Fyziologie působení farmak a toxických látek

Přednáška č.9
Endokrinní disrupce u obratlovců II. Modulace funkcí RAR/RXR, TR a PPAR – deregulace vývoje organismu, modulace endokrinních signálů a karcinogenní účinky;

Efekty spojené s deregulací hladiny retinoidů:

- Funkce RA;
- Vznik končetin;
- Vývoj nervové soustavy;
- Vývojové abnormality obojživelníků;
- Narušení hladin vitaminu A;

Struktura a syntéza kyseliny retinové

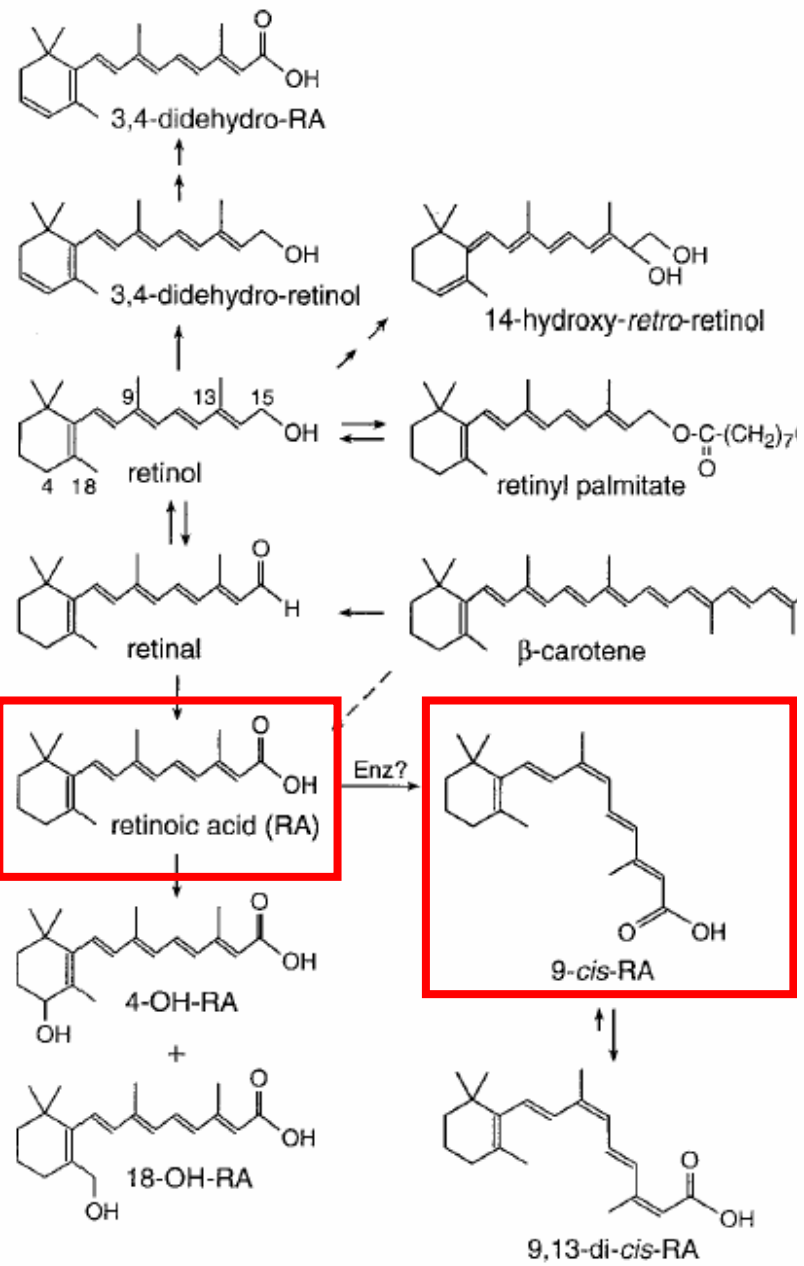


FIG. 2. Structures of naturally occurring retinoids.

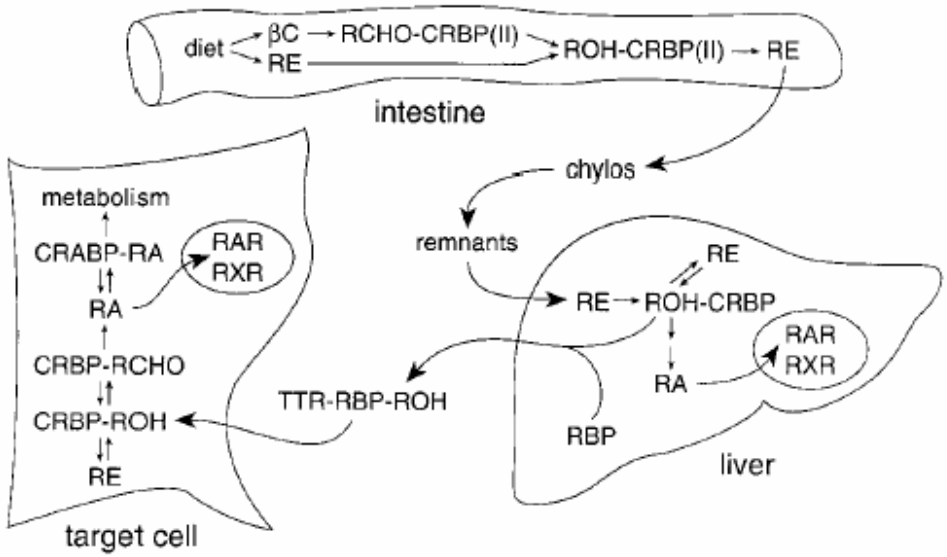


FIG. 3. Absorption, distribution, and metabolism of naturally occurring retinoids.

