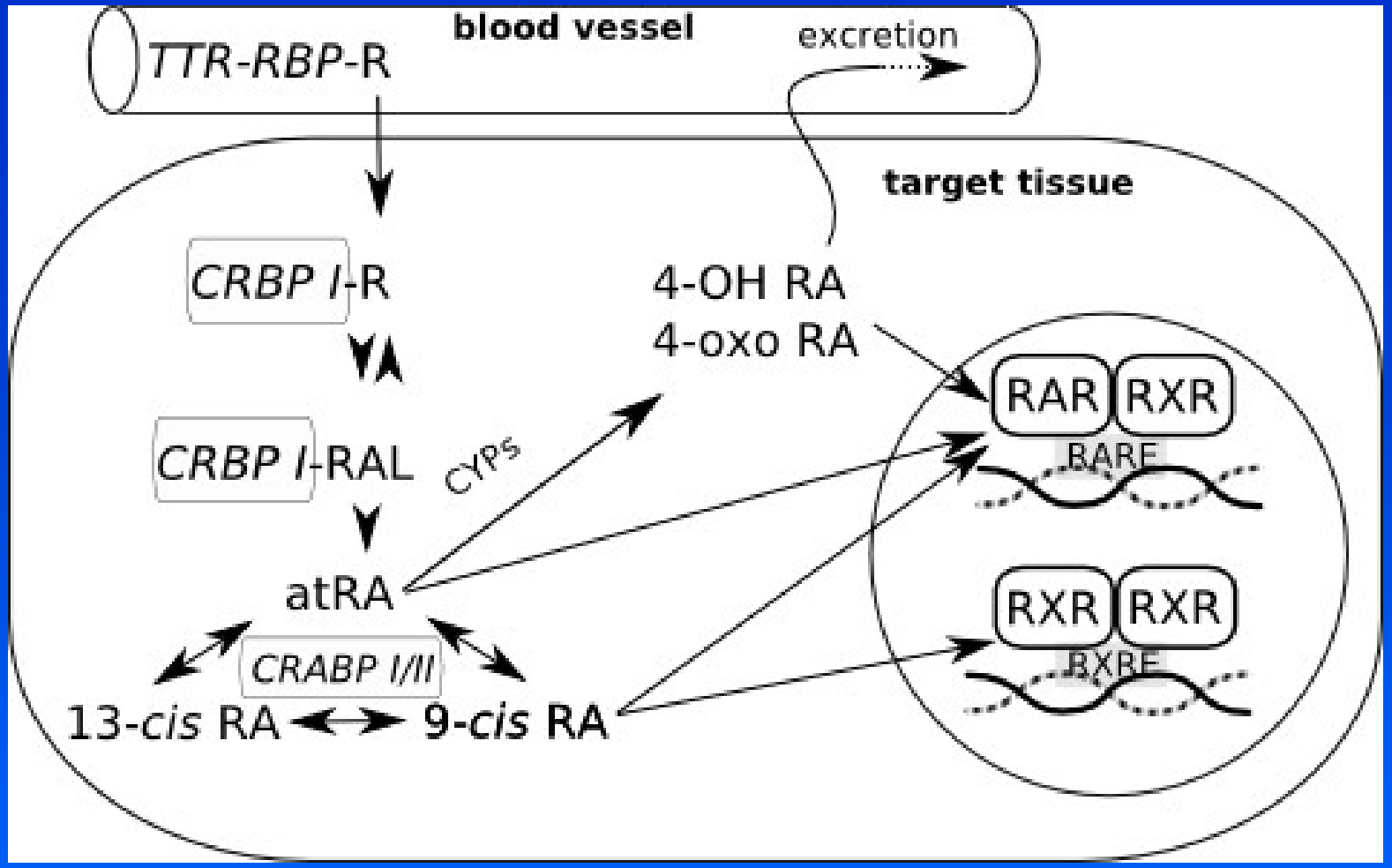
RE: Retinol-Ester

R: Retinol

RBP: Retinol Binding Protein (*LMW*)

TTR: Transthyrethin (*HMW*)





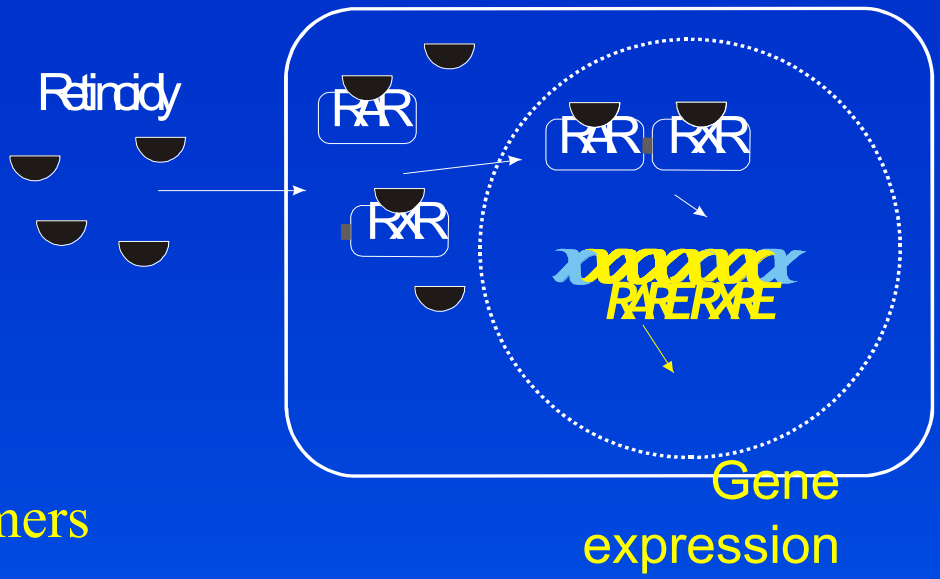
Retinoid binding proteins

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

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

RAL - Retinal

Mode of action



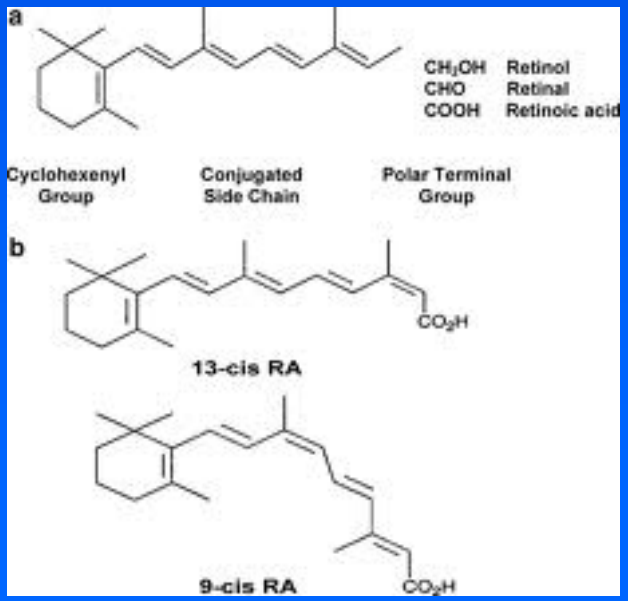
- Isoforms of RAR a RXR
- Both have isoforms α, β and γ, each of them several subtypes
- Formation of homo- and heterodimers
- 48 possible RAR-RXR heterodimers

