Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

09b –Nuclear Receptors

AHR – Arylhydrocarbon Receptor

Luděk Bláha, PřF MU, RECETOX
www.recetox.cz

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

EVROPSKÁ UNIE esf

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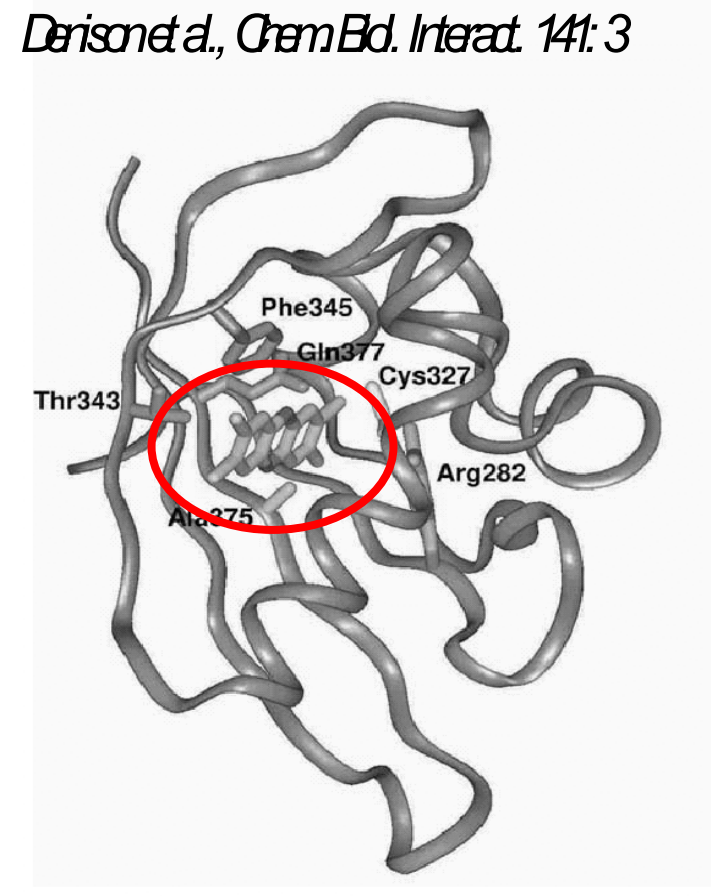
OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

AhR (Arylhydrocarbon receptor)

AhR structure



2,3,7,8-TCDD
(dioxin) bound to AhR

AhR

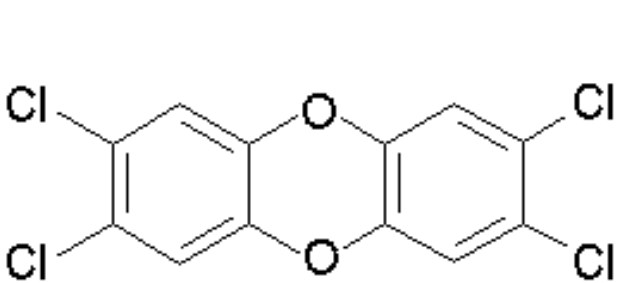
- Also known as „dioxin-receptor“ (and its modulation leads to so called „dioxin-like“ activity or toxicity)

- Ligand-activated transcription factor
  - Similar to all NRs
- AhR has effects on many different genes

- important mediator of toxicity of POPs – primary target of **planar aromatic substances**
  - regulator of xenobiotic metabolism and activation of promutagens

- Crossactivation/crosstalk with other NRs

- **Strongest known ligand - TCDD**
  - (not endogeneous !)



AhR regulated genes

- Many genes contain xenobiotic response elements (XRE) or dioxin responsive elements (DRE) in their promoter region:
  - **Detoxification genes** phase I enzymes (CYP 1A1, CYP 1A2, CYP 1B1) and phase II enzymes (UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase)
    → **Detoxification after toxicant exposure**
    … also with possible toxic consequences (oxidative stress, activation of promutagens accelerated clearance of hormones)
  - **Other genes** - regulation of cell cycle and apoptosis
    - Bax (apoptosis control), p27Kip1, Jun B (MAP-kinase), TGF-b (tumor growth factor)
      → Various adverse toxic effects

