TOXICOLOGY

Nuclear Receptor-Mediated Toxicity

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LEARNING OBJECTIVES

After this lecture you will be able to:

- explain why nuclear receptors mediate toxicity without genotoxicity
- describe the domain structure and activation mechanism of nuclear receptors
- name the principal xenobiotic receptors (AhR, PXR, CAR) and endocrine receptors (ER, AR, PPAR, RAR/RXR)
- give examples of NR-mediated toxicity: TCDD, xenoestrogens, antiandrogens, peroxisome proliferators, retinoids
- explain species differences, receptor cross-talk and their impact on risk assessment

Evidence for Nuclear Receptor-Mediated Toxicity:

1. Tissue specific effect
2. Predictable effect
3. Transactivation of specific genes is increased
4. Rapid transcriptional response
5. Reversible binding of compound to intracellular molecules
6. Stereospecific effects

Molecular Properties of Nuclear Receptors:

1. Soluble (intra-cellular) receptor that binds a ligand, migrates to cell nucleus and interacts with specific genomic response elements
2. No second messengers in NR signalling
3. Effector is DNA

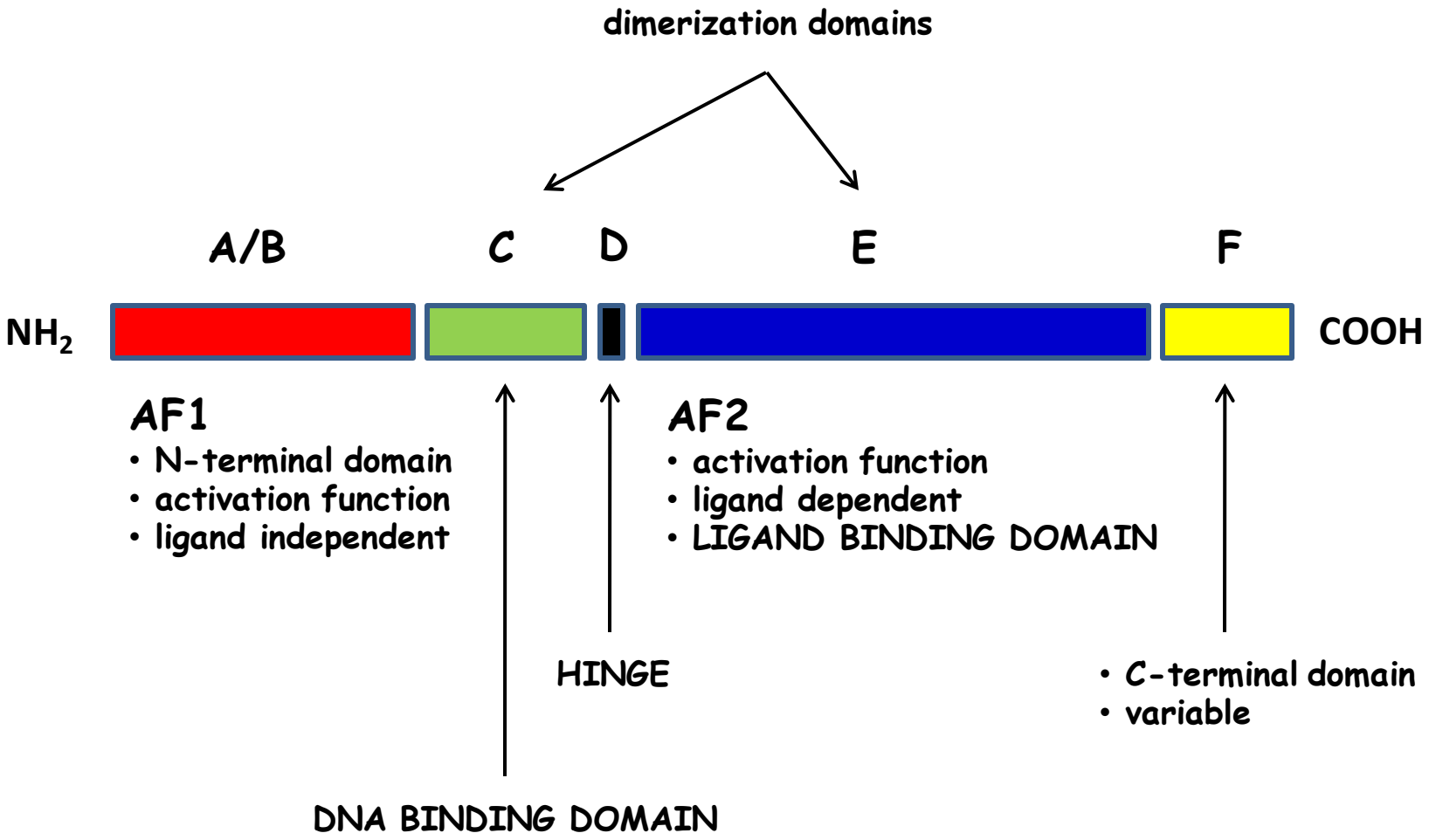
STEROID/THYROID/RETINOID FAMILY

- **Steroid receptors (ER, PR, AR, GR, MR)**
- **Vitamin D Receptor (VDR)**
- **Retinoid Receptors (RARs, RXRs)**
- **Thyroid hormone receptor (TR)**
- **Peroxisome proliferator-activated r. (PPARs)**

PAS (Per/ARNT/Sim) FAMILY

- **AhR = Aryl Hydrocarbon Receptor**
- **ARNT = AhR Nuclear Translocator**
- **HIF1α = Hypoxia-Inducible Factor**