=>sensitive regulation of gene expression

- RXR – heterodimers even with other receptors like VDR, TR, PPAR

Retinoic acid

- 3 basic subtypes
- all-trans-, 9-cis- and 13-cis-retinoic acid
- All-trans RA binds selectively to RAR
- Cis RA bind to both receptor types



Disruption of retinoid signalling by xenobiotics

- **Relatively little known**

- Possible modes of action:
  - Metabolization of retinoids by detoxication enzymes
  - Disruption of binding retinoids to retinoid binding proteins
  - Retinoids as antioxidants may be consumed cause of oxidative stress caused by xenobiotics
  - Interference of chemicals (binding to RAR/RXR)

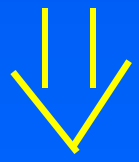
Consequences of retinoid signalling disruption

Decreased retinoid levels in organisms

- Downregulation of growth factors
- Xerophtalmia, night blindness
- Embryotoxicity, developmental abnormalities

X

Increased ATRA concentration – teratogenic effect



Change may cause severe developmental anomalies (both excess and deficiency)

Disruption of retinoid signalling by xenobiotics

Polluted areas – mostly decrease of retinoid levels 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
- in kidney, concentration of all forms elevated

How to assess retinoid signalling disruption?

In vivo

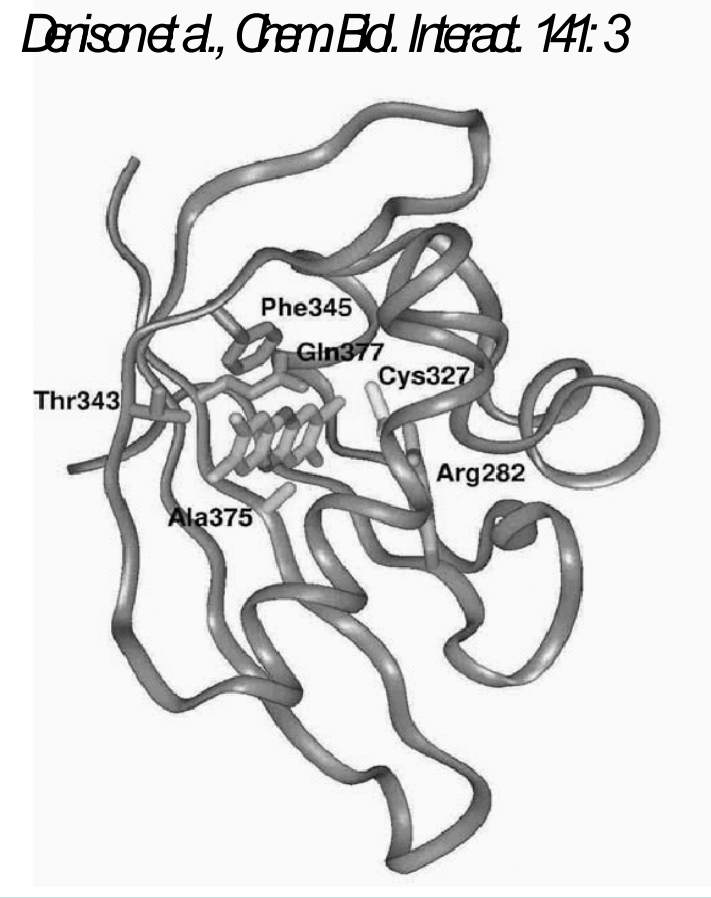
- Mostly derived from classical toxicity tests, particularly of developmental toxicity
- Direct measurements of various retinoid forms in living organisms (laboratory and wildlife)

In vitro

- Mostly epithelial cell lines (keratinocytes)
- Mouse embryonic cell lines P19
  pluripotent cells
  differentiation dependent on circumstances, triggered by ATRA
  - reporter gene assay P19/A15
  - Other cell lines – rainbow trout gonads, human salivary gland, breast or prostatic carcinomas etc.

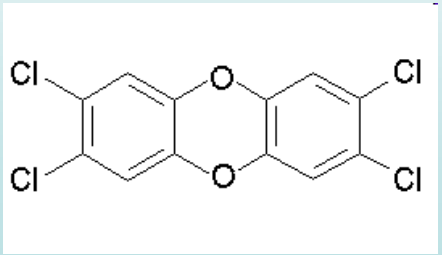
AhR (Arylhydrocarbon receptor)