Physiological role of AhR

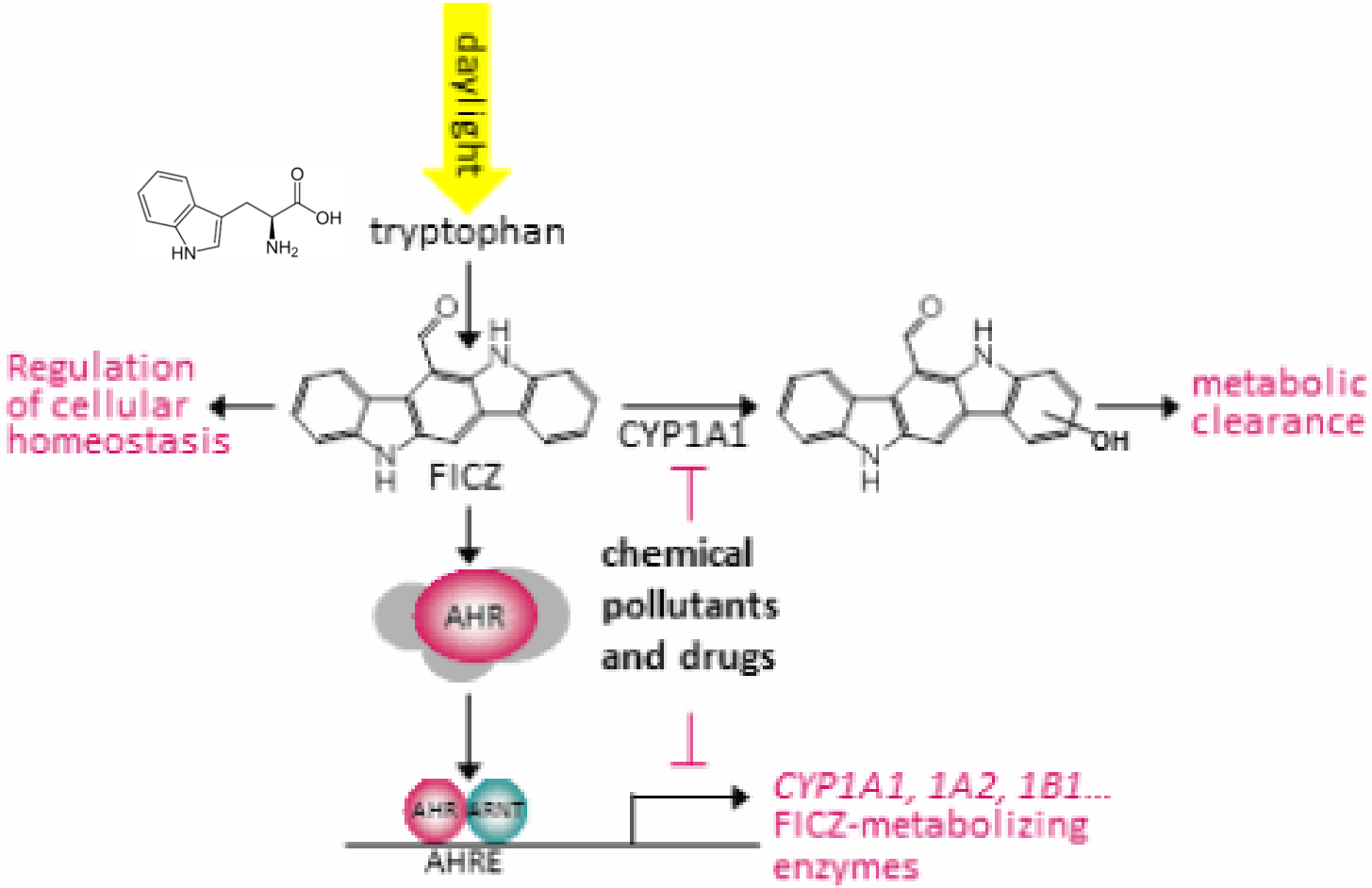
- **Physiological role for AhR still not known completely (?)**
  - Most likely – "protection" against toxicants → induction of detoxification

- Many adverse effects documented 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.

→ this implies presence of **natural endogeneous ligand(s)** (not only exogeneous toxicants can bind AhR)