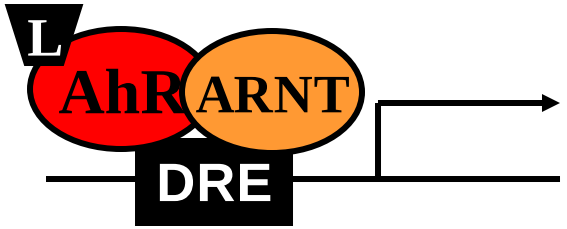
STRUCTURE OF NUCLEAR RECEPTORS



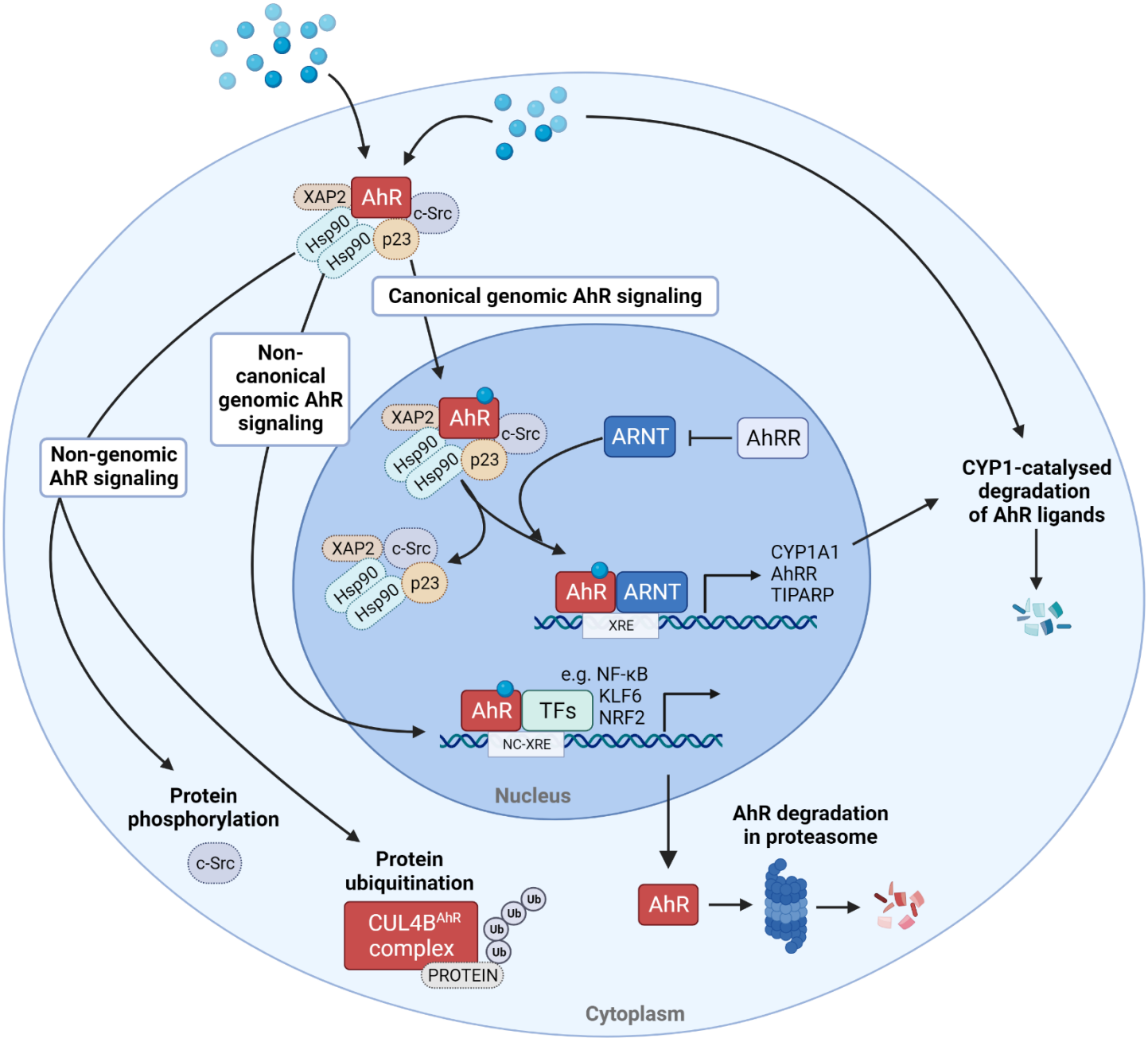
ARYL HYDROCARBON RECEPTOR

- dioxin receptor
- ubiquitously expressed (lung, thymus, placenta, liver, kidney, heart, spleen)
- ligand-(in)dependent activation
- ligands/activators:
  - NATURAL (flavonoids, isoflavones, stilbenes, anthocyanins)
  - DRUGS (omeprazole, lansoprazole, primaquine, ketoconazole)
  - CHEMICALS (SP600125, U0126)
  - ENDOGENOUS (indoles)
  - **ENVIRONMENTAL POLLUTANTS (PCBs, PAHs….)**
- target genes – CYP1A1, CYP1A2, CYP1B1, phase II enzymes
- AHRR = AhR repressor – forms AHRR/ARNT heterodimer, which binds to XRE but does not trigger gene expression
- AHRR is up-regulated by AhR = negative regulatory feed-back

DRE = dioxin-responsive element
XRE = xenobiotic-responsive element
AHRE = AhR responsive element

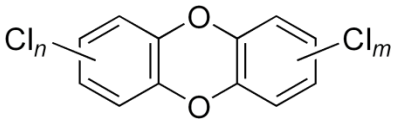


core sequence 5'-GCGTG-3'
consensus sequence 5'-T/GNGCGTGA/CG/CA-3'

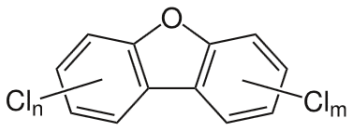

XAP2
AhR
c-Src
Hsp90
Hsp90
p23
Canonical genomic AhR signaling
Non-canonical genomic AhR signaling
Non-genomic AhR signaling
ARNT
AhRR
CYP1-catalysed degradation of AhR ligands
CYP1A1
AhRR
TIPARP
XRE
e.g. NF-κB
KLF6
NRF2
TFs
NC-XRE
Nucleus
Protein phosphorylation
Protein ubiquitination
CUL4B^AhR complex
Ub
PROTEIN
AhR degradation in proteasome
Cytoplasm

AHR-MEDIATED TOXICITY

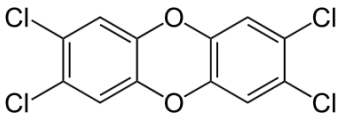
- AhR binds many polychlorinated aromatic compounds with differing affinity
- **pleiotropic toxicological effects**; multitude transcriptional and phenotypic effects
- PCDDs, PCDFs, PCBs = the **most toxic** environmental chemicals
- highly **lipophilic persistent** compounds = **long biological $t_{½}$** (7 – 11 years!)
- different degree of chlorination = **many congeners**; variable toxic potential
- often **mixtures** → **relative measure of toxicity** = **TOXICITY EQUIVALENTS (T.E.)**
- T.E.s calculated for the **most potent congener TCDD**; T.E. = 1.0
- relative toxic potential of PCBs, PCDDs and PCDFs correlates with their affinity to AhR
- binding to AhR is optimal when ligand is coplanar



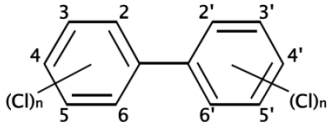
polychlorinated dibenzodioxins
PCDDs (75 congeners)



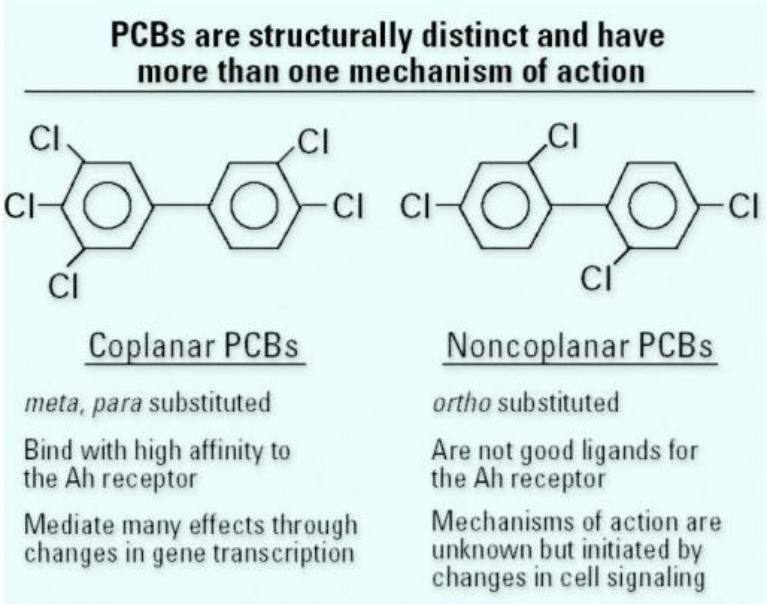
polychlorinated dibenzofurans
PCDFs (75 congeners)