AhR structure

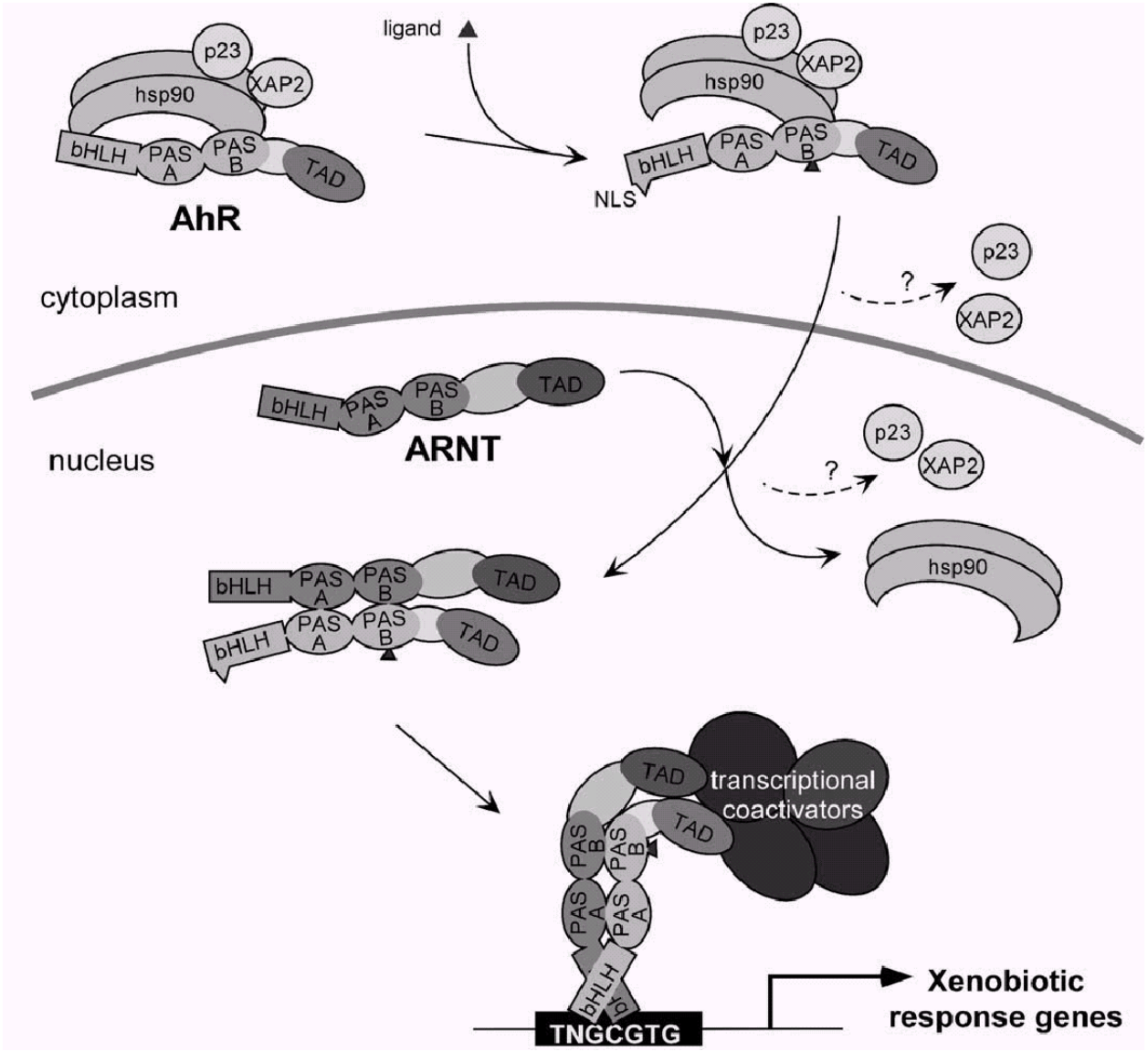


AhR

- ligand-activated transcription factor
- activation of different responsive elements (genes)
- important mediator of toxicity of POPs – primary target of coplanar aromatic substances
- regulator of xenobiotic metabolism and activation of promutagens
- crossactivation/crosstalk with other receptors
- strongest known ligand TCDD



AhR activation:

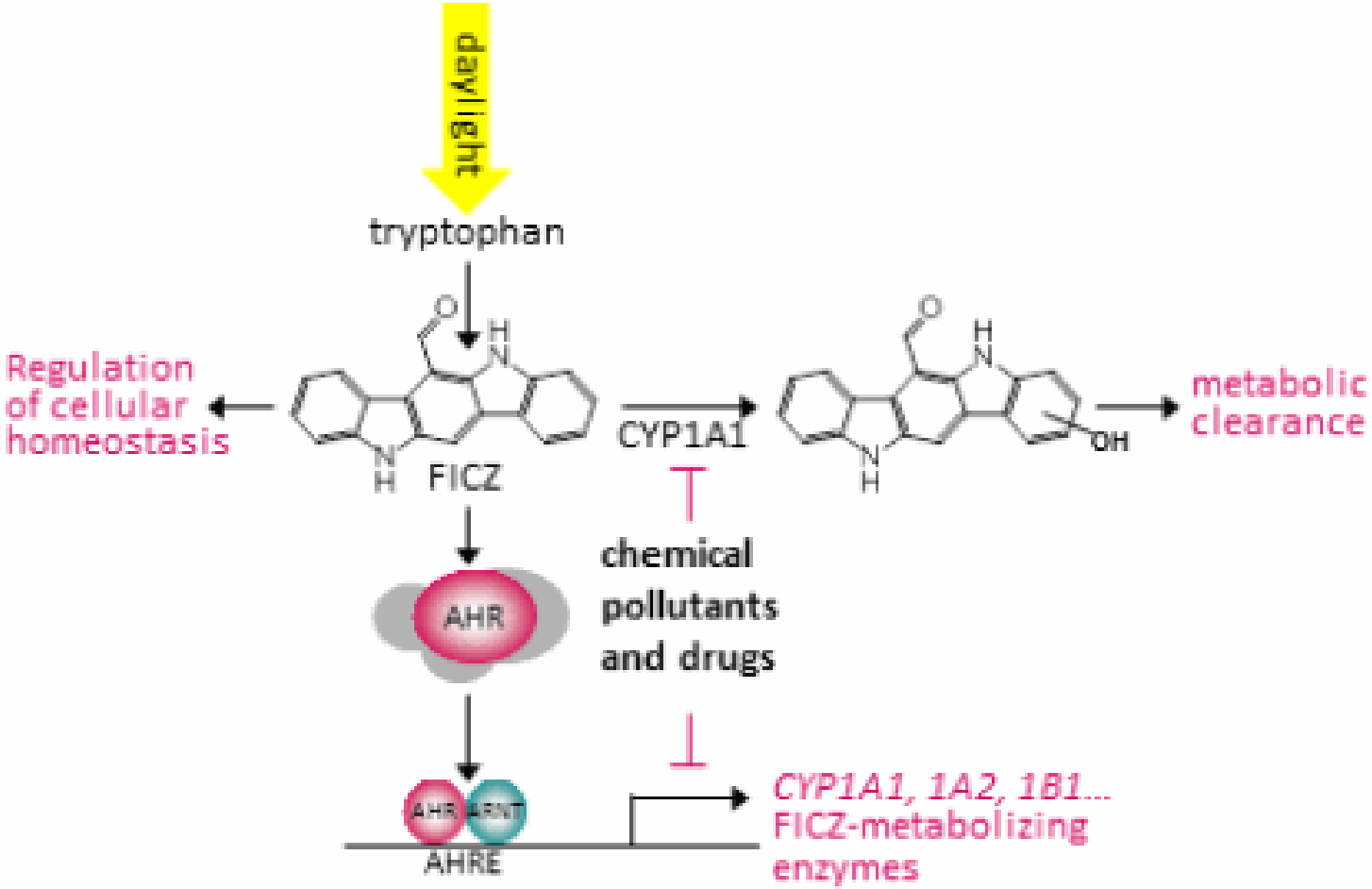


AhR regulated genes:

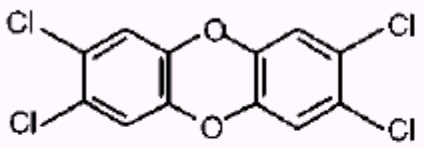
contain xenobiotic response elements (XRE) or dioxin responsive elements (DRE) in their promoter region:

- **phase I enzymes - *CYP 1A1, CYP 1A2, CYP 1B1;***
- **phase II enzymes - *UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase;***
- **other genes - *Bax, $p27^{Kip1}$, Jun B, TGF-β* - regulation of cell cycle and apoptosis;**

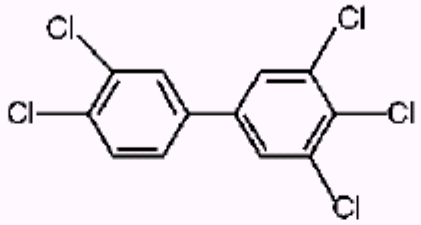
6-formylindolo[3,2-b]carbazole (FICZ)
potent endogenous physiological (natural) ligand of AhR



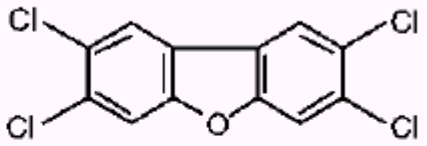
"Classical" AhR Ligands and CYP1A1 Inducers