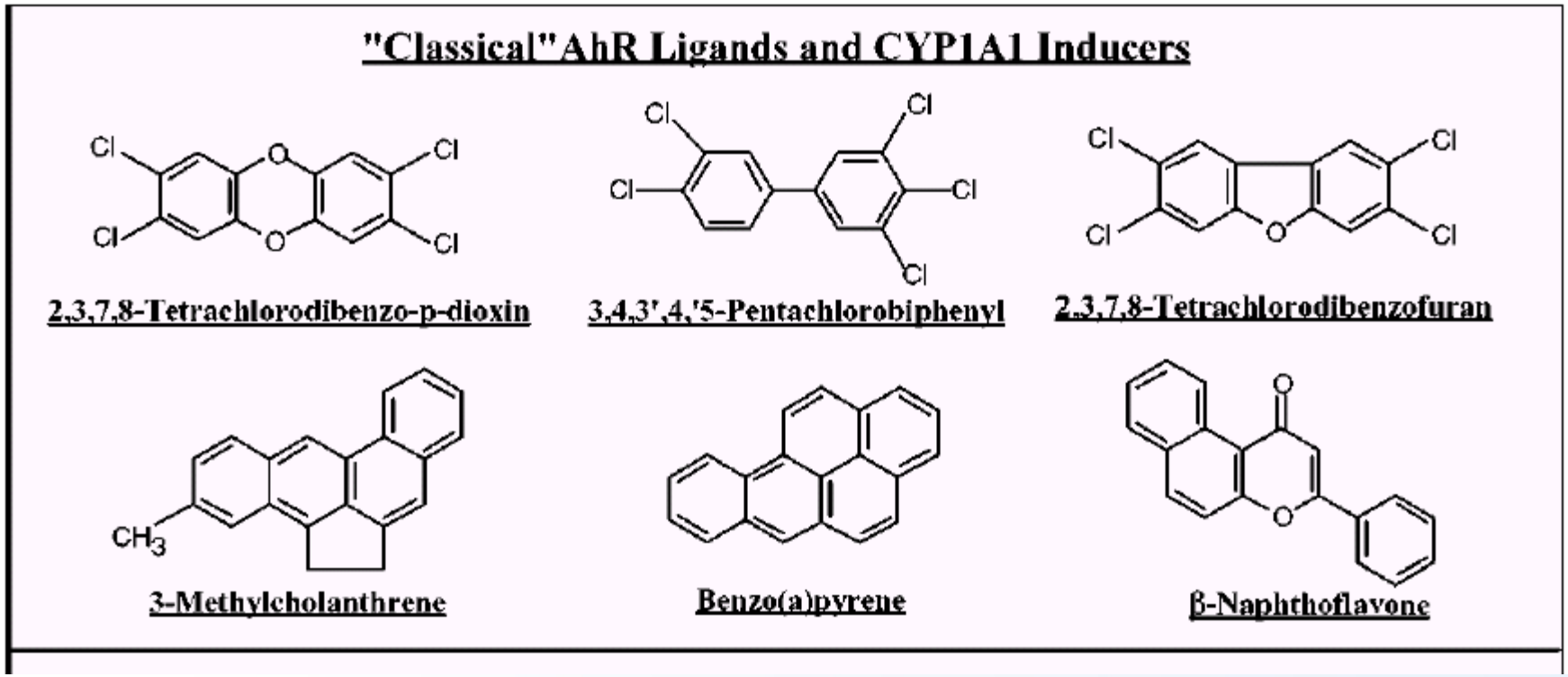
What is the natural (endogenous) physiological ligand of AhR ?

Potential candidate: 6-formylindolo[3,2-b]carbazole (FICZ)



Classical and “non-classical” AhR ligands

Classical = planar structures → direct binding to AhR

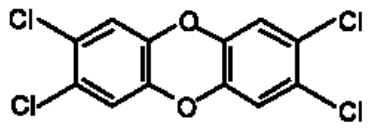


Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

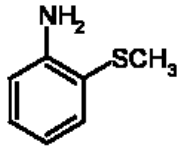
„Non-classical“ AhR ligands – various structures

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

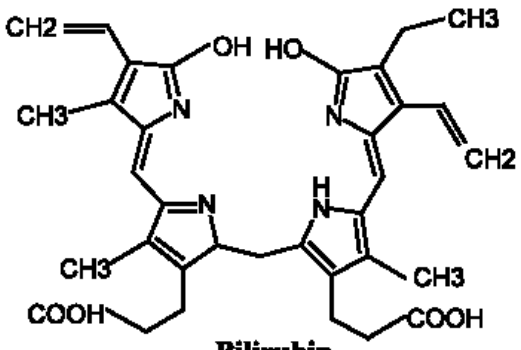
“Classical” ligand



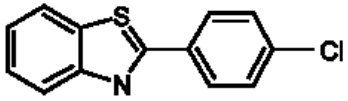
2,3,7,8-Tetrachlorodibenzo-p-dioxin



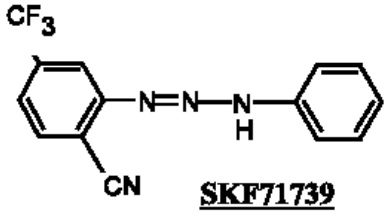
2-(Methylmercapto)aniline



Bilirubin



2-(4'-Chlorophenyl)benzothiazole



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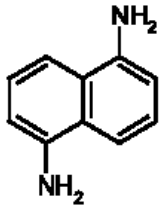
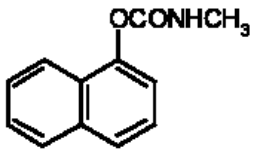
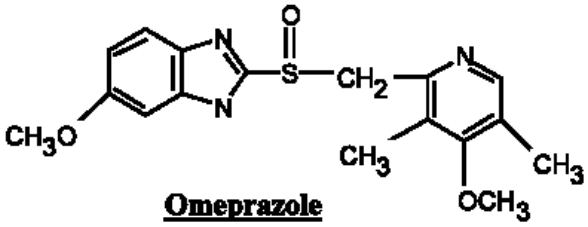