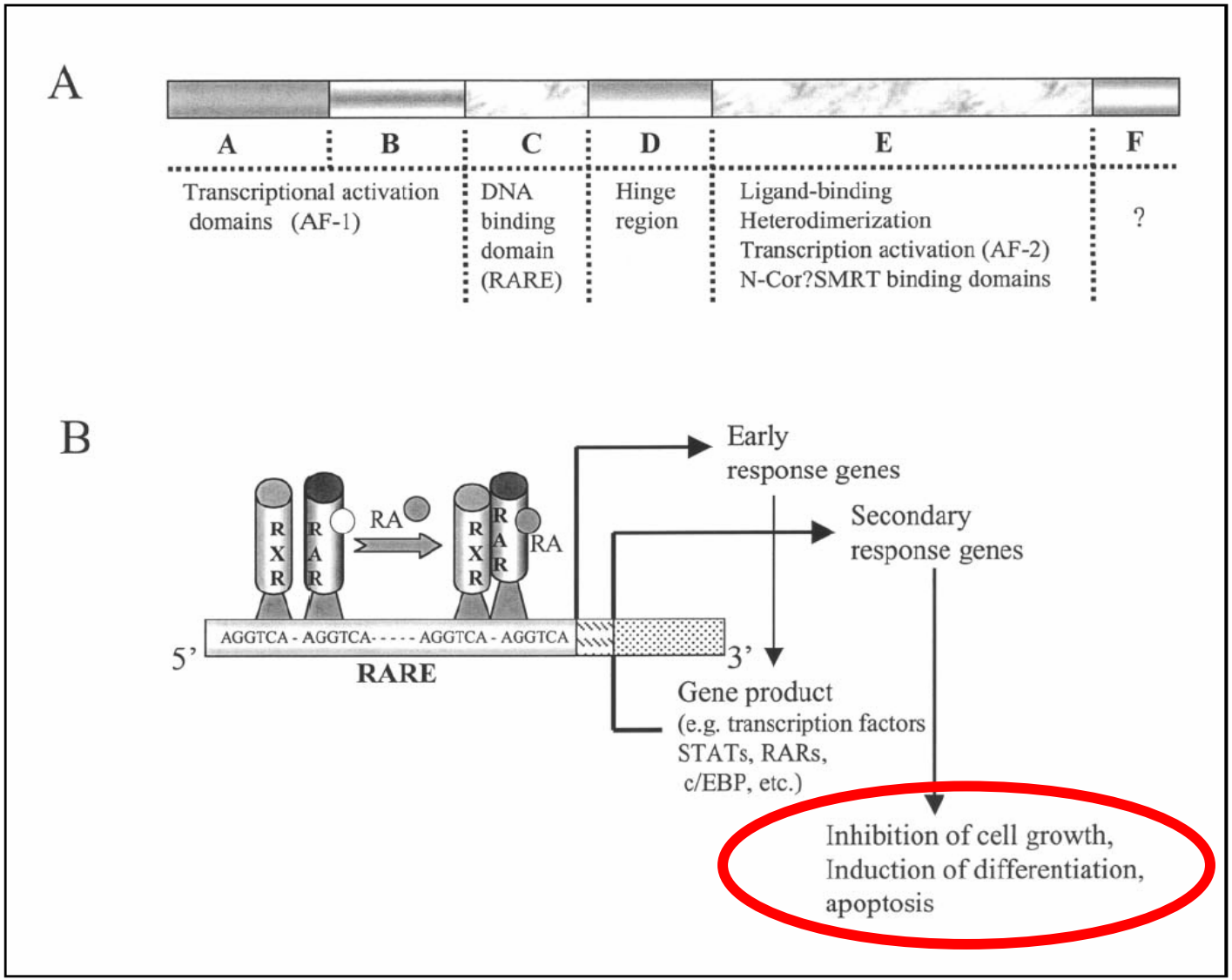


Fig. 1 - Structure and functions of retinoid receptors. A) Schematic representation of retinoid receptor protein depicting various functional domains. B) A molecular model for retinoid action. The liganded RAR forms heterodimer with RXR, binds to specific regulatory sequences (RARE) in the promoter region of target genes. Transactivation of such early response genes is a primary event of retinoid action. In addition to this, the products of early response genes can activate the transcription of secondary genes. Transactivation of these genes therefore represents secondary action of retinoids since their transcription requires protein synthesis. This cascade of gene events leads to secondary and tertiary events that eventually produce a phenotype that is characteristic of retinoid action.

• Abnormalities caused by exogenous agents (certain chemicals or viruses, radiation, or hyperthermia) are called **disruptions**. The agents responsible for these disruptions are called **teratogens**. Most teratogens produce their effects only during certain critical periods of development. The most critical time for any organ is when it is growing and forming its structures. Different organs have different critical periods, but the time from period from day 15 through day 60 of gestation is critical for many human organs.

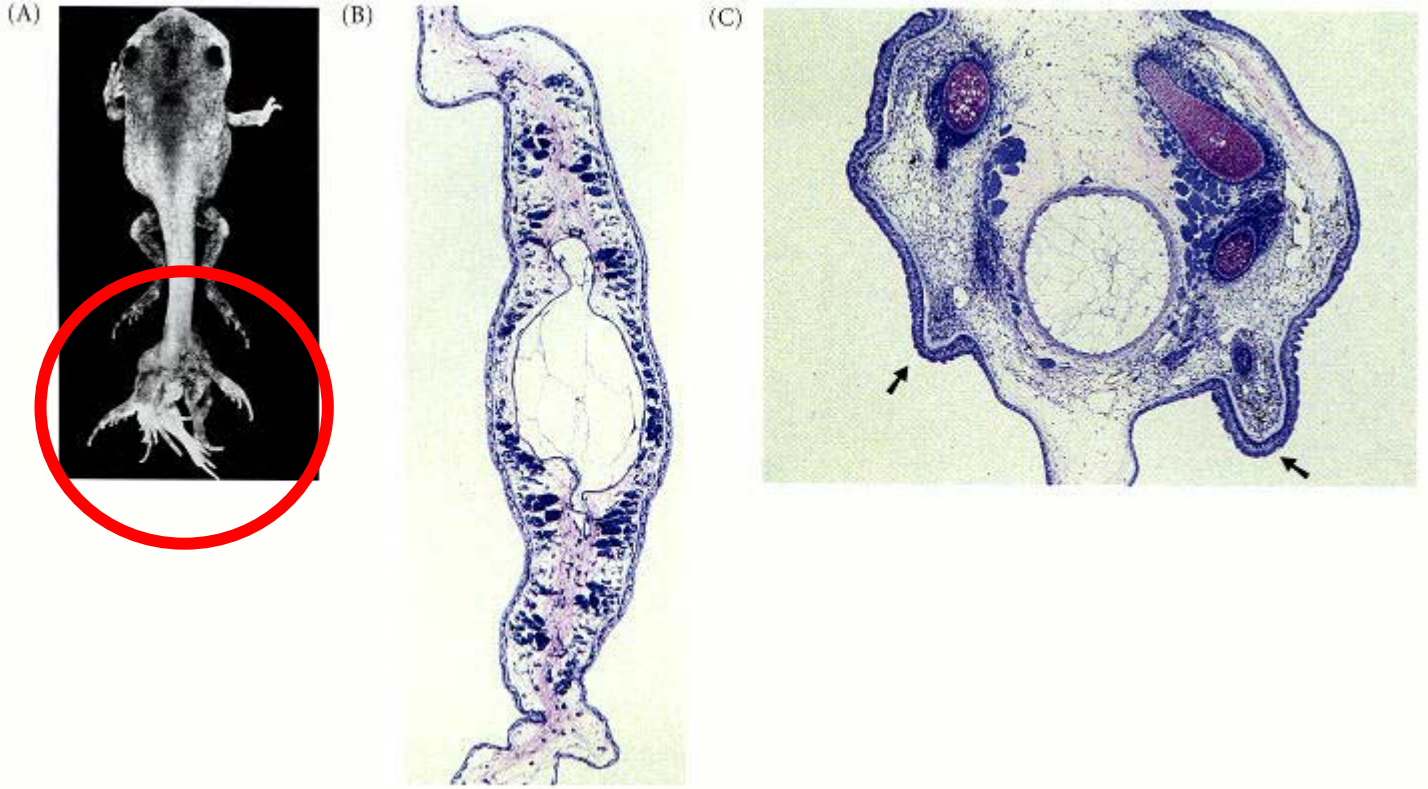
• **Retinoic acid is important in forming the anterior-posterior axis of the mammalian embryo and also in forming the limbs**. In these instances, retinoic acid is secreted from discrete cells and works in a small area. However, if retinoic acid is present in large amounts, cells that normally would not receive such high concentrations of this molecule will respond to it. **Inside the developing embryo, vitamin A and 13-*cis*-retinoic acid become isomerized to the developmentally active forms of retinoic acid, all-*trans*-retinoic acid and 9-*cis*-retinoic acid. Some of the Hox genes have retinoic acid response elements in their promoters.**

• The disastrous consequences of exposure to exogenous retinoids during human pregnancy were underscored in the early 1980s when the drug Accutane® (the trade name for isoretinoin, or 13-*cis*-retinoic acid) was introduced as a treatment for severe acne. Women who took this drug during pregnancy had an increased number of spontaneous abortions and children born