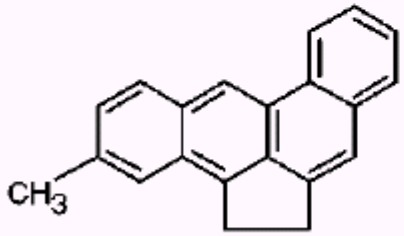
2,3,7,8-Tetrachlorodibenzo-p-dioxin



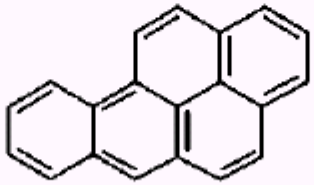
3,4,3',4,'5-Pentachlorobiphenyl



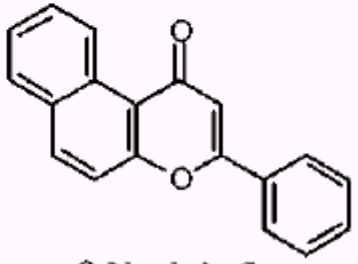
2,3,7,8-Tetrachlorodibenzofuran



3-Methylcholanthrene



Benzo(a)pyrene

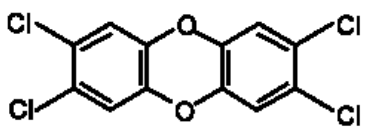


β-Naphthoflavone

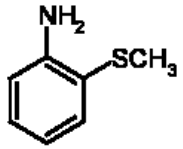
Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

„Non-classical“ AhR ligands

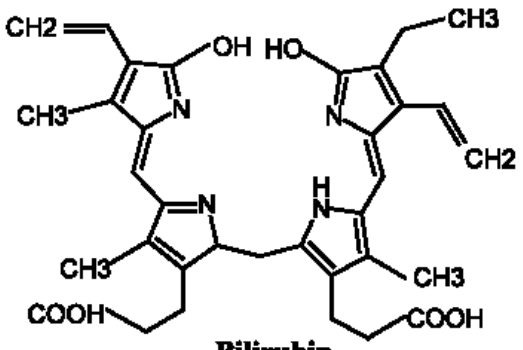
M.S. Denison et al. / Chemico-Biological Interactions 141 (2002) 3–24



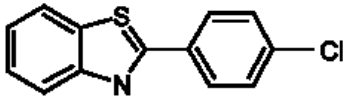
2,3,7,8-Tetrachlorodibenzo-p-dioxin



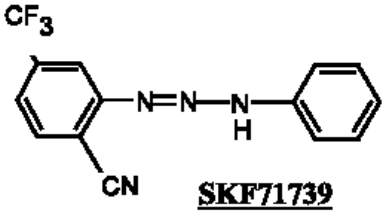
2-(Methylmercapto)aniline



Bilirubin



2-(4'-Chlorophenyl)benzothiazole



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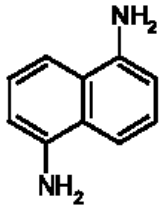
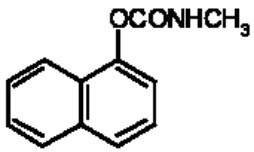
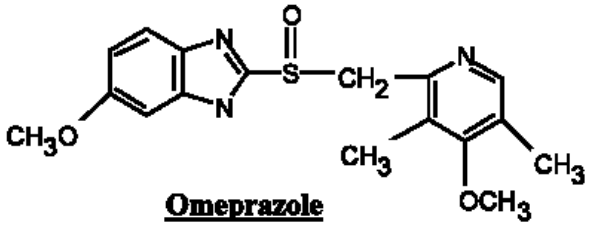


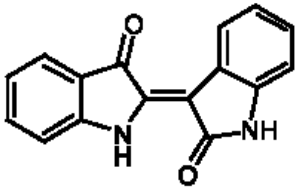
1,5-Diaminonaphthalene



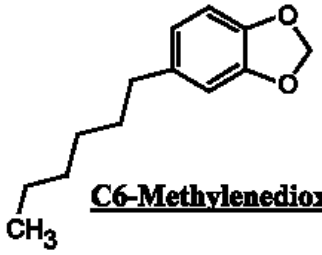
Carbaryl



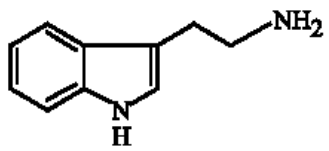
Omeprazole



Indirubin



C6-Methylenedioxybenzene



Tryptamine

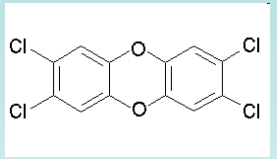
Physiological role for AhR not known (?)

→ Effects in AhR-deficient mice:

- significant growth retardation;
- defective development of liver and immune system;
- retinoid accumulation in liver;
- abnormal kidney and hepatic vascular structures.

- resistant to BaP-induced carcinogenesis and TCDD-induced teratogenesis;
- no inducible expression of CYP 1A1 and 2.

Biological responses & effects of TCDD (mostly related to AhR activation)