2,3,7,8-tetrachloro-*p*-dibenzodioxin
TCDD

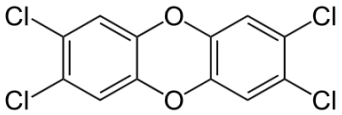


polychlorinated biphenyls
PCBs (209 congeners)



Ganey PE, Boyd SA (2005) An approach to evaluation of the effect of bioremediation on biological activity of environmental contaminants: dechlorination of polychlorinated biphenyls. Environ Health Perspect. 113(2):180-5

AHR-MEDIATED TOXICITY - TCDD



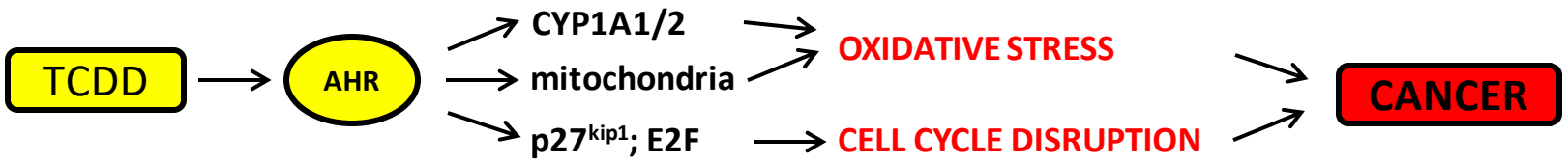
- environmental pollutant with a very high toxic potential featuring pleiotropic activity
- immunotoxicity, endocrine toxicity, embryotoxicity, teratogenesis (in aminals)
- human **carcinogen** Class 1 *via* non-genomic mechanisms
- acute toxicity = maximal effects after 2-4 weeks after single exposure
- **species-specific sensitivity**; e.g. guinea pigs (LD_{50} 500 ng/kg) vs hamster (LD_{50} 5 mg/kg)
- in humans – **chloracne** = persistent disturbance of epithelial cells differentiation in skin
- high affinity of TCDD to AhR ($K_d = 10^{-11}$ M) – species-specific(!)
- TCDD-activated AhR triggers transcriptional response of many genes, but one single „*toxicity gene*" dysregulated by AHR is unlikely

Evidence for AHR-mediated toxicity of TCDD:

- Responsiveness to TCDD positively correlates and segregates with Ah^{b-l} allele
- The Kd for TCDD of nonsensitive mouse strains is 10-20 x higher than of sensitive ones
- AhR-null mice are resistant against toxic effects of TCDD

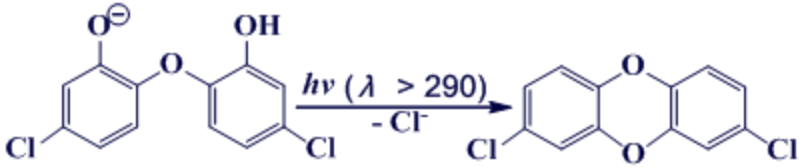
AHR-MEDIATED TOXICITY – TCDD&CANCER

- mechanism is probably combined, **involving disruption of cell cycle and oxidative stress**
- TCDD alters transcription factors (E2F) and cyclin-dependent kinases inhibitors ($p27^{kip1}$), resulting in **CELL CYCLE DISRUPTION**
- TCDD induces extensive **OXIDATIVE STRESS** by induction of CYP1A1/2 and in mitochondria by interfering with electron transport chain (oxidation of SH- groups, increase of Ca^{2+}, ROS production, DNA damage)
- ROS production in mitochondria is low in AhR-knock-out mice = mitochondrial oxidant stress depends rather on AhR than on CYP1A1/2



TRICLOSAN

- antifungal, antibacterial
- tooth pastes, soaps, deodorants
- gets to environment where it undergoes photochemical degradation to 2,8-DCDD



AHR-MEDIATED TOXICITY - TCDD

AhR-mediated toxicity: historical TCDD exposures

all caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) — the most potent AhR ligand among environmental pollutants

Agent Orange, Vietnam 1962–1971



- mass defoliation herbicide, contaminated with TCDD as a by-product of synthesis
- ~80 million liters sprayed
- dioxin hotspots (Da Nang, Bien Hoa) persist for decades
- **health effects** in veterans and subsequent generations

Seveso, Italy 1976



- ICMESA plant explosion — TCDD cloud over populated area
- the highest-known residential TCDD exposure in history
- **chloracne** in ~190 children
- zones A, B, R evacuated; soil decontamination took years

Yushchenko, Ukraine 2004



- presidential candidate poisoned with pure TCDD
- blood level ~100,000 pg/g lipid — among the highest ever recorded in a human
- **severe chloracne** on the face; recovery took years
- criminal poisoning case — TCDD identified by mass spectrometry

Germany 2010–11: dioxin in eggs



- industrial fatty acids (biodiesel residue) diverted into animal feed
- dioxin up to 77× the legal limit in feed fat
- ~4,700 farms closed, eggs recalled across the food chain
- led to stricter EU feed-monitoring rules

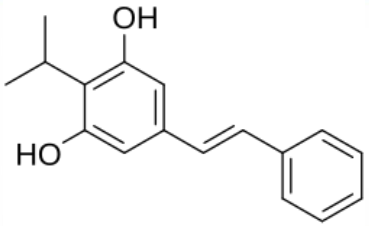
Common thread: chloracne is the hallmark of TCDD toxicity — and dioxin monitoring of the food chain remains a permanent task

AHR-THERAPEUTIC TARGET

AhR: from toxicology to pharmacology

the same receptor that mediates dioxin toxicity is now a validated drug target — translation took ~30 years

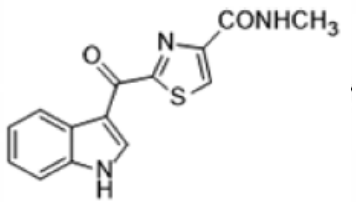
Tapinarof (VTAMA) — first AhR agonist drug



tapinarof — bacterial stilbene derivative

FDA approved for plaque psoriasis (2022)
FDA approved for atopic dermatitis (2024)
mechanism: ↓ IL-17A/IL-13 signaling, ↑ skin barrier proteins, Nrf2 activation

AhR ligands in inflammatory bowel disease



ITE-CONHCH$_3$ — indole-thiazole bioisostere of ITE

EC_{50} = 1.6 nM, 10× more stable than ITE
alleviates DSS-induced colitis in mice
Dvořák *et al.* 2026, *Front. Pharmacol.* 17:1931941 — *in vitro* profiling supports preclinical development

Clinical pipeline — both directions

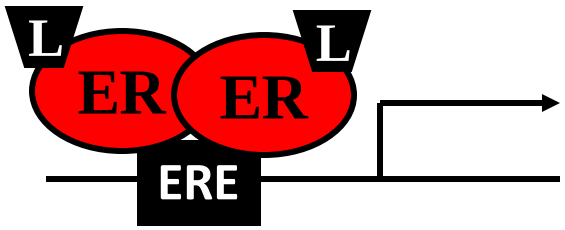
AGONISTS	ANTAGONISTS
EQ504 (Equillium) — phase I, ulcerative colitis, colon-targeted oral	**BAY 2416964 (Bayer)** — phase I ± pembrolizumab, solid tumors
AT-177 (Azora/Adial) — oral colon-targeted indirubin prodrug, ulcerative colitis	**IK-175 (Ikena)** — phase I + nivolumab, FDA Fast Track, urothelial carcinoma
AT-193 (Azora/Adial) — topical indirubin, hidradenitis suppurativa, nail psoriasis	
ATB102 (Allianthera/Falk) — phase I, gut-enriched, ulcerative colitis	
AQ312 (Aqilion), DMVT-506 — phase I–II dermatology	
indigo naturalis (qing-dai) — indirubin, UC trials; limited by **hypertension** and **hepatotoxicity**	

agonism for barrier-tissue inflammation (skin, gut) — antagonism to restore tumor immunosurveillance; direction depends on tissue and disease context.