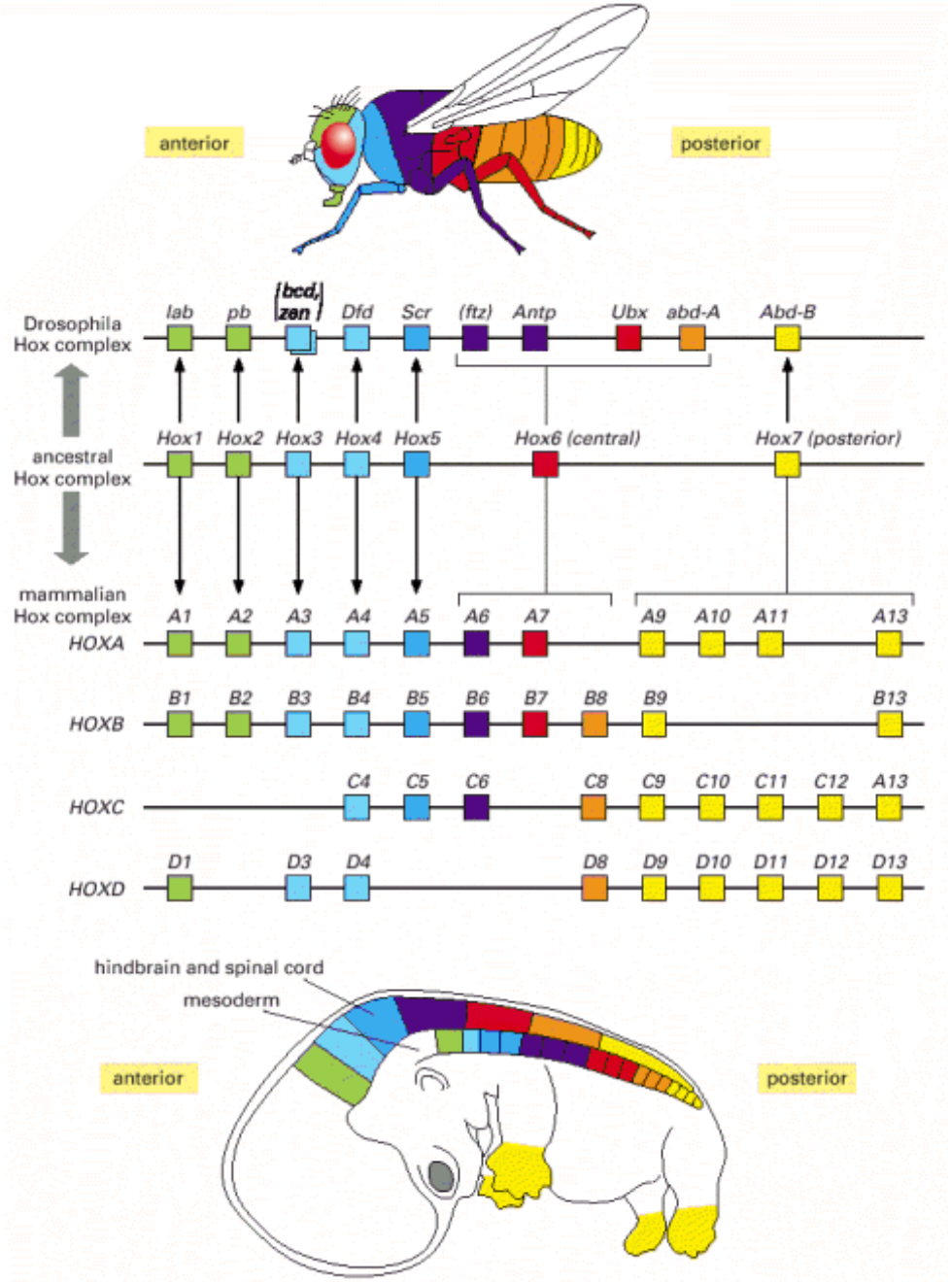
RA reguluje vznik a vývoj končetin

There are discrete positions where limb fields are generated. Researchers have precisely localized the limb fields of many vertebrate species. Interestingly, in all land vertebrates, there are only four limb buds per embryo, and they are always opposite each other with respect to the midline. **Although the limbs of different vertebrates differ with respect to which somite level they arise from, their position is constant with respect to the level of Hox gene expression along the anterior-posterior axis.** For instance, in fishes (in which the pectoral and pelvic fins correspond to the anterior and posterior limbs, respectively), amphibians, birds, and mammals, the forelimb buds are found at the most anterior expression region of *Hoxc-6,* the position of the first thoracic vertebra.

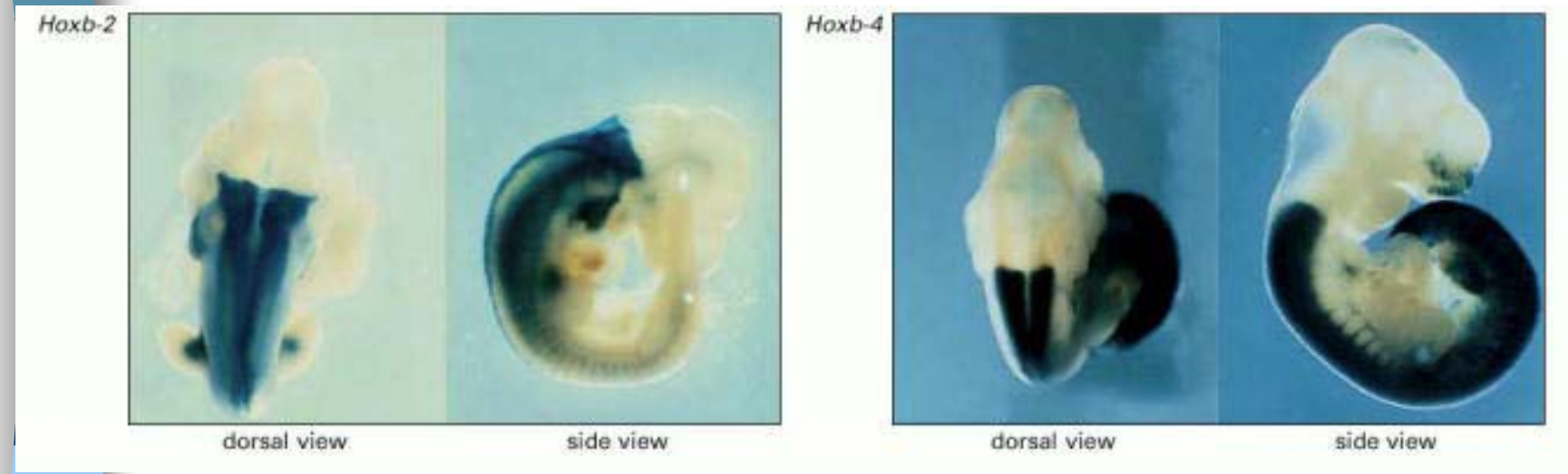
Retinoic acid appears to be critical for the initiation of limb bud outgrowth, since blocking the synthesis of retinoic acid with certain drugs prevents limb bud initiation, suggested that a gradient of retinoic acid along the anterior-posterior axis might activate certain homeotic genes in particular cells and thereby specify them to become included in the limb field.