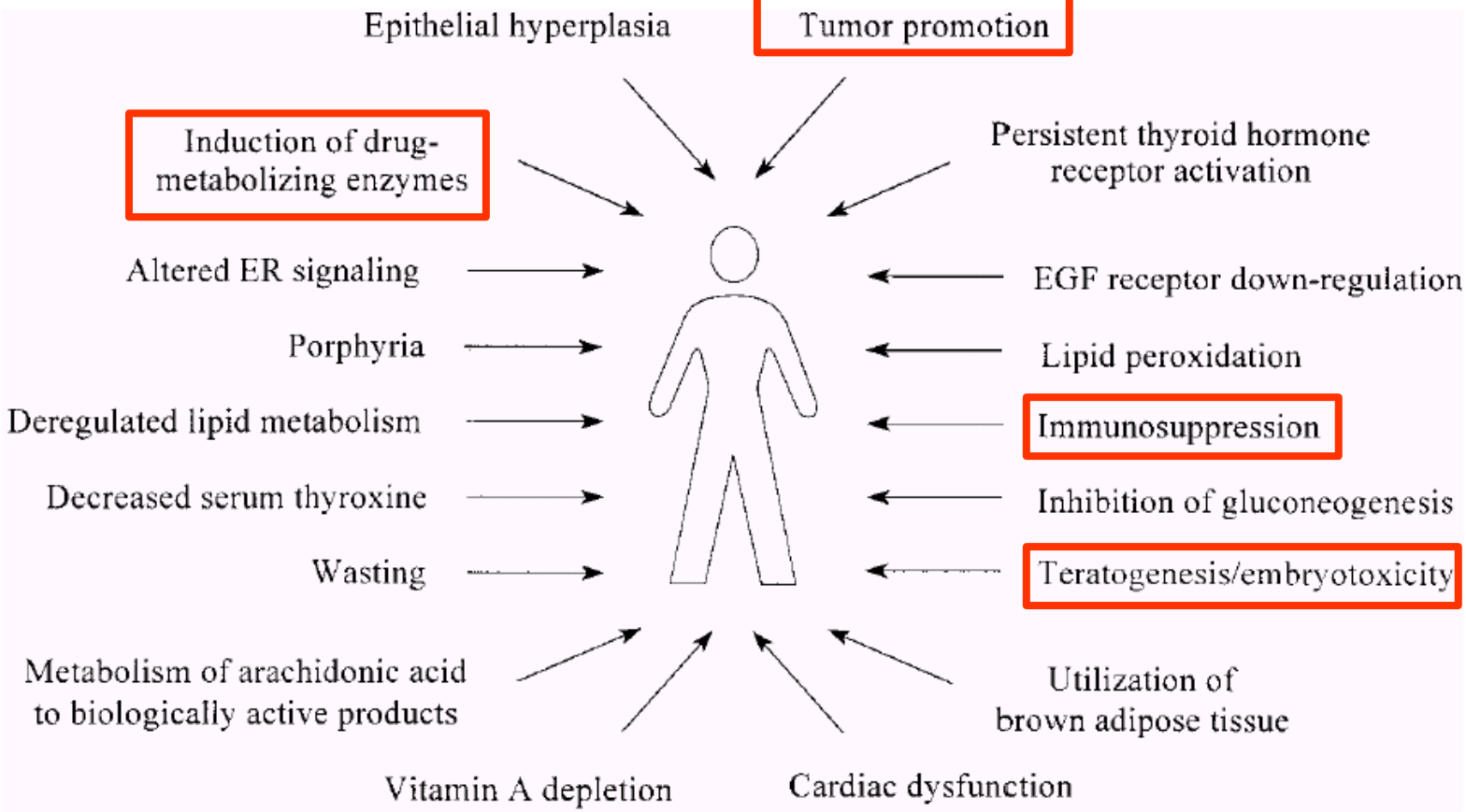


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

Schmidt & Bradfield, Annu. Rev. Cell Dev. Biol. 12:55

Toxic equivalency factors (TEF)/TEQ concept:

Compounds having similar toxicological properties as TCDD (strongest AhR ligand) may be evaluated by TEF/TEQ concept

TEF = Toxic Equivalency Factor (characteristic of the Chemical)
TEQ = Toxic Equivalent (sum of TEFs x concentrations)

TEFs are consensus values based on REPs (relative potencies) across multiple species and/or endpoints. TEFs are based upon a number of endpoints, from chronic in vivo toxicity to in vitro toxicity with the former having the greatest importance in determining overall TEF.

TEQs provide a simple, single number that is indicative of overall toxicity of a sample containing a **mixture of dioxins and dioxin-like compounds.**

The total potency of a mixture can be expressed in TCDD TEQ concentration:

$$\mathrm{TEQ} = \Sigma\{\mathrm{compound_1} \times \mathrm{TEF_1} + \ldots$$

$$+ \mathrm{compound_n} \times \mathrm{TEF_n}\}$$

Toxic equivalency factors for PCDDs, PCDFs and PCBs:

Table 4. Toxic Equivalent Factors established by the WHO (WHO-TEFs) for dioxins and dioxin-like PCBs [4]

PCDD Congener	WHO-TEF	PCDF Congener	WHO-TEF	PCB Congener	WHO-TEF
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	*Non-ortho*	
12,3,7,8-PeCDD	1	12,3,7,8-PeCDF	0.05	PCB#81	0.0005
123478-HxCDD	0.1	23478-PeCDF	0.5	PCB#77	0.0005
123678-HxCDD	0.1	123478-HxCDF	0.01	PCB#126	0.1
12,3,7,89-HxCDD	0.1	123678-HxCDF	0.1	PCB#169	0.01
1234678-HpCDD	0.01	234678-HxCDF	0.1	*Mono-ortho*	
OCDD	0.0001	12,3,7,89-HxCDF	0.1	PCB#105	0.0001
		1234678-HpCDF	0.01	PCB#114	0.0005
		1234789-HpCDF	0.01	PCB#118	0.0001
		OCDF	0.0001	PCB#123	0.0001
				PCB#156	0.0005
				PCB#157	0.0005
				PCB#167	0.00001
				PCB#189	0.0001

Eljarrat & Barceló, Trends Anal. Chem.22: 655

Final concentration is expressed as „Equivalents of TCDD“ (e.g. ng TEQ / kg = ng TCDD / kg)

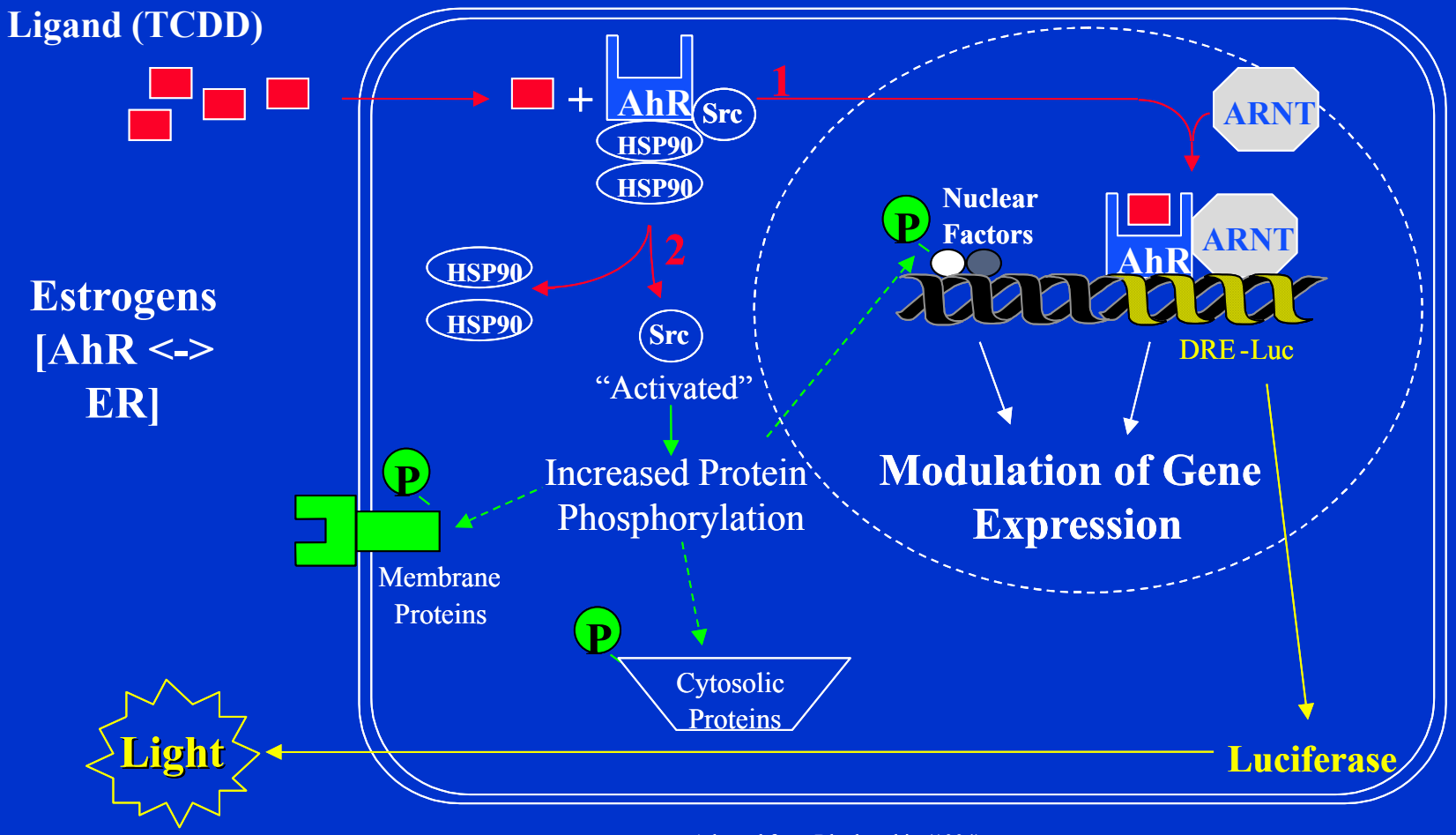
Biomarkers/bioanalytical methods for AhR toxicity