Lesson: 30 years ago AhR was the 'dioxin receptor' — today it is a drug target in dermatology, gastroenterology and oncology.

ESTROGEN RECEPTOR - ER

- exists in two forms ERα and ERβ – distinct proteins differing in transcriptional activity
- tissue-specific expression
- forms homodimers ERα/ERα and ERβ/ERβ
- ERα form preferentially heterodimer ERα/ERβ
- ER binds large variety of chemically unrelated compounds = „promiscuity"



Estrogen Response Element – ERE

XENOESTROGENS = environmental estrogens

- xenobiotics that can elicit agonistic (enhancing) response mediated by ER
- responsible for hormonally-controlled cancers (mammary, testicular, prostate)
- interfere with normal reproduction in wildlife
- DDT, hydroxy-PCBc, alkylphenols, bisphenol A, diethylstilbestrol (anti-abortive)

complicated risk assessment

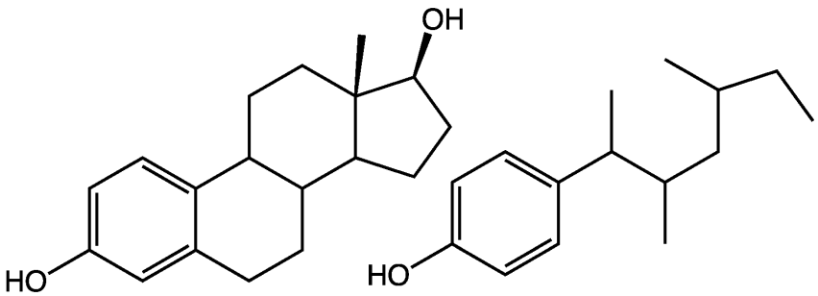
A) xenoestrogens are usually **weak ER agonists**, i.e. they have **low relative estrogenic potency** as compared to endogenous estrogens = rather high concentrations of xenoestrogens are required to elicit adverse effect

B) **environmental stability, bioaccumulation, high lipophilicity** = increased danger

ER-MEDIATED TOXICITY: ALKYLPHENOLS

- **alkylphenol ethoxylates** – industrial and household use – detergents, emulsifiers
- massive commercial production – nonionic surfactants, stabilizers in plastics…
- bacterial degradation to alkylphenols
- **alkylphenols** – very low acute toxicity *vs* accumulation in organisms and sediments
  – estrogenic activity !!!

- the most known ***p*-nonylphenol (PNP)**
- PNP binds to and activates ER
- transactivation of estrogen-responsive genes



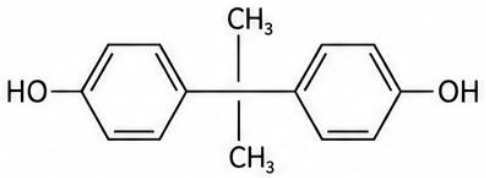
estradiol *p*-nonylphenol

Risk of PNP is low or high?

- PNP has 30,000× lower affinity to ER than E2
- PNP is inactivated by glucuronidation
- PNP does not cross placental barrier
- PNP accumulates in fat tissue
- **risk assessment must be very complex**

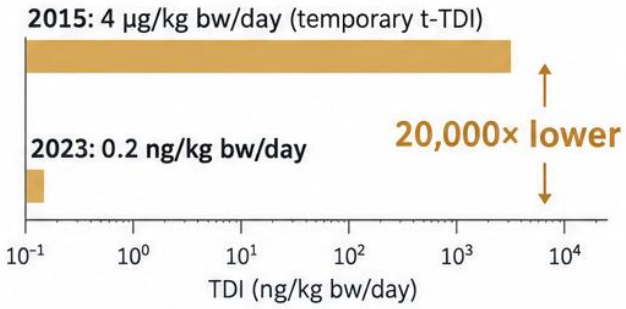
ER-mediated toxicity — update: BPA and PFAS

BPA: EFSA re-evaluation 2023



bisphenol A (BPA) — weak ER agonist

Tolerable daily intake (TDI)



critical effect: increased Th17 cells (immune system)

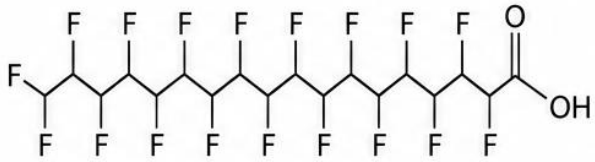
dietary exposure exceeds the TDI in ALL age groups by **2–3 orders of magnitude**

EU ban 2024



EU ban of BPA in food-contact materials (2024)

PFAS — 'forever chemicals'



perfluoroalkyl acids (e.g. PFOA)

extreme persistence (C–F bonds)

emerging **endocrine disruptors**

ER-mediated effects on **reproduction** and **development**

Lesson: the xenoestrogen story moves from 'weak agonists, complex risk assessment' to regulatory action — potency is not the only variable that matters

ER-MEDIATED TOXICITY: ER&AHR

- cross-talk between nuclear receptors, e.g. ER *vs* AHR
- biphasic effects of TCDD on ER: positive and negative

Monostory K., Pascussi J.M., Kobori L., **Dvorak Z**. (2009) Hormonal regulation of CYP1A expression. *Drug Metab Rev* **41(4)**:547-572.