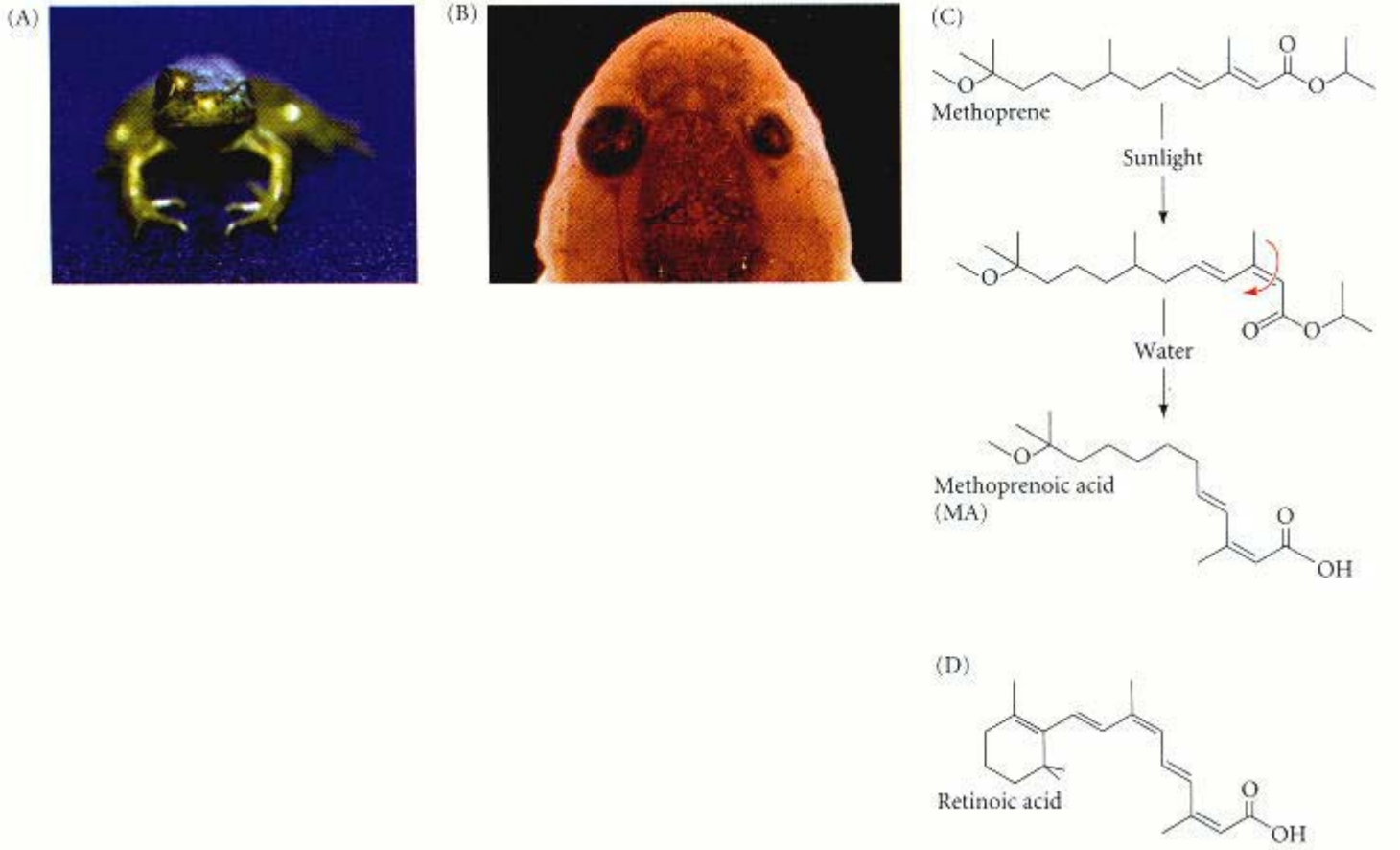
Legs regenerating from retinoic acid-treated tadpole tail. (A) The tail stump of a balloon frog tadpole treated with retinoic acid after amputation will form limbs from the amputation site. (B) Normal tail regeneration in a *Rana temporaria* tadpole 4 weeks after amputation. A small neural tube can be seen above a large notochord, and the muscles are arranged in packets. No cartilage or bone is present. (C) A retinoic acid-treated tadpole tail makes limb buds (arrows) as well as pelvic cartilage and bone. The cartilaginous rudiment of the femur can be seen in the right limb bud.



The Hox complex of an insect and the Hox complexes of a mammal compared and related to body regions.

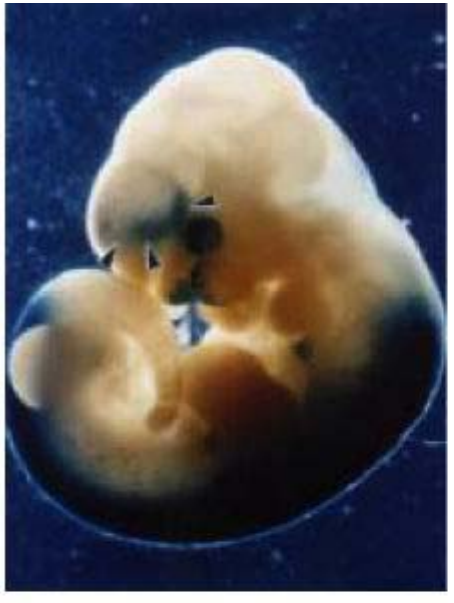


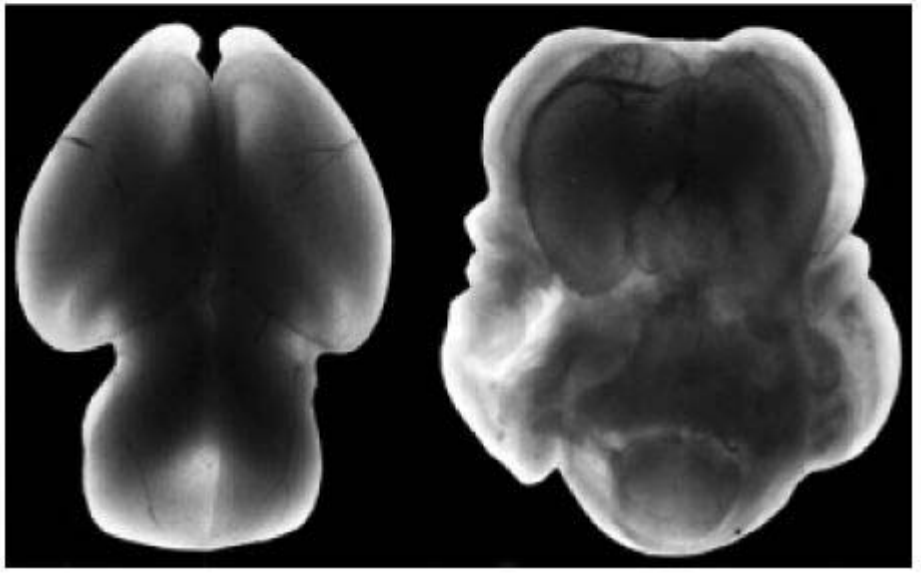
Expression domains of Hox genes in a mouse. The photographs show whole embryos displaying the expression domains of two genes of the HoxB complex *(blue* stain). These domains can be revealed by *in situ* hybridization or, as in these examples, by constructing transgenic mice containing the control sequence of a Hox gene coupled to a *LacZ* reporter gene, whose product is detected histochemically. Each gene is expressed in a long expanse of tissue with a sharply defined anterior limit. The earlier the position of the gene in its chromosomal complex, the more anterior the anatomical limit of its expression. Thus, with minor exceptions, the anatomical domains of the successive genes form a nested set, ordered according to the ordering of the genes in the chromosomal complex.



Teratogenesis in frogs. (A) Wild green frog (*Rana clamitans*) with an eye deformity, collected in New Hampshire in 1999 by K. Babbitt. (B) *Xenopus* tadpole with eye deformities caused by incubating newly fertilized eggs in water containing methoprenic acid, a by-product of methoprene. (C) One of several pathways by which methoprene can decay into teratogenic compounds such as methoprenic acid. (D) An isomer of retinoic acid showing the structural similarities to methoprenic acid.