- ***in vivo***: liver enlargement, reduction of thymus weight, wasting syndrome, reproductive and developmental disorders
- ***in vivo*** biomarkers: EROD activity, CYP 1A1 and 1B1 expression

- ***in vitro:***
  - ➔ EROD (ethoxyresorufin-O-deethylase activity)
    in H4IIE rat hepatoma cells;
  - ➔ CALUX/CAFLUX assays;
  - ➔ GRAB assay (AhR-DNA binding)
  - ➔ yeast bioassay;
  - ➔ immunoassays;
  - ➔ detection of CYP1A mRNA or protein

In vitro CALUX/CAFLUX assays

AhR-mediated effects
luciferase reporter assay - H4IIE.luc cells



Adapted from Blankenship (1994)

Detection of EROD activity:

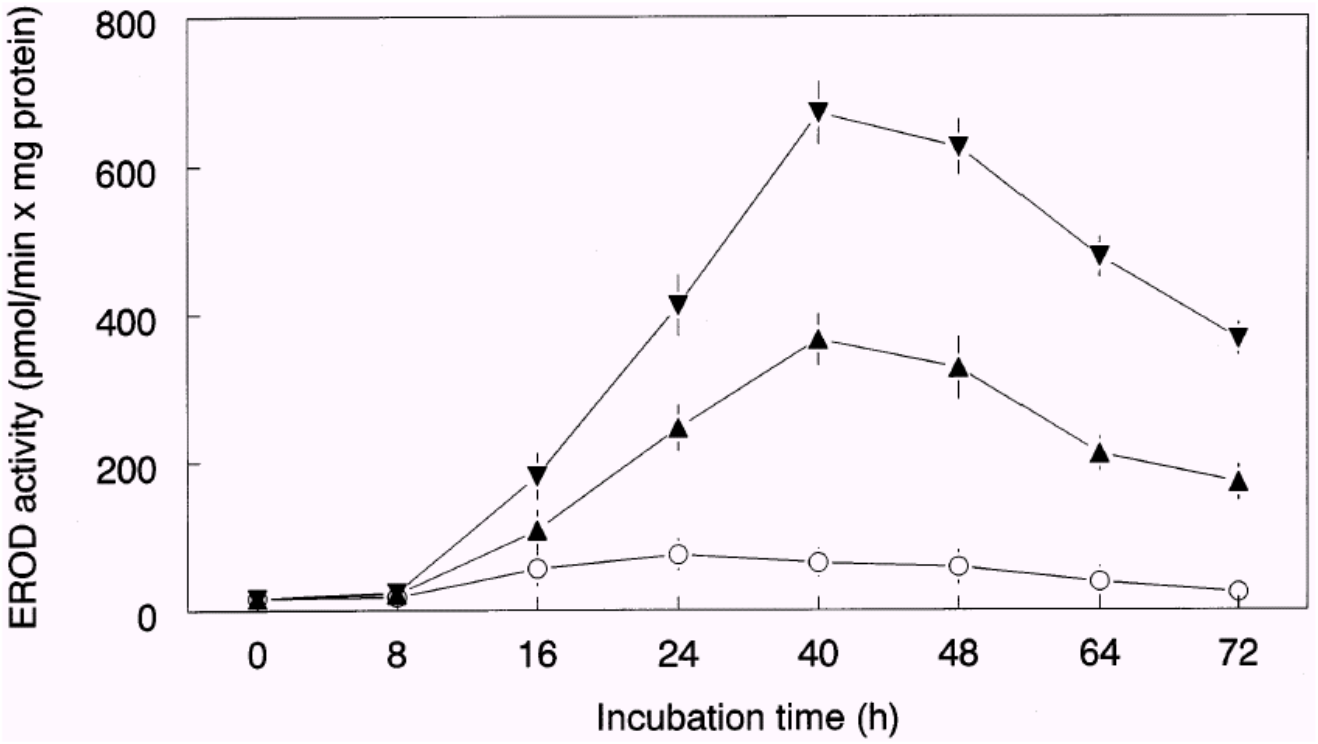
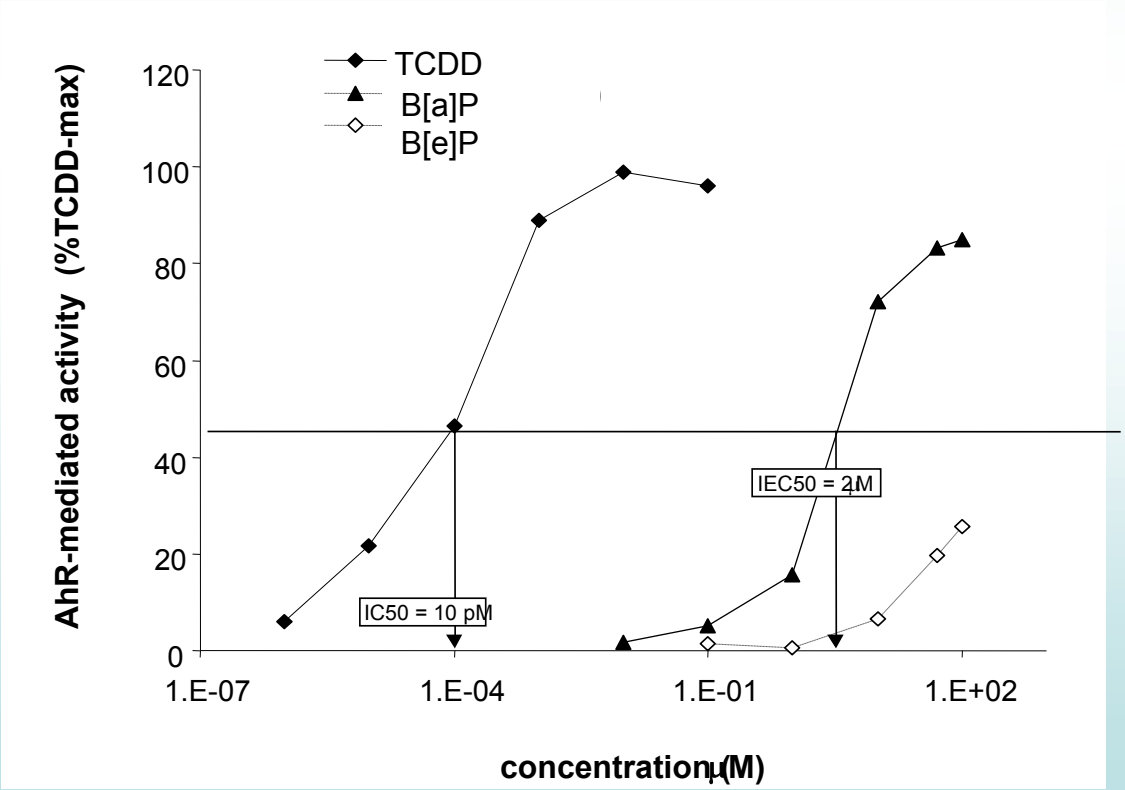