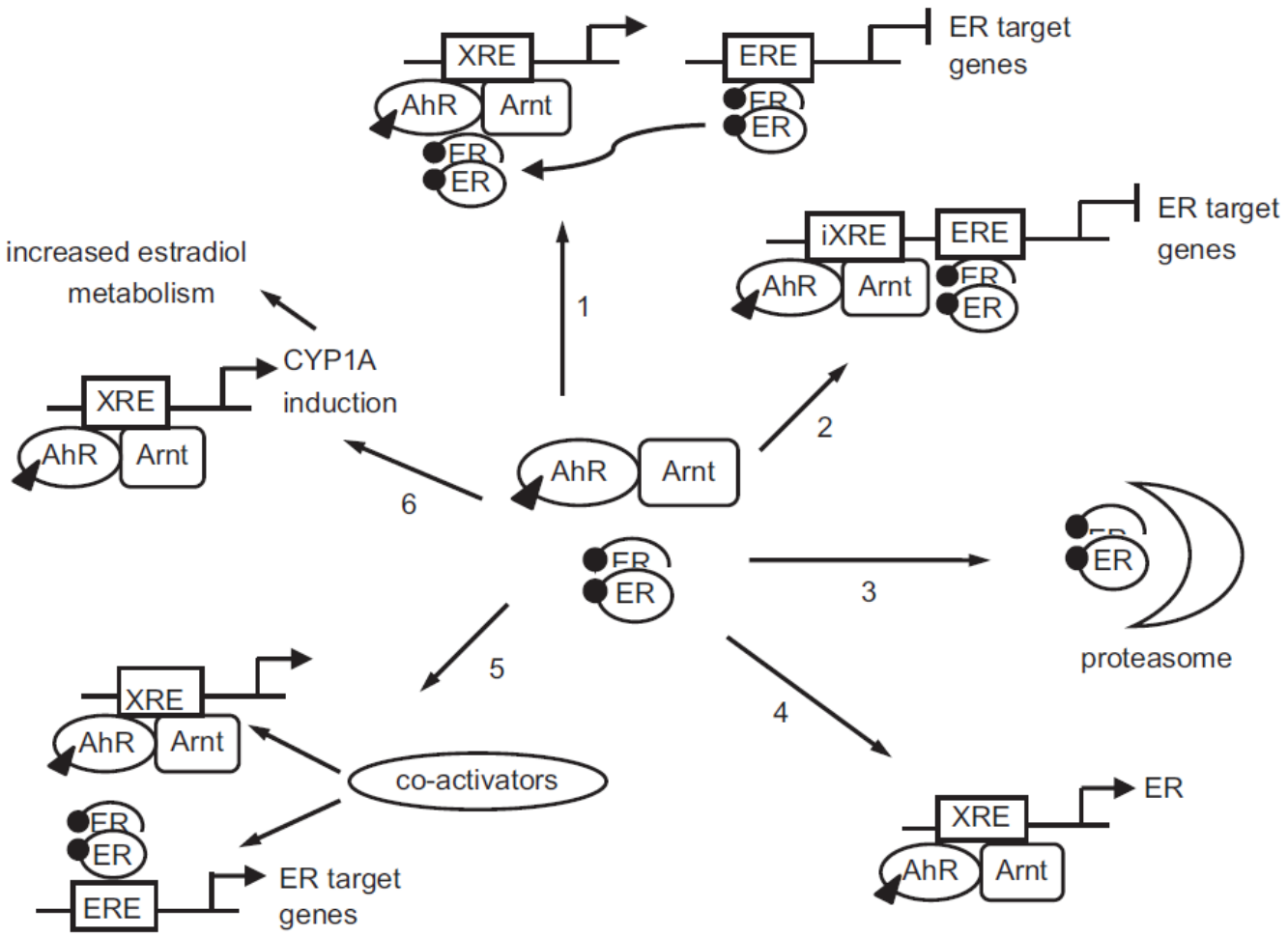
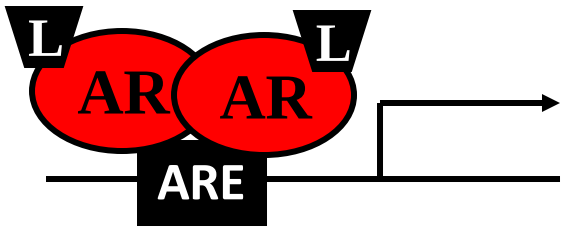


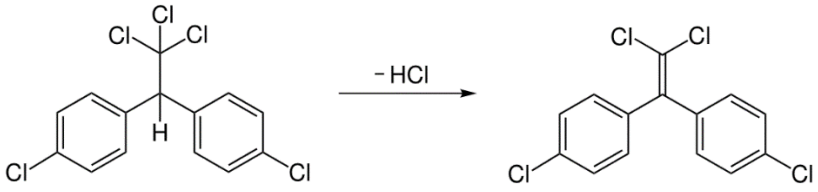
Figure 3. Crosstalk between the AhR- and ER-signaling pathways. 1: Liganded ER is recruited to AhR target genes; 2: AhR complex interacts with iXRE in the promoter of ER target genes; 3: AhR induces the proteasomal downregulation of ER; 4: AhR downregulates the expression of ER; 5: AhR signaling competes with ER signaling for the common coactivator pool; and 6: induction of CYP1A increases the metabolism of estrogen.

ANDROGEN RECEPTOR - AR

- pivotal role in development of male gonadal tissues
- implicated in development of tumors (prostate cancer)
- present in most tissues; abundant in male reproductive organs; low levels in female reproductive organs
- endogenous ligands – testosterone, DHT
- xenobiotics causing endocrine-disruption through AR = **ANTIANDROGENS** (e.g. Vinclozolin, DDT)



Androgen Response Element – ARE



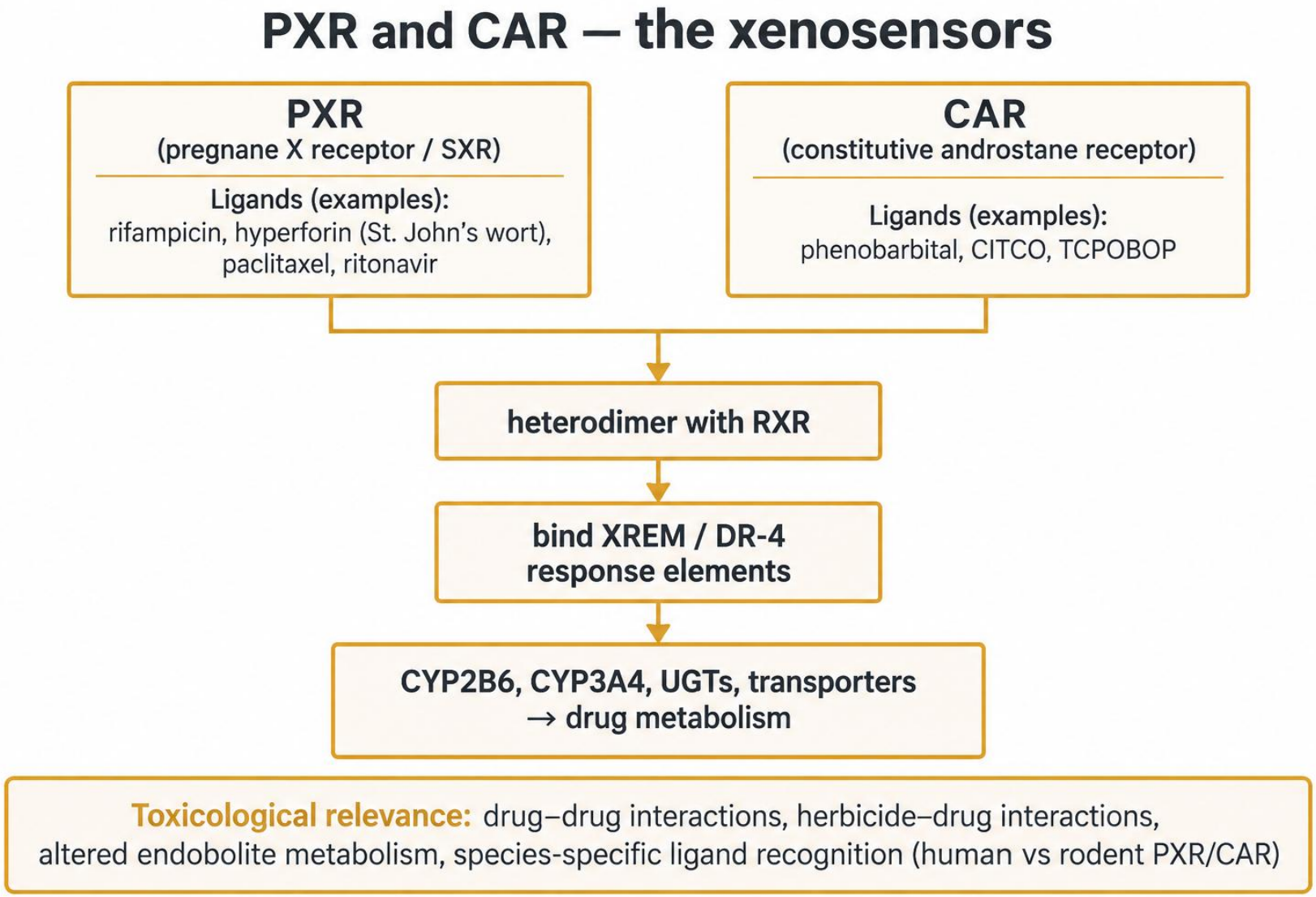
p,p´-DDT *p,p´*-DDE

- DDT = massively used pesticide in the past; DDE = breakdown product of DDT
- DDE = **antiandrogen**, persistent, lipophilic, widespread in environment
- DDE blocks AR functions in multiple ways: ↓ AR interaction with coactivators
  ↓ androgen binding to AR
  ↓ AR nuclear translocation → ↓ DNA binding

Some xenobiotics are dual ECD: XENOESTROGENS and ANTIANDROGENS

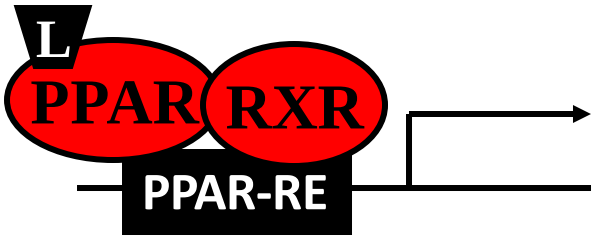
e.g. bisphenol A, p-nonylphenol

PREGNANE X RECEPTOR & CONSTITUTIVE ANDROSTANE RECEPTOR



PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR - PPAR

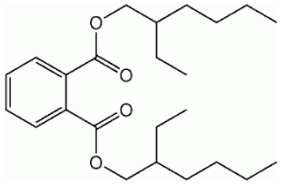
- PPARs – endogenous ligands: **fatty acids and eicosanoids+metabolites**
- subtypes: PPARα, PPARβ (= PPARδ), PPARγ → **different tissue distribution and function**
- name PPAR – historic reasons = ligands for PPAR caused proliferation of peroxisomes in rodents ; but in humans it is not true; better ***„fatty acid-activated receptor***"
- **peroxisomes** = subcellular organelles that function as sites of fatty acyl-β-oxidation, cholesterol metabolism glycerolipid synthesis and other lipids pathways



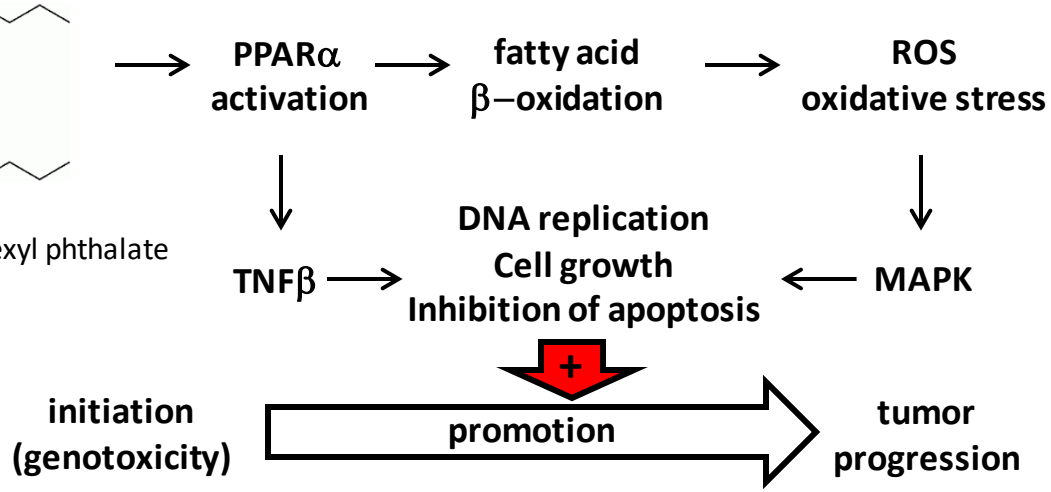
- **peroxisome proliferators** = chemically diverse compounds (clofibrate, ciprofibrate, gemfibrozil, nafenodipin, plasticizers – phtalates, solvents
- PPs induce proliferation of peroxisomes and cause liver tumors in rodents
- the common for PPs is that they are ligands for PPARs = chicken&egg problem
- all subtypes of **PPAR form heterodimer with RXR**

PPARα-MEDIATED TOXICITY

- PPARα – ligands/activators cause hepatocarcinogenesis
- PPs stimulate replicative DNA synthesis and liver growth = **tumor-promoting characteristics**
- PPs inhibit hepatocyte apoptosis *in vivo* and *in vitro* → **tumor-promotion**
- involvement of tumor necrosis factor beta (**TNFβ**) → it suppresses apoptosis and stimulates DNA synthesis in hepatocytes
- role for mitogen-activated protein kinases (**MAPKs; p38**) → activated by oxidative stress
- PPs increase fatty acyl-β-oxidation (<u>in rodents not in man</u>) → increased production of H_2O_2 → **Reactive Oxygen Species = oxidative stress**
- sustained oxidative stress leads to nongenotoxic liver carcinogenesis
- demonstrated in rodents not in man = perhaps irrelevant mechanism in man?

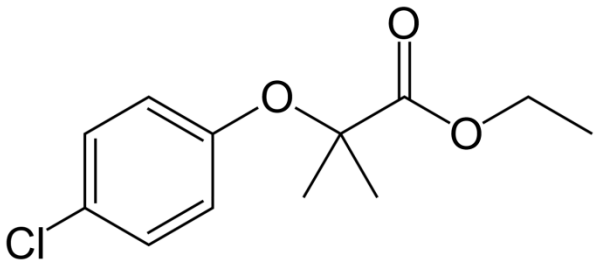


DEHP; diethyl hexyl phthalate

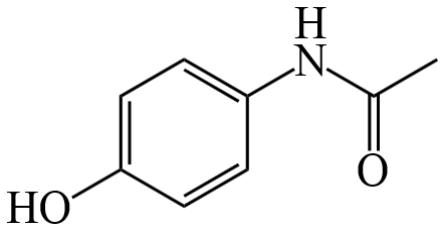


PPARα-MEDIATED TOXICITY

- PPARα interestingly participates in **cytoprotection** against chemically different and mechanistically distinct hepatotoxicants
- **clofibrate** (hypolipidemic drug – class „fibrates") has hepatoprotective effect against liver injury induced by acetaminophen APAP, carbon tetrachloride, bromobenzene, chloroform
- **mechanism of protection is unrelated to mechanism of liver injury**
- PPARα–null mice had lost the ability to be resistant against APAP when given clofibrate; they also showed impaired liver regeneration after partial hepatectomy
- PPARα controls **growth regulatory genes** (c-Ha-ras, fos, jun, egr-1) that are involved in the progression of cell cycle ($G_0 \rightarrow$ S transition)
- **the mechanism of PPARα-mediated hepatoprotection may involve stimulation of mitogenic response → increased hepatocellular proliferation → regeneration**



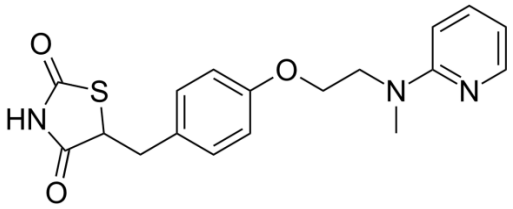
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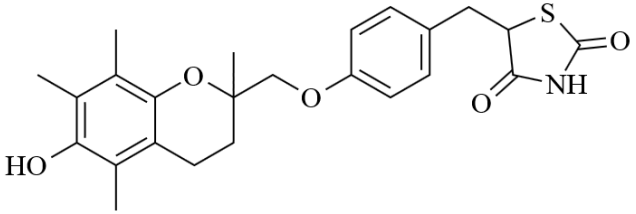
acetaminophen (paracetamol)

PPARγ-MEDIATED TOXICITY

- PPARγ – "*opposing receptor*" = mediates biological functions opposite to those by PPARα
- antitumor effects, increase the storage of lipids
- PPARγ – encoded by single gene, highly conserved structure in mice, rats, humans
- alternative promotor use and alternative splicing = PPARγ1, PPARγ2
- two forms of proteins identified: PPARγ1, PPARγ2 → distinct tissue distribution
- extra-adipose tissues (PPARγ1) *vs* adipose tissue (PPARγ2; 10-100x more)
- expression of PPARγ is influenced epigenetically (↑nutrition, ↑obesity, ↑type II diabetes)
- activation of PPARγ drastically differs under **normal and pathophysiological conditions**!
- several drugs (e.g. thiazolidinediones, TZD) are ligands for PPARγ
- **TZD can induce fat accumulation in the bone marrow → anemia**
- **TZD** cause fatty changes in mice liver – **enlargement of liver, microvesicular steatosis**
- **occured only in obese mice with type II diabetes!! – extrapolation to humans??**



rosiglitazone



troglitazone

PPARγ-MEDIATED TOXICITY

• TZD→ increased transcription of adipocyte-specific PPARγ–regulated genes in bone marrow stromal cells

• OBESE mice have highly up-regulated expression of PPARγ in liver

•TZD induced transcription of genes involved in lipid metabolism (fatty acid-binding protein, fatty acid translocase) in liver of **OBESE but not LEAN mice**!! → translation to humans??

FUNCTIONAL CHANGES BY TZD-PPARγ:

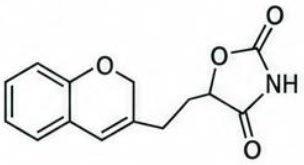
- Increased flux of non-esterified fatty acids NEFA from adipose tissue to liver in obese animals = continuous exposure of hepatocytes to fatty acids
- Increased *de novo* synthesis of fatty acids from glucose in liver (through PPARγ)
- Up-regulation of fatty acid trasporters and binding proteins (PPARγ–regulated genes)
- Inhibition of fatty acids β-oxidation by TZD

- AhR-PPARγ cross-talk? TCDD down-regulates PPARγ
- Relation between TCDD exposure and development of type II diabetes in humans

PPARγ-MEDIATED TOXICITY

PPARγ agonists (glitazones): two toxicity stories

Troglitazone (Rezulin, Warner-Lambert/Pfizer)



Troglitazone
(thiazolidinedione, chromane ring)

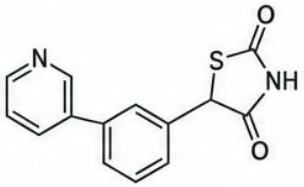


Hepatotoxicity

- **1997** — FDA approval
- **Dec 1997** — withdrawn in UK
- **Mar 1999** — FDA: liver-enzyme monitoring NOT sufficient
- **Mar 2000** — withdrawn in USA — 63 liver-failure deaths, ~430 liver failures, 1.9M users
- **2001–2009** — 35,000+ lawsuits settled; payouts > $1 billion



Rosiglitazone (Avandia, GSK)



Rosiglitazone
(thiazolidinedione, pyridine ring)



Cardiovascular toxicity

- **1999** — FDA approval
- **2007** — NEJM meta-analysis: **+43% myocardial infarction risk**
- **Sep 2010** — EMA suspends in EU; FDA restricts use
- **2010–2011** — ~13,000 lawsuits; $460M settles 10,000 suits
- **2012** — GSK pays $3 billion DOJ settlement — largest healthcare fraud settlement in US history (withheld CV safety data)



1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

Lesson: receptor promiscuity + incomplete safety data = delayed detection of organ toxicity (liver vs heart).

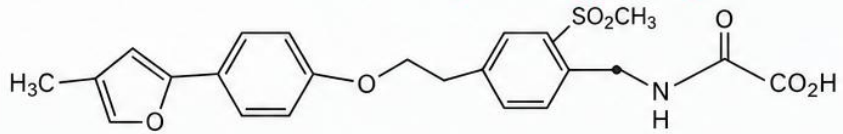
Dual and pan-PPAR agonists (glitazars): promiscuity cuts both ways

designed to combine PPARα (fibrate-like lipid effects) + PPARγ (insulin sensitizing) — one receptor switch for glucose AND lipids

2002 — **Ragaglitazar** (Novo Nordisk / Dr Reddy's) — **suspended:** urinary bladder tumors in rodents

2003 — **Farglitazar** (GSK) — **discontinued:** edema

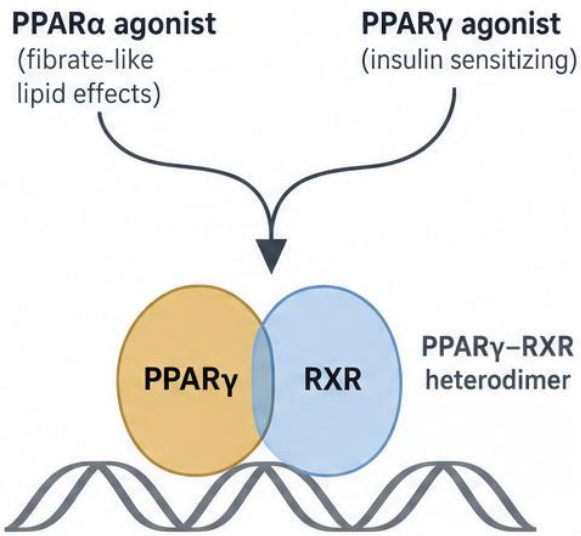
2005–2006 — **Muraglitazar** (BMS/Merck, Pargluva) — FDA advisory committee voted 8:1 for approval, but JAMA analysis (Nissen) showed **2.2×** death/MI/stroke; **development stopped May 2006**



muraglitazar

2006 — **Tesaglitazar** (AstraZeneca, Galida) — **discontinued:** decreased glomerular filtration (kidney effects)

2013 — **Aleglitazar** (Roche) — **abandoned in phase III:** safety signals

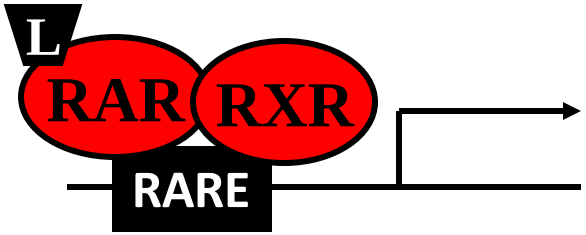


dual activation = dual toxicity:
edema, heart failure, CV events, rodent tumors

Lesson: activating two nuclear receptors at once doubles the transcriptional side-effect surface — **none of the glitazars ever reached the market.**

RETINOIC ACID RECEPTOR - RAR

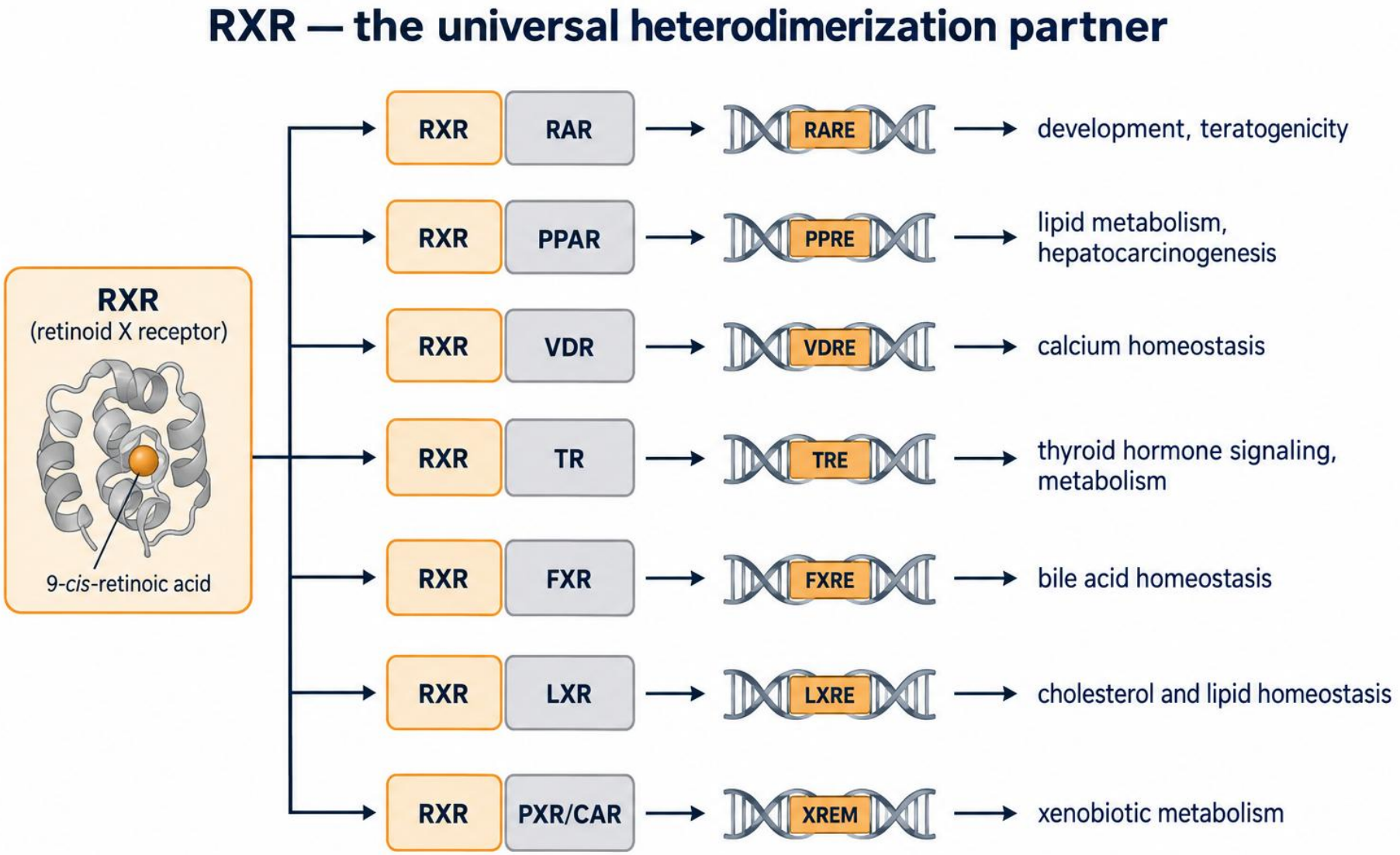
- physiological ligands for RAR: **retinol, *all-trans*-retinoic acid**
- RAR – expressed abundantly in **liver**, also testes and epididymis (Sertoli cells)
- perturbation of RAR (agonist/antagonist) → **testicular toxicity and embryotoxicity**
- **BMS189453** – retinoid analogue (treatment of skin diseases; RARβ agonist, RARα/γ antagonist) induced testicular atrophy and hypospermia in rats – symptoms of vitamin A deficiency
- retinoids are important in embryonic development = xenobiotics that interfere with RAR during embryonic development cause developmental abnormalities
- **Isotretinoin (Accutane)** (13-cis-retinoic acid) – classic human teratogen; absolute contraindication in pregnancy



PHENYTOIN:

- Anticonvulsant
- Causes embryopathy and teratogenic effects in animals and humans (craniofacial abnormalities, microcephaly)
- Up-regulates RARα,β,γ in embryonic tissues

RETINOID X RECEPTOR



Environmental obesogens: organotins hijack PPARγ/RXR

Sources of exposure



tributyltin in ship antifouling paints, IMO ban 2008



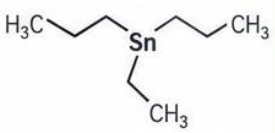
PVC stabilizers



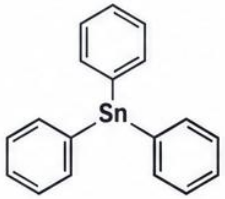
pesticides: triphenyltin

Mechanism

Organotins:
tributyltin (TBT), triphenyltin (TPT)

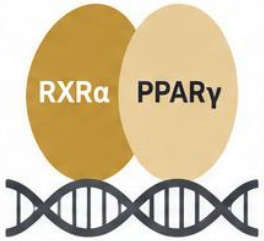


Tributyltin (TBT)



Triphenyltin (TPT)

bind **RXRα** and **PPARγ** at nanomolar concentrations
—
RXR/PPARγ heterodimer activated



PPAR response element (PPRE)

↑ adipocyte differentiation and lipid accumulation (adipogenesis)



Adipocytes

Evidence

Newborn mice exposed to TBT → **increased adiposity** in adulthood, transmitted to F2–F3 generations *(Grün et al. 2006)*

Classic ecotoxicology: TBT causes **imposex** in marine snails at picomolar levels

Obesogen hypothesis: endocrine-disrupting chemicals promote obesity by redirecting precursor cells toward adipocyte fate

Lesson: the same receptor promiscuity that causes drug toxicity lets environmental chemicals hijack metabolic regulators — nuclear receptors as targets of pollution.

TAKE-HOME MESSAGES

- Nuclear receptors mediate toxicity by altering gene transcription – no genotoxicity required.
- Ligand promiscuity (AhR, ER, PXR) means many unrelated chemicals can trigger the same receptor.
- Species differences in receptor biology (PPARα, PXR, CAR, AhR affinity) make extrapolation the central problem of risk assessment.
- Receptors cross-talk – toxicity rarely comes from a single receptor in isolation.
- NR toxicology is clinically relevant (troglitazone, rosiglitazone, isotretinoin) and therapeutically exploitable (tapinarof, AhR ligands in IBD).