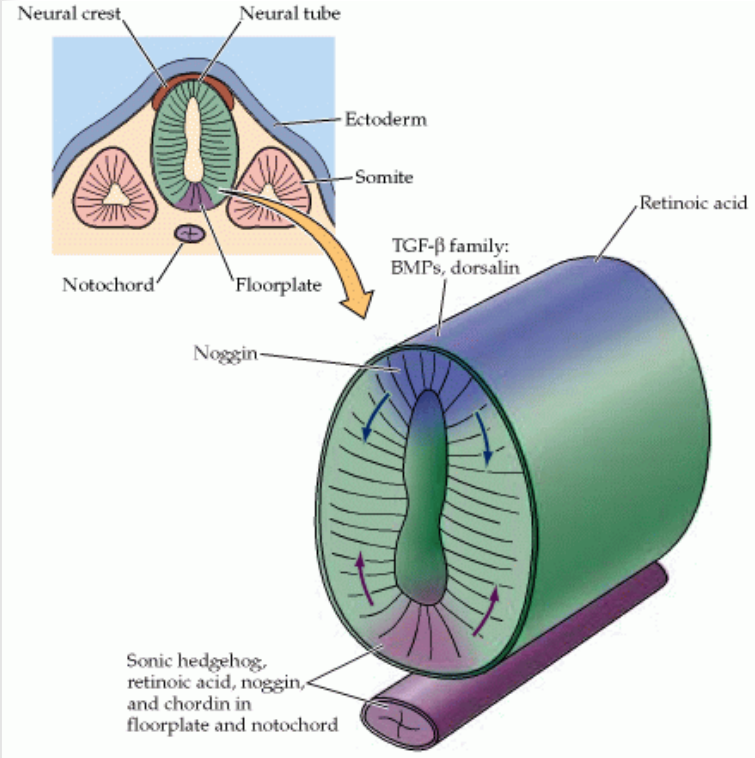
RA reguluje vývoj CNS





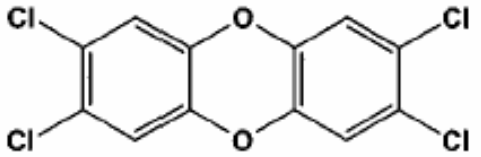
Top panel: At left, retinoic acid activates gene expression in a subset of cells in the normal developing forebrain of a midgestation mouse embryo (blue areas indicate β-galactosidase reaction product, an indicator of gene expression in this experiment); at right, after maternal ingestion of a small quantity of retinoic acid (0.00025 mg/g of maternal weight), gene expression is ectopically activated throughout the forebrain.

Bottom panel: At left, the brain of a normal mouse at term; at right, the grossly abnormal brain of a mouse whose mother ingested this same amount of retinoic acid at mid-gestation.

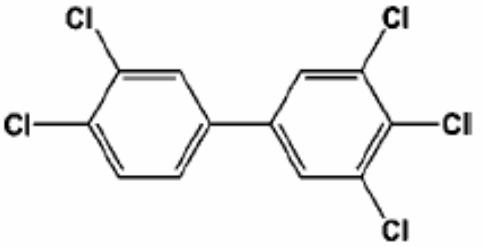


Location of some inductive signals in the developing neural tube. Inductive signals are provided by either the notochord, the floorplate, the roofplate and dorsal ectoderm, or the somites. These signals act locally on either the ventral or dorsal neuroepithelium of the developing spinal cord and hindbrain to elicit distinct patterns of gene expression and, ultimately, differentiation of specific classes of neurons. The peptide hormone sonic hedgehog (shh) is the most important ventral signal and is produced by both the notochord and floorplate. In addition, noggin, chordin, and retinoic acid are produced either by the notochord or floorplate. In contrast, a variety of signals including dorsalin and other members of the TGF family as well as noggin and retinoic acid are provided by the roofplate and dorsal ectoderm. These signals influence the differentiation of several dorsal cell types including the neural crest

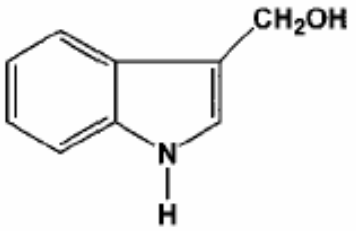
PHAHs a PAHs narušují funkci a strukturu štítné žlázy a hladiny thyroidních hormonů a retinoidů



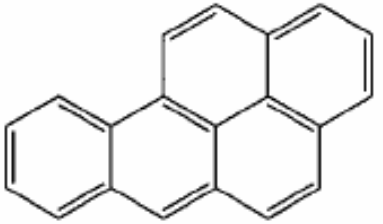
2,3,7,8-TCDD



3,3',4,4',5-pentaCB



I3C



BaP

Figure 5 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds that bind to the AhR.

TABLE 1. Alterations in thyroid gland morphology, thyroid hormones and retinoid levels in marine mammals associated with exposure to polyhalogenated aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thyroid/retinoid changes	References
Harbor seal[a] (*Phoca vitulina*)	North Sea	PCBs[b]	thyroid gland	thyroid colloid depletion interfollicular fibrosis	Schumacher et al., (1993)
Harbor porpoise (*Phocoena phocoena*)					
Beluga whale (*Delphinapterus leucas*)	St. Lawrence Estuary, Quebec	PCBs other OCs[c]	thyroid gland	thyroid abscesses thyroid adenoma	De Guise et al., (1995)
Northern elephant seal (*Mirounga angustirostris*)	California	PCBs p,p′-DDE[d]	plasma	↓ retinol ↓ TT_4[e], TT_3[f]	Beckmen et al., (1997)
Harbor seal (*Phoca vitulina*)	captive	PCBs p,p′-DDE	plasma	↓ retinol ↓ TT_4, FT_4[g], TT_3	Brouwer et al., (1989)
Harbor seal (*Phoca vitulina*)	captive	PCBs dioxin TEQ's[h]	plasma	↓ retinol ↓ TT_4, TT_3[i]	De Swart et al., (1994, 1995)
Grey seal[j] (*Halichoerus grypus*)	Norway	PCBs	plasma	↓ TT_4, FT_4	Jenssen et al., (1995)
Grey seal[k] (*Halichoerus grypus*)	United Kingdom	PCB 169	plasma	↓ TT_3:TT_4	Hall et al., (1998)

TABLE 3. Alterations in thyroid gland morphology and retinoid levels in fish associated with exposure to polyhalogenated aromatic hydrocarbons and polynuclear aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thyroid/retinoid changes	References
Salmon species (*Oncorhynchus* sp.)	Great Lakes	unknown factor	thyroid gland	thyroid hypertrophy, hyperplasia	Sonstegard et al., (1976)
White sucker (*Catostomus commersoni*)	Montreal, Quebec	coplanar PCBs[a]	liver	↓ retinol ↓ retinyl palmitate	Spear et al., (1992) Branchaud et al., (1995)
Lake sturgeon (*Acipenser fulvescens*)	Montreal, Quebec	PCBs	intestine	↓ retinyl palmitate ↓ dehydroretinyl palmitate	Ndayibagira et al., (1994)
Lake sturgeon (*Acipenser fulvescens*)	St. Lawrence River, Quebec	coplanar PCBs	liver	↑ RA[b] metabolism ↓ retinoids	Doyon et al., (1999)
Brown bullheads (*Ameiurus nebulosus*)	Great Lakes	PAHs[c]	liver	↓ retinyl palmitate ↓ dehydroretinyl palmitate	Arcand-Hoy et al., (1999)