Fig. 2. Time course of induction of CYP1A1-catalyzed 7-ethoxyresorufin *O*-deethylase (EROD) activity in primary cultures of rat hepatocytes, after addition of 1.7×10^{-5} M benzo[*a*]pyrene (-▼-), 1.9×10^{-6} M benzo[*k*]fluoranthene (-▲-) or 9.4×10^{-5} M acenaphthylene (-○-). EROD activity was determined in cell homogenates. The data represent means ± S.D. from four independent experiments.

Comparing compounds -> Application in Risk Assessment

- Quantification of effects (EC_{50}) - relative potencies
- Comparison with the effect of reference toxicant (2,3,7,8-TCDD)
  - Expression as Equivalency Factors (~ TEFs)



TCDD: IC_{50}

PAH: IEC_{50}

Induction Equivalency Factor

$IEF = IC_{50} / IEC_{50}$

How many times is the compound "weaker" inducer than TCDD ?

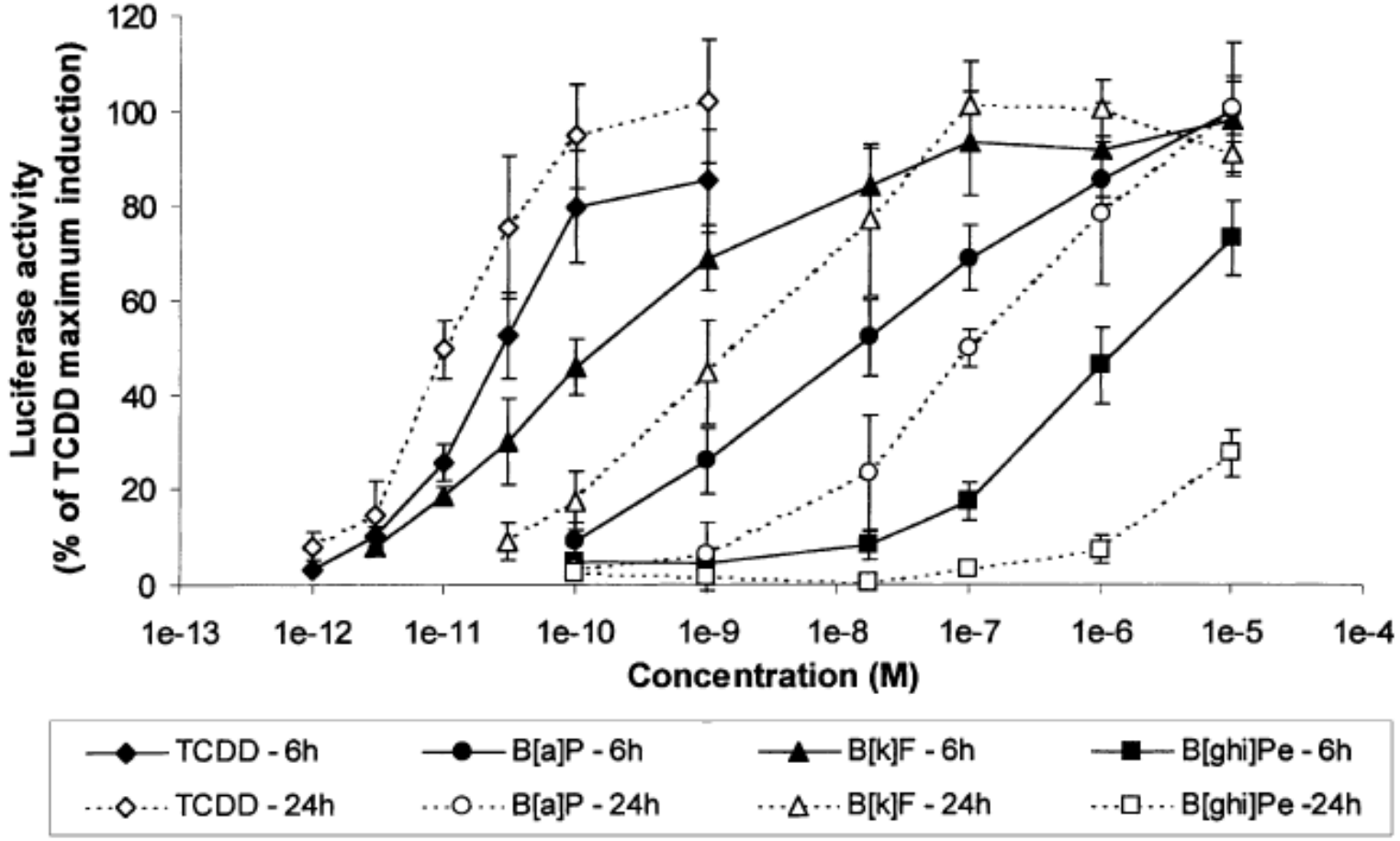

Luciferase activity
(% of TCDD maximum induction)
120
100
80
60
40
20
0
1e-13
1e-12
1e-11
1e-10
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
Concentration (M)
TCDD - 6h
B[a]P - 6h
B[k]F - 6h
B[ghi]Pe - 6h
TCDD - 24h
B[a]P - 24h
B[k]F - 24h
B[ghi]Pe -24h

Table 2
IEFs of PAHs relative to TCDD or B[a]P derived from EC50 or EC25 values in 24 and 6 h exposure assays