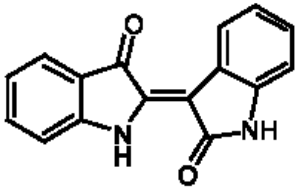
1,5-Diaminonaphthalene



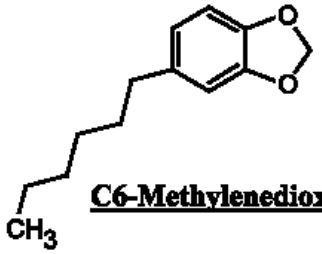
Carbaryl



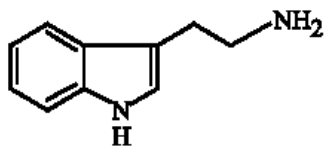
Omeprazole



Indirubin

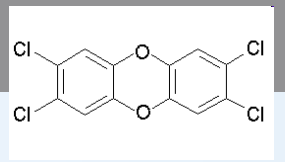


C6-Methylenedioxybenzene



Tryptamine

Biological responses to TCDD & AhR ligands



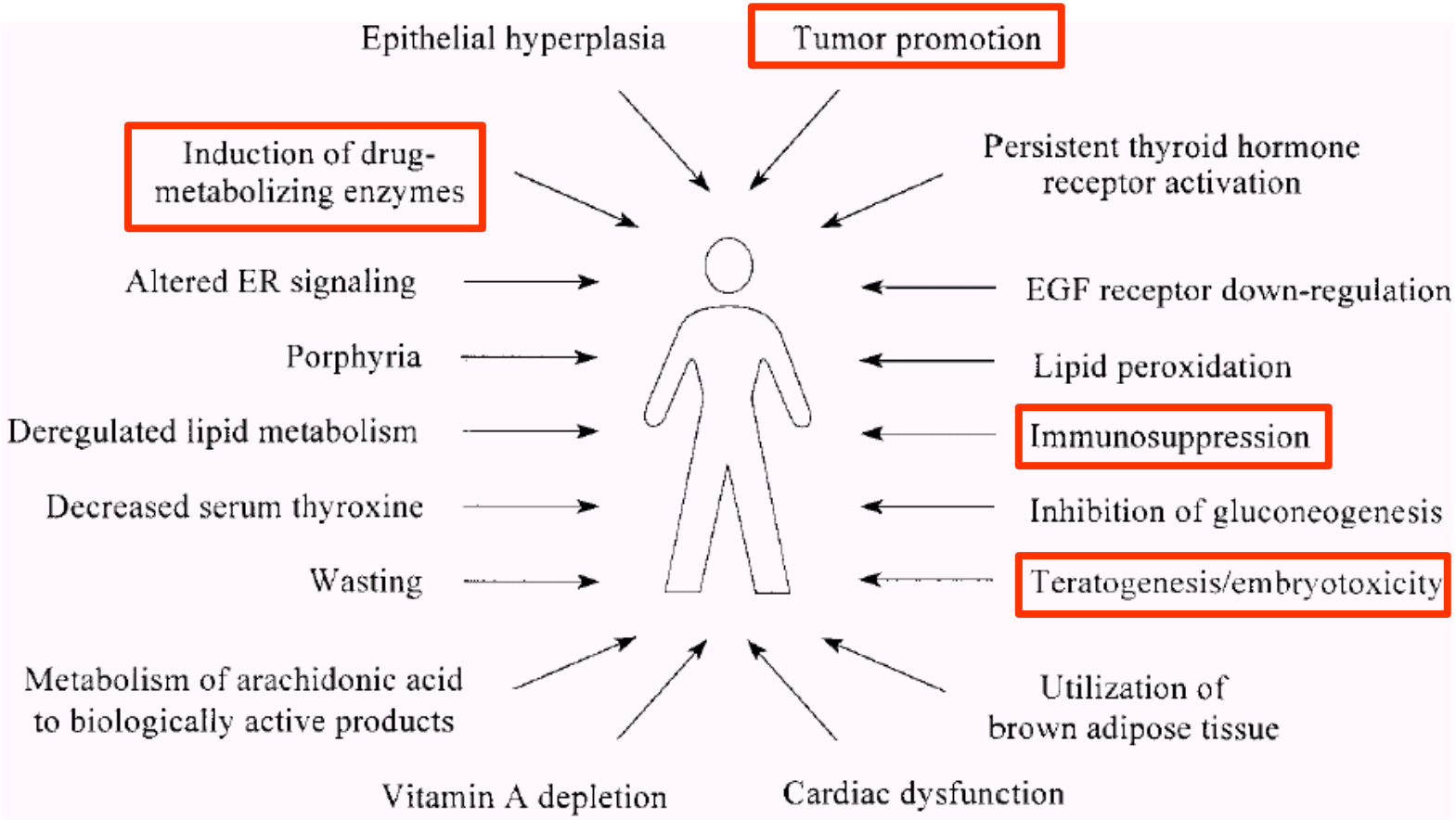


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

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

Toxic equivalency factors (TEF)/TEQ concept

- Toxicity of compounds with similar toxicological properties as TCDD (activating AhR) 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** (water, sediment, food) containing a mixture of dioxins and dioxin-like compounds.

- The total potency of a mixture can be expressed in TCDD TEQ concentration
  - i.e. TEQ = concentration corresponding to the effect that would be induced by TCDD

$$\mathrm{TEQ} = \Sigma\{\mathrm{compound}_1 \times \mathrm{TEF}_1 + \ldots$$

$$+ \mathrm{compound}_n \times \mathrm{TEF}_n\}$$

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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)

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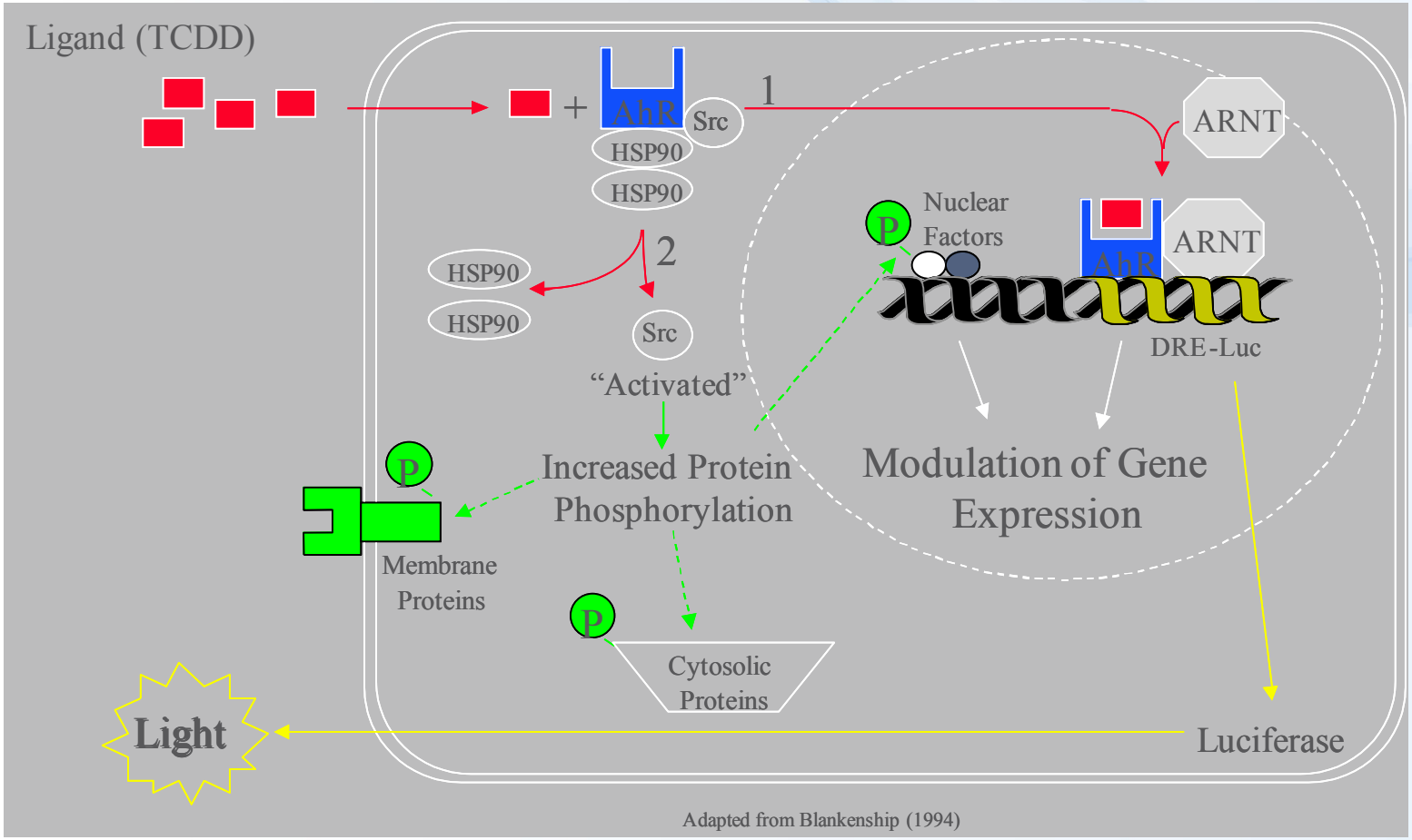
Biomarkers/bioanalytical methods for AhR toxicity

- In vivo studies
  - liver enlargement, reduction of thymus weight, wasting syndrome, reproductive and developmental disorders
- In vivo biomarkers
  - **EROD** activity, CYP 1A1 and 1B1 expression (discussed in biomarker section)

- in vitro assessment of chemical potencies
  - EROD (ethoxyresorufin-O-deethylase activity) in cell cultures;
  - **CALUX/CAFLUX assays** (luciferase expression – reporter gene assays)
  - GRAB assay (AhR-DNA binding)
  - yeast bioassay;
  - immunoassays;
  - detection of CYP1A mRNA (qPCR) or AhR protein (western blotting)

In vitro CALUX/CAFLUX assays

CALUX – Chemical Assisted Luciferase Expression
DR-CALUX (Dioxin Responsive CALUX)
(i.e. Luciferase Reporter Gene Assay with H4IIE.luc cells)



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DETECTION of EROD activity - example

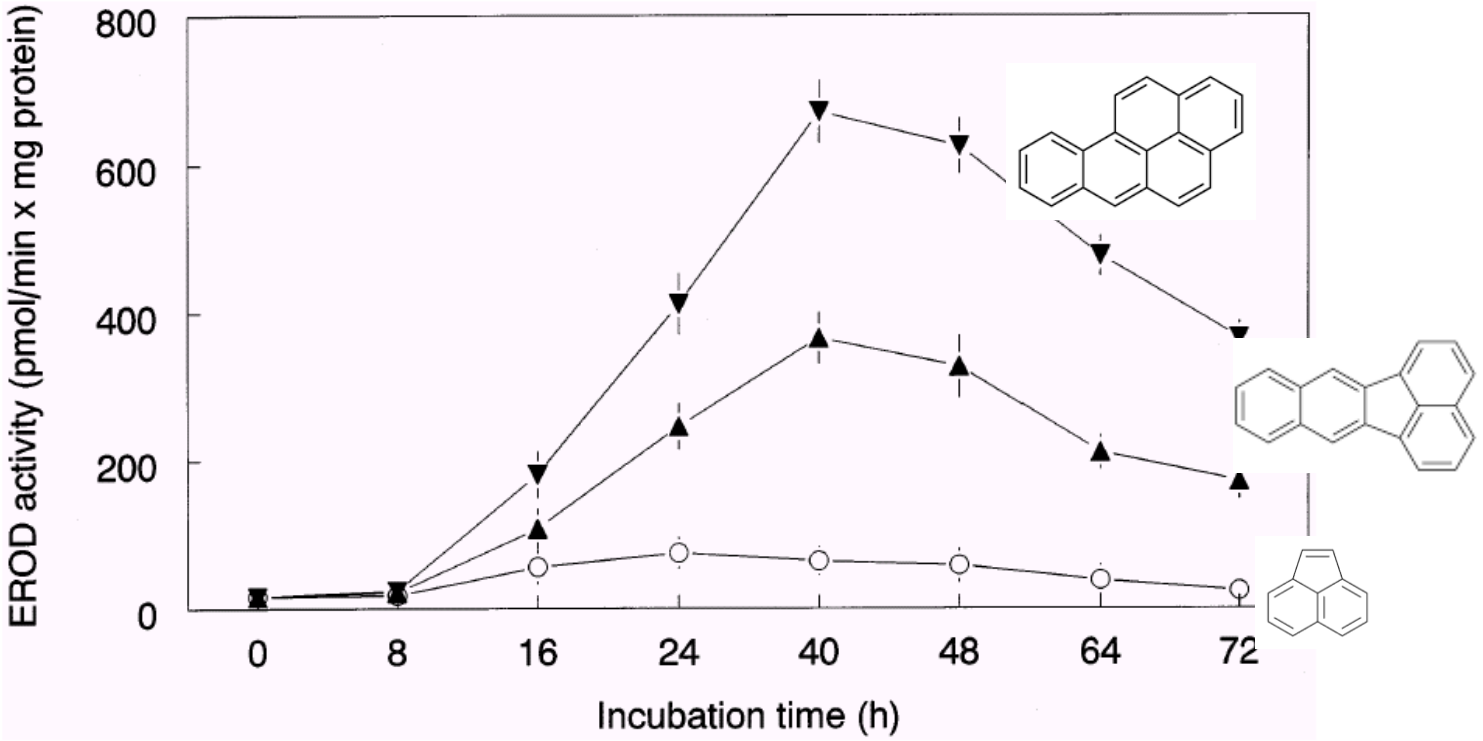
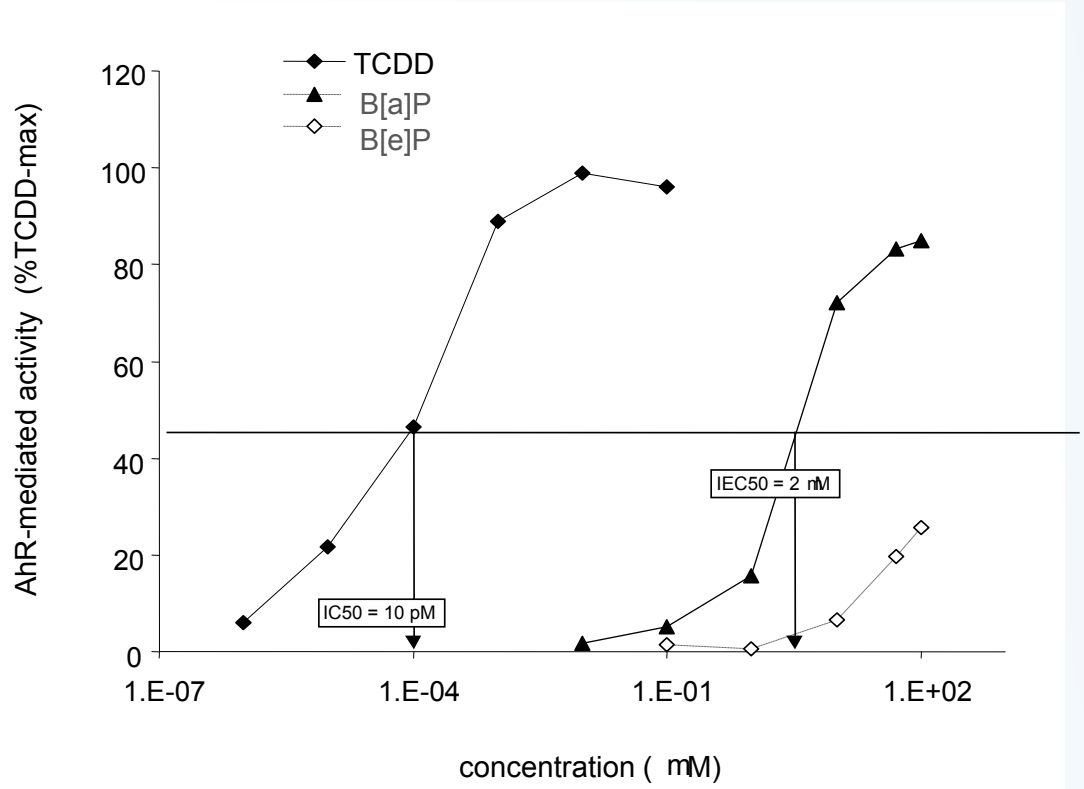


Fig. 2. Time course of induction of CYP1A1-catalyzed 7-ethoxyresorufin *O*-deethylase (EROD) activity in primary cultures of rat hepatocytes, after addition of 1.7×10^{-5} M benzo[*a*]pyrene (-▼-), 1.9×10^{-6} M benzo[*k*]fluoranthene (-▲-) or 9.4×10^{-5} M acenaphthylene (-○-). EROD activity was determined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

Comparing toxicity of compounds → Application in Risk Assessment

- Quantification of effects (EC_{50})
- Comparison with the effect of reference toxicant (2,3,7,8-TCDD)
  - → relative potencies (REPs) to TCDD
    (= in vitro "Toxic Equivalency Factors" ~ TEFs)



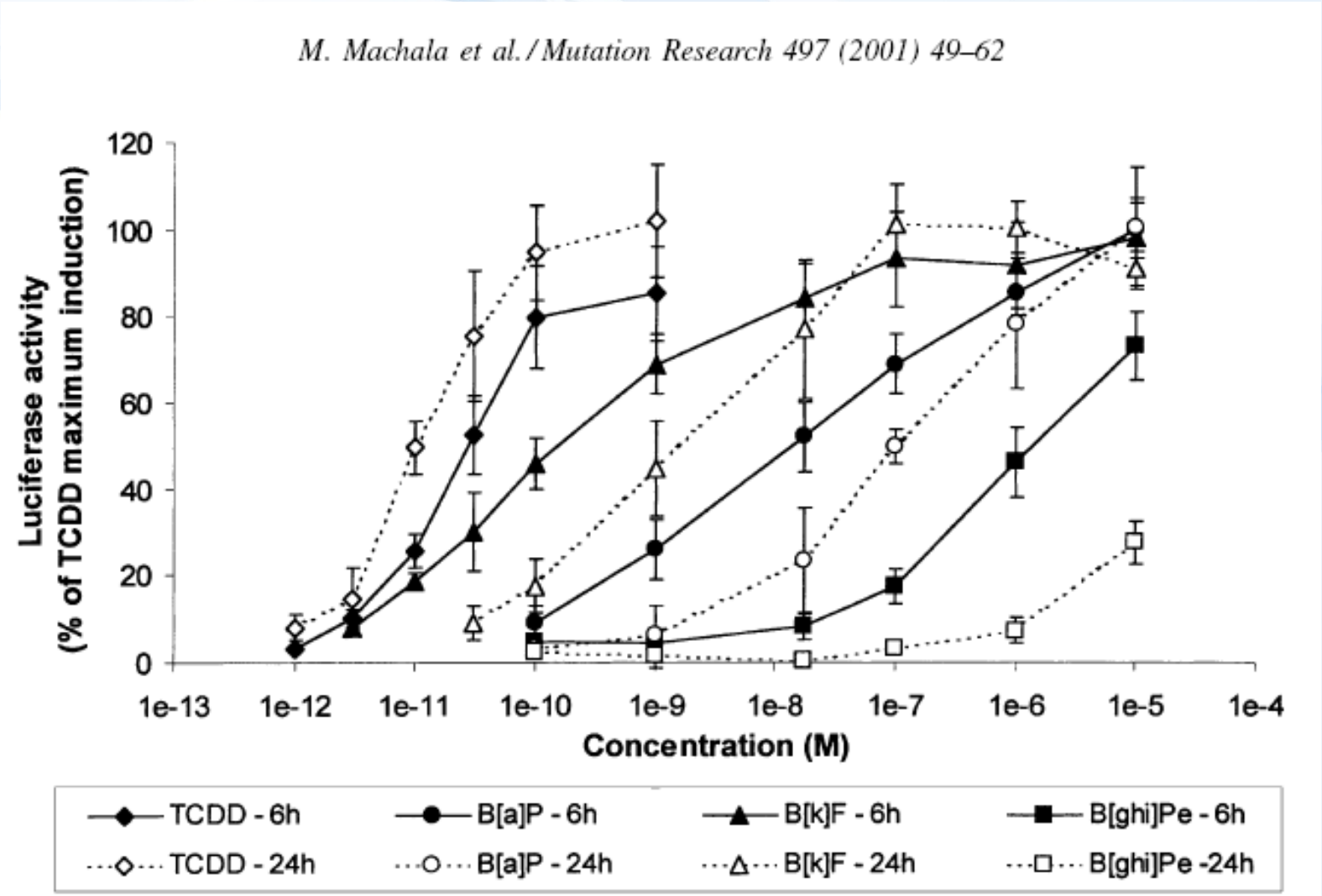
TCDD: IC_{50}
PAH: IEC_{50}

Relative Potency (REP)
= Induction Equivalency Factor

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

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

Example - relative potencies of PAHs (two exposure periods) „CALUX“ assay



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Longer period: lower induction due to (partial) metabolization of PAHs (CYPs, oxidation) to products less potent to AhR

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.

Summary – Nuclear receptors

- Important physiological functions,
- Important roles in pathologies and chemical toxicity (ENDOCRINE DISRUPTION)

- NRs with well studied roles in toxicity: **ER and AhR**
  - Other NRs (AR, RAR/RXR, ThR) – important but less explored

- All NRs share similar structure and mechanisms of action
  - Act as direct transcription factors on DNA
- Natural ligands of NRs are small lipophilic hormones
  - steroids, thyroids, retinoids
  - Various regulatory functions
  - Role in toxicity: NR interact with **structurally similar xenobiotics**
- Various mechanisms beyond the toxicity
  - Adverse are both STIMULATIONS and INHIBITIONS directly at the receptor site (e.g. "anti-androgenicity)
  - Additional mechanisms –in blood (Thyroids), metabolism (Thyroids) clearance (Retinoids), heterodimerization and transport of hormones, "crosstalk" of different NRs

- **Other key information to remember**
  - REPORTER GENE ASSAYS (principle, use, what is CALUX?)
  - Characterization of chemical "toxic potentials"
    - General concept of "REPs" (valid for activation of all NRs)
    - Specifically for AhR - concept of TEFs / TEQs