TABLE 2. Alterations in thyroid gland morphology, thyroid hormones and retinoid levels in free-ranging avian species associated with exposure to polyhalogenated aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thryoid/retinoid changes	References
Herring gulls (*Larus argentatus*)	Great Lakes	PHAHs[a]	thyroid	↑ thyroid mass thyroid hyperplasia	Moccia et al., (1986)
Herring gulls (*Larus argentatus*)	Great Lakes	PHAHs	liver	↓ retinyl palmitate	Government of Canada, (1991)
Herring gulls (*Larus argentatus*)	Great Lakes	2,3,7,8-TCDD[b]	liver	↓ retinol ↓ retinyl palmitate	Spear et al., (1986, 1992)
Herring gulls (*Larus argentatus*)	Lakes Huron, Erie, Ontario	2,3,7,8-TCDD ΣPCDDs + PCDFs[c] dioxin TEQs[d]	egg yolk	↑ retinol : retinyl palmitate	Spear et al., (1990)
Great blue herons (*Area herodias*)	St. Lawrence River, Quebec	ΣPCBs 105 + 118[e] ΣPCBs 105 + 118 TEQs[f]	egg yolk	↓ retinyl palmitate	Boily et al., (1994)
Cormorants (*Phalacrocorax carbo*)	Netherlands	PCBs[g] PCDDs PCDFs	egg yolk liver plasma	↓ FT_4[h] ↑ EROD[i]	van den Berg et al., (1994)
Herring gulls (*Larus argentatus*) Caspian terns (*Sterna caspia*)	Great Lakes	PCBs[j]	plasma[k]	↓ retinol	Grasman et al., (1996)
Common terns (*Sterna hirundo*)	Belgium Netherlands	mono-ortho PCBs PCDDs PCDFs	egg yolk plasma liver	↓ retinyl palmitate[l] ↓ TT_3[m], TT_4[n], FT_4 ↑ plasma retinol to yolk sac retinyl palmitate	Murk et al., (1996)
Herring gulls (*Larus argentatus*)	Great Lakes	PCBs, DDE,[o] dieldrin, mirex	liver	↓ retinyl palmitate	Fox et al., (1998)
Tree swallows[p] (*Tachycineta bicolor*)	Great Lakes St. Lawrence River basin	Ah-inducing chemicals[q]	liver	↓ retinol ↑ EROD	Bishop et al., (1999)

PHAHs modulují hladiny retinoidů – mobilizace zásob vitaminu A v játrech

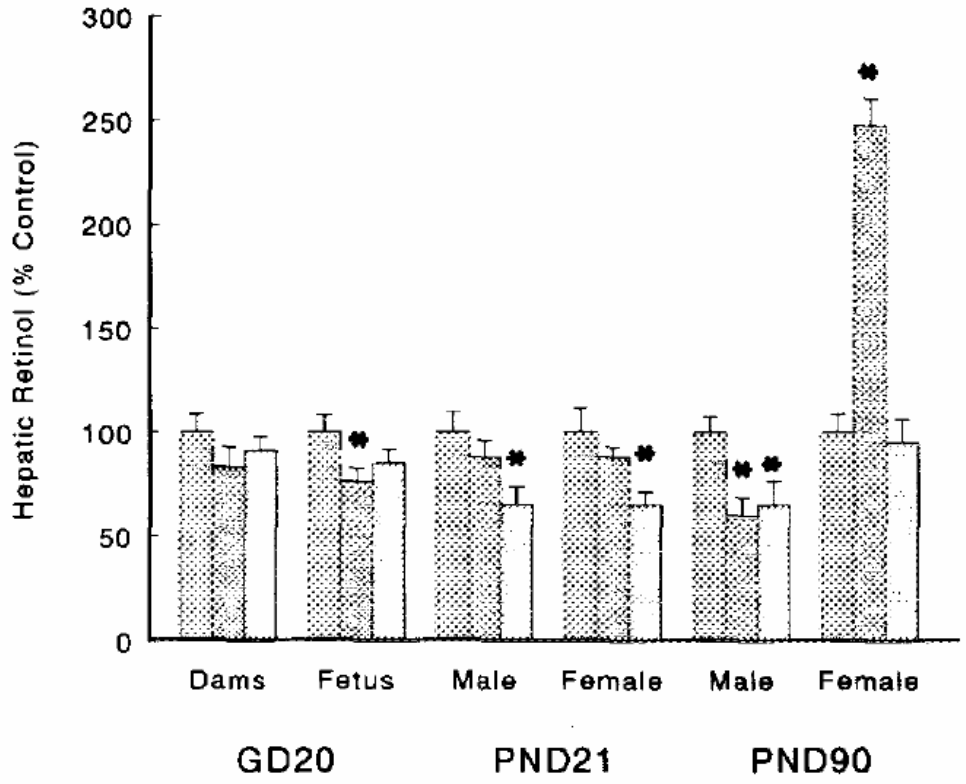


FIG. 3. Hepatic retinol concentrations, expressed as percentage of control values, mean ± SEM, from dams, their fetuses (N = 6), male and female neonates (N = 8–10), and adult offspring (N = 10) following maternal exposure to 0 □, 5 ▩, or 25 □ mg Aroclor 1254/kg on Days 10–16 of gestation. *Indicates a significant difference from controls, $p < 0.05$. GD20, Gestation Day 20; PND21, Postnatal Day 21; PND90, Postnatal Day 90.

BPA moduluje hladiny retinoidních receptorů v průběhu embryogeneze - myši

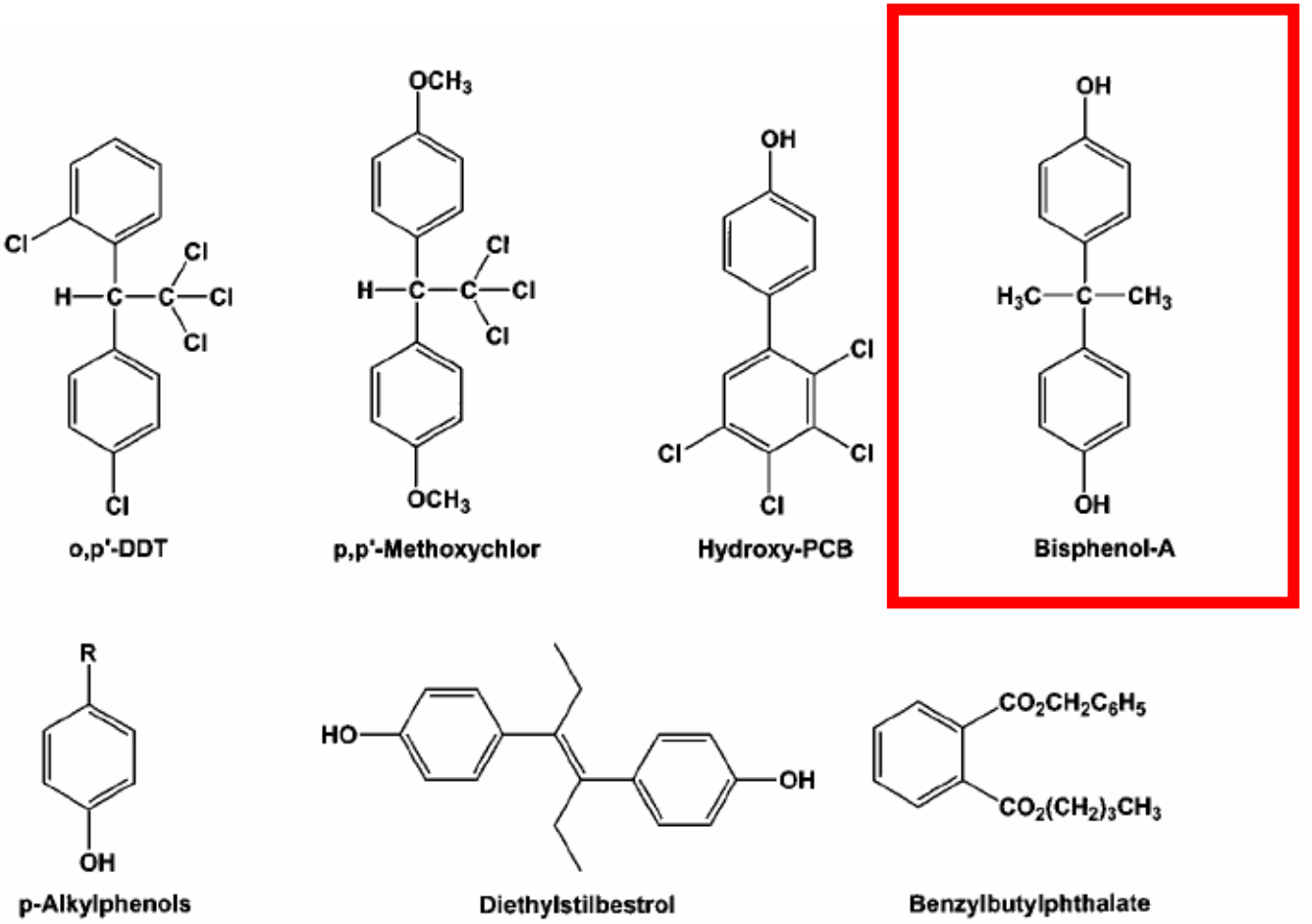


Figure 2 Structures of some xenoestrogens.

Funkce thyroidních hormonů v ontogenezi a vliv organických polutantů:

- Funkce thyroidních hormonů v metamorfóze
- Funkce thyroidních hormonů ve vývoji nervové soustavy;

Hypotéza – environmentální polutanty jako kauzální faktor neurologických poruch (autismus, poruchy učení, hyperaktivita, nádorová onemocnění, juvenilní formy diabetes);

- Toxické látky narušující thyroidní regulace;

T3 a T4 mají zásadní význam pro iniciaci metamorfózy obojživelníků

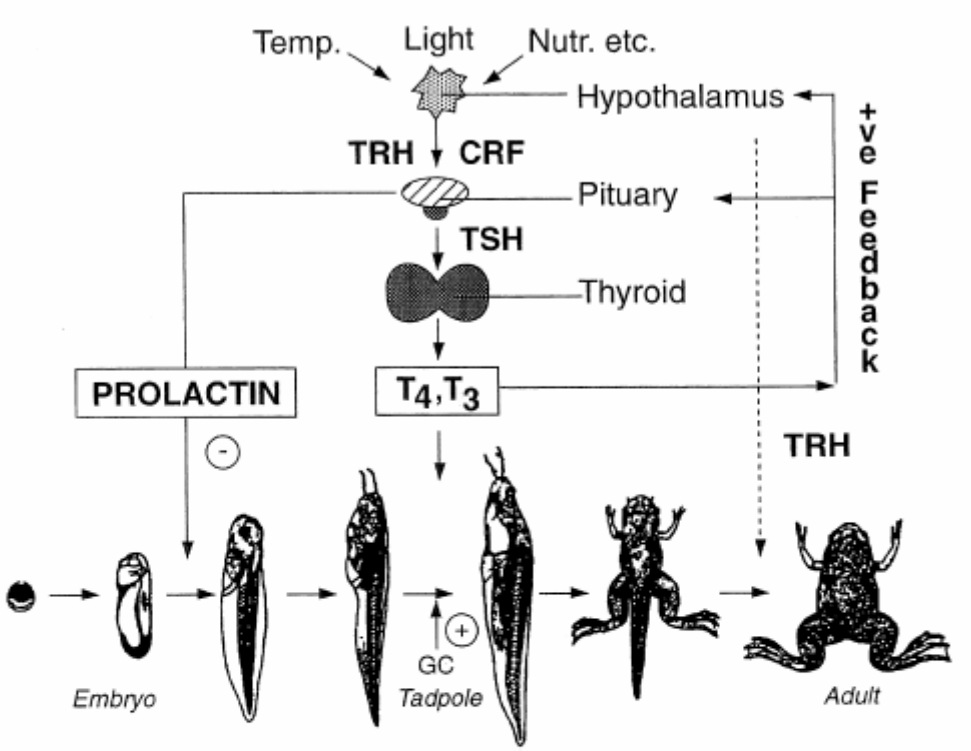


Fig. 1. Schematic representation of the hormonal regulation of amphibian metamorphosis. In response to environmental cues, the dormant thyroid gland of the tadpole is activated to produce the thyroid hormones T_4 and T_3 by the hypothalamic and pituitary hormones TRF, CRF and TSH. Thyroid hormone (TH) is obligatorily required to initiate and maintain the metamorphosis, its action being potentiated by glucocorticoid hormone and retarded by prolactin. Nutr., nutritional factors; TRH, thyrotrophin-releasing hormone; CRF, cortcotrophin-releasing factor; TSH, thyoid-stimulating hormone; T_4, L-thyroxine; T_3, triiodo-L-thyronine; GC, glucocorticoid hormone.

Table 1
Diversity of morphological and biochemical responses to thyroid hormone during amphibian metamorphosis

Tissue	Response	
	Morphological	Biochemical
Brain	Restructuring; axon guidance and growth; cell turnover	Cell division; apoptosis; protein synthesis
Liver	Functional differentiation; restructuring	Induction of albumin and urea cycle enzymes; larval–adult haemoglobin switch
Eye	Repositioning; new retinal neurones; altered lens	Visual pigment switch; induction of β-crystallin
Skin	Restructuring; keratinisation; granular gland formation	Induction of collagen, 63 kDa keratin, magainin
Limb bud, lung	De novo morphogenesis of bone, skin, muscle, nerve, etc.	Cell proliferation; gene expression
Tail, gills	Total tissue regression and removal	Programmed cell death; induction of lytic enzymes
Intestine, pancreas	Major remodelling of tissues	New structural and functional constituents
Immune system	Redistribution of immune cell populations	Aquisition of new immunocompetence
Muscle	Growth, differentiation, apoptosis	Induction of myosin heavy chain

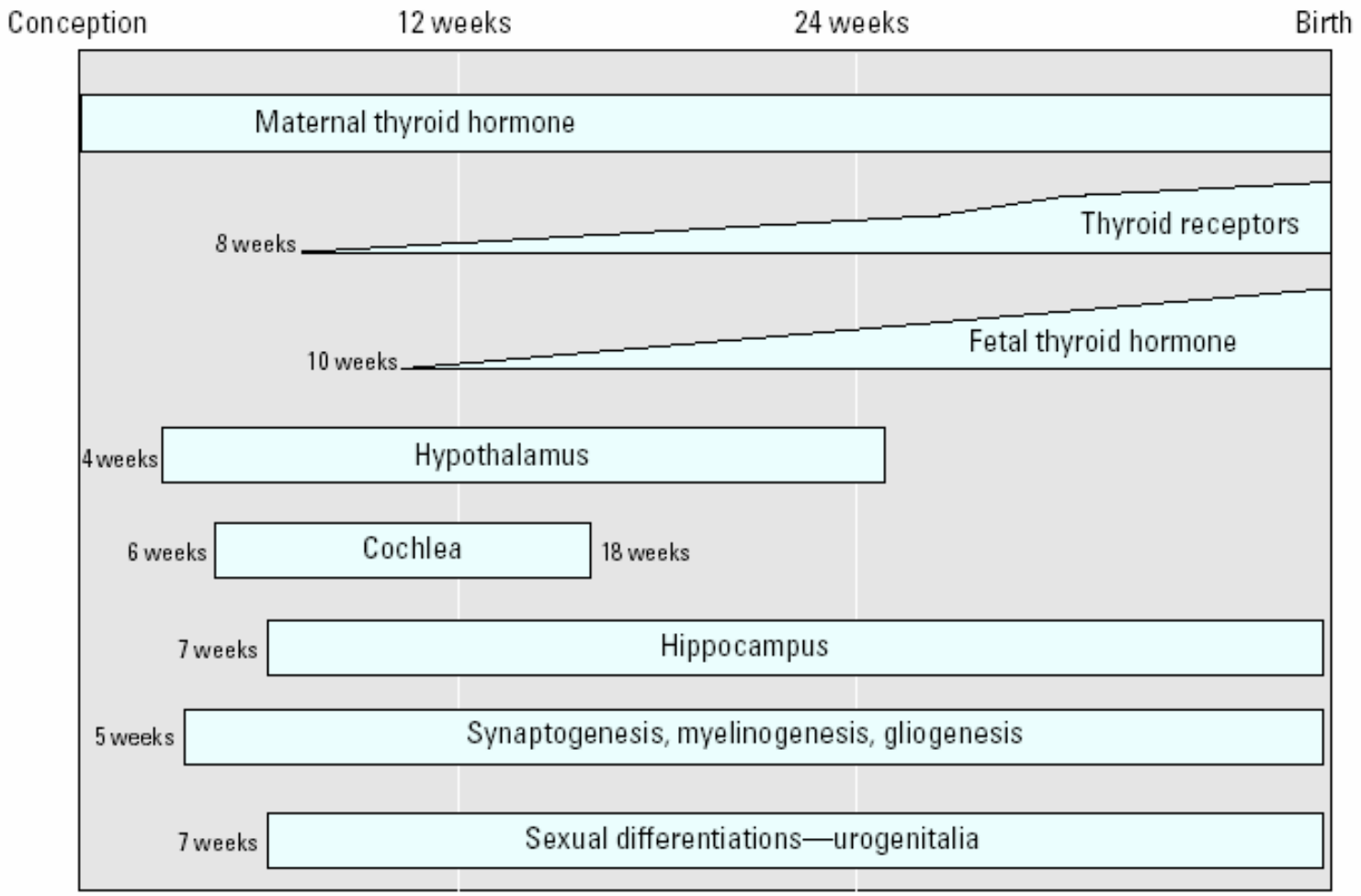


Figure 1. Role of thyroid hormones in fetal neurologic development in relation to timing of several landmark stages of development. Figure adapted from Howdeshell (2002).

Although it has been known for a century that hypothyroidism leads to retardation and other serious developmental effects, the role of thyroid hormones in brain development is still not completely understood. It is also accepted that thyroid hormones transferred from the mother to the embryo and fetus are critical for normal brain development, even though the thyroid gland of a fetus starts producing thyroid hormones at about 10 weeks.

We now recognize that only a slight difference in the concentration of thyroid hormones during pregnancy can lead to significant changes in intelligence in children.

Možné mechanismy disrupce funkce thyroidních hormonů

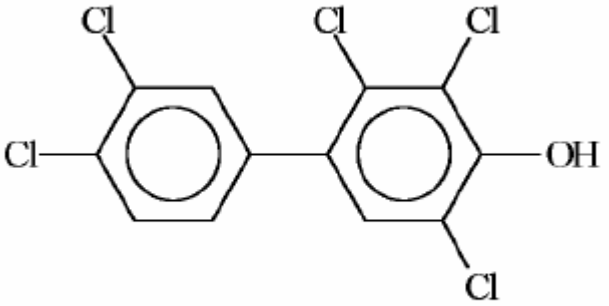
- Inhibition of active transport of inorganic iodide into the follicular cell
- Interference with the sodium/iodide transporter system
- Inhibition of thyroid peroxidases to convert inorganic iodide into organic iodide to couple iodinated tyrosyl moieties into thyroid hormone
- Damage to follicular cells
- Inhibition or enhancement of thyroid hormone release into the blood
- Inhibition or activation of the conversion of T4 to T3 by 5´-monodeiodinase at various sites in the body, for example, the fetal brain
- Enhancement or interference of the metabolism and excretion of thyroid hormone by liver uridine diphosphate
- Interference with transport of thyroid hormones
- Vitamin A (retinol) disturbances
- Blocking of or interfering with thyroid receptors

Mechanisms of Action of Thyroid-Disrupting Chemicals

The complexity of the development of both the neurologic and thyroid systems offers numerous opportunities for chemicals to interfere as the systems develop, mature, and function. Briefly, there are chemicals that interfere with iodine uptake (the herbicides 2,4-D and mancozeb, several PCB congeners, and thiocyanates) and peroxidation at the molecular level (the herbicides aminotriazole and thioureas, the insecticides endosulfan and malathion, and PCBs). They also interfere with the protein transporter that provides a pathway for iodine to enter the cell (military and aerospace chemicals, perchlorates). Certain antagonists (PCBs, the herbicides aminotriazole and dimethoate, and the insecticide fenvalerate) prevent the release of thyroid hormone from the cell and inhibit conversion of T4 to triiodothyronine (T3). Various chemicals enhance excessive excretion of thyroid hormones, some through activation of the cytochrome P450 system (dioxin, hexachlorobenzene, and fenvalerate). Some PCBs, phthalates, and other widely used chemicals compete for sites on the thyroid transport proteins that deliver thyroid hormones throughout the body. New research focuses on the role of chemicals as they interfere with vitamin A (retinols). retinols, a process essential for thyroid hormone expression. There is still no evidence that environmental chemicals directly block the thyroid receptor.

Hydroxylované PCB

During normal enzyme detoxification of PCBs in the maternal liver, certain PCB congeners are hydroxylated. This metabolic step enhances the binding affinity of the hydroxylated PCBs to TTR. Through their high-affinity binding the hydroxylated congeners displace essential fT4 that must get to the fetal brain to be converted to fT3. Hydroxylated PCBs also interfere with the normal excretion of thyroid hormones by inhibiting their sulfation. PCB hydroxylates also have estrogenic and antithyroid properties.



4-OH-PCB107

Thyroidní disrupce u volně žijících obratlovců:

Obojživelníci

Gutleb and co-workers did a series of exposure studies with *Xenopus laevis* and *Rana temporaria.* They found increased incidence of mortality in tadpoles weeks after they ceased dosing the animals. Over an 80-day period, 47.5% of the tadpoles died. The *X. laevis* exposed to 7.7 pM and 0.64 nM PCB 126 exhibited swimming disorders prior to death. Both increased mortality and reduced T4 concentrations occurred in a dose- response manner in *X. laevis*. Severe eye and tail malformations increased in the froglets in a dose-response manner after approximately 60–68 days.

Ptáci

Thyroid hormones in birds have been investigated for their role in migration and courtship. Preventing migrating species from breeding out of season is especially critical for their survival. From the 1950s through to the 1970s, fish-eating birds in the Great Lakes were experiencing very poor reproductive success. Keith suggested that the high embryo mortality and low chick survival in herring gulls nesting in upper Green Bay in the mid 1960s was both the result of
a) the effects of the chemical residues from the mother on the embryo and b) the effects of the adult's contamination on its parental

Ryby

Migration of salmonids is linked with THs effecting a sequence of behaviors. In the laboratory, increases in T4 led to less display of aggressive behavior such as territoriality. Elevated concentrations of both T3 and T4 reduced the fishes' preference for shade to more open areas (phototaxis). T3 treatment caused the fish to swim with the current rather than against the flow (rheotaxis).

Savci

PCBs and dioxins have been shown to alter thyroid function in rodents by multiple mechanisms, including direct toxic effects on the thyroid gland, induction of thyroid hormone metabolism via the UDP-glucuronyl transferases, and interactions with thyroid hormone plasma transport proteins, particularly transthyretin. A number of investigators have evaluated the effects of maternal PCB exposure on thyroid function of rat pups. Pup serum thyroxine (T4) levels are markedly reduced by PCB or dioxin exposure, but the levels of the active form of the hormone, triiodothyronine (T3), are generally unchanged, or only slightly reduced. A relationship between exposure to dioxins and PCBs and alterations in thyroid hormones has also been reported in human infants. Infants exposed to higher levels of PCBs and dioxins had lower free T4 levels and higher thyroid-stimulating hormone levels.

PPAR

- Deregulace PPAR a reprodukce
- PPAR a karcinogenita