Derived from	$IEF_{TCDD(24h)}$		$IEF_{TCDD(6h)}$		$IEF_{B[a]P(6h)}$	
	EC50	EC25	EC50	EC25	EC50	EC25
Flu	ni[a]	ni	ni	ni	ni	ni
Ant	ni	ni	ni	ni	ni	ni
Fla	2.27E−8	9.31E−7	9.84E−5	1.11E−4	1.05E−2	5.59E−3
Py	1.78E−6	3.38E−6	2.59E−5	4.45E−5	7.57E−3	6.21E−3
B[a]A	7.04E−6	9.60E−6	7.64E−7	2.40E−6	0.39	0.50
Chry	1.01E−4	1.07E−4	1.41E−2	3.26E−2	3.25	2.04
B[b]F	3.35E−5	4.82E−5	4.90E−2	2.32E−1	8.83	12.81
B[k]F	1.64E−3	2.94E−3	0.28	0.57	67.76	36.33
B[a]P	9.01E−5	1.99E−4	1.11E−2	2.02E−2	1.0	1.0
DB[ah]A	1.17E−3	1.52E−3	0.06	0.20	11.46	11.72
I[123-cd]P	2.96E−4	5.01E−4	0.86	1.24	44.20	29.70
B[ghi]Pe	ni	ni	2.27E−5	4.68E−5	5.47E−3	2.99E−3
DB[al]P	4.90E−6	1.13E−6	2.52E−5	3.26E−5	2.36E−2	1.88E−2
NPyr	2.05E−4	3.83E−4	5.80E−3	1.31E−2	1.10	0.88
CPP	2.48E−7	6.53E−7	6.20E−6	1.72E−5	4.23E−3	3.38E−3
B[a]Pe	6.19E−6	6.28E−6	2.27E−4	3.05E−4	3.37E−2	1.68E−2
DB[ae]F	9.30E−6	1.18E−5	2.75E−5	1.33E−4	1.74E−3	6.74E−3
DB[ai]P	1.65E−4	4.41E−4	4.29E−2	3.82E−2	2.59	1.75
DB[ae]P	1.80E−5	3.90E−5	1.08E−3	3.90E−3	0.49	0.13
DB[ah]P	7.14E−5	3.70E−4	2.65E−2	5.43E−2	2.80	2.68
DB[ak]F	1.23E−3	1.37E−3	1.55E−2	2.02E−2	2.69	1.65
5-MeChry	9.48E−5	1.59E−4	4.05E−2	5.08E−2	3.07	2.46
DB[aj]A	3.70E−4	5.21E−4	3.07E−2	4.04E−2	2.16	2.16
B[j]F	3.68E−4	7.40E−4	4.05E−2	6.33E−2	2.25	2.51
B[c]Phe	4.49E−7	1.07E−6	6.21E−5	7.51E−5	4.64E−3	3.76E−3
B[e]P	5.15E−7	6.30E−7	3.71E−5	8.17E−5	2.27E−3	2.86E−3
DMBA	5.41E−6	1.30E−5	4.71E−2	3.98E−2	0.46	0.9
1-MePyr	2.07E−6	2.82E−6	4.80E−5	7.20E−5	8.54E−3	6.33E−3
DB[ac]A	1.92E−4	4.23E−4	3.53E−2	7.80E−2	1.75	2.78
Pic	4.11E−5	5.54E−5	1.90E−3	5.20E−3	0.12	0.25

[a] ni, no induction observed.

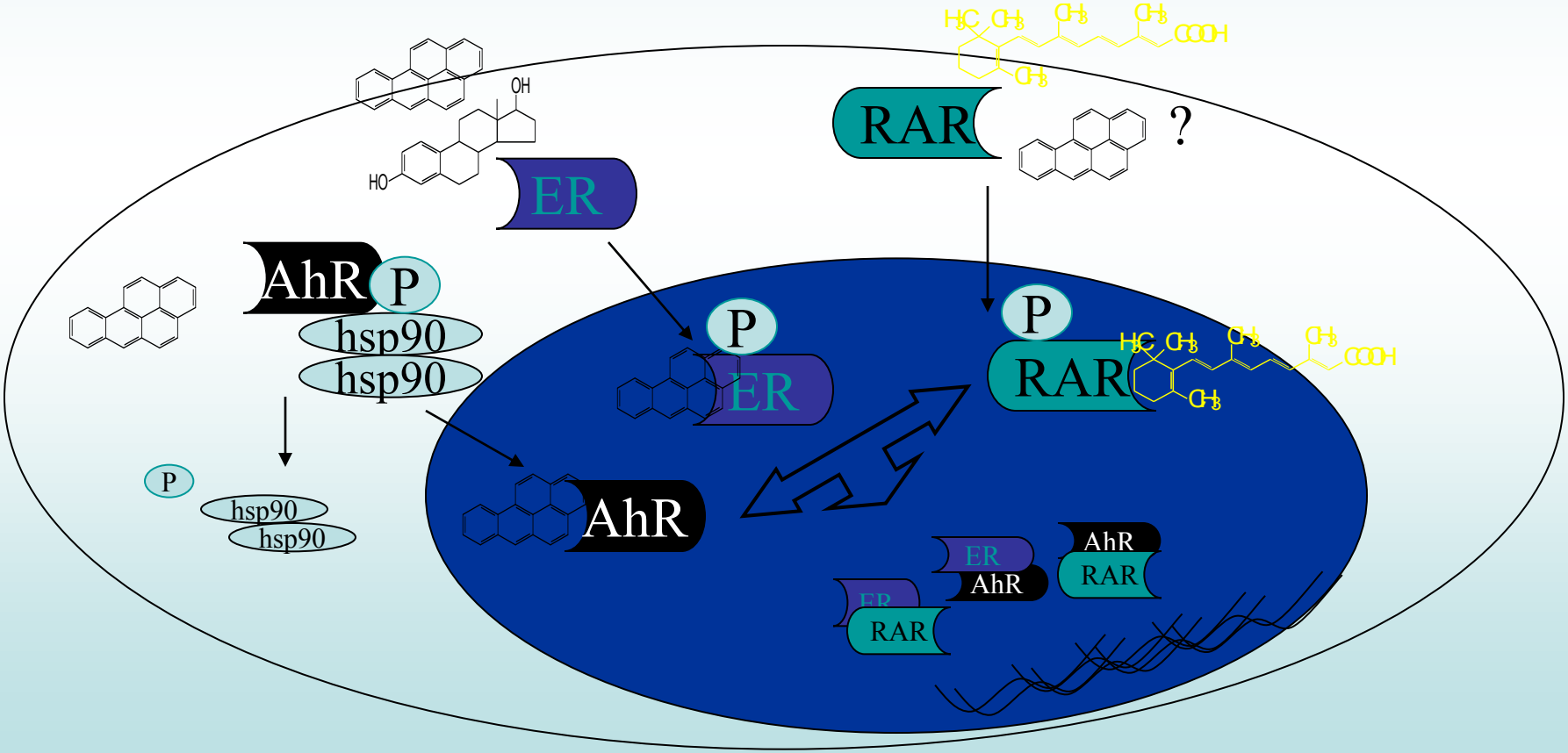
Crosstalk in signalling of nuclear receptors

In vitro assays for nongenotoxic effects

Nuclear Receptors & Signalling Crosstalk

poorly characterized (toxicity) mechanisms

Nuclear receptors (AhR, ER, RAR/RXR ...) = Transcription factors with numerous cofactors and interactions (crosstalk)



Cross-talk between estrogen signalling pathways and other receptors

- estrogen signalling pathways and
  other members of nuclear receptor superfamily
- estrogen signalling pathways and AhR
- estrogen signalling pathways and receptors for EGF and insuline

=> Many effects observed in vivo
(higher cancer incidence, allergies ...)
without known mechanisms ... ? complex toxicity / crosstalk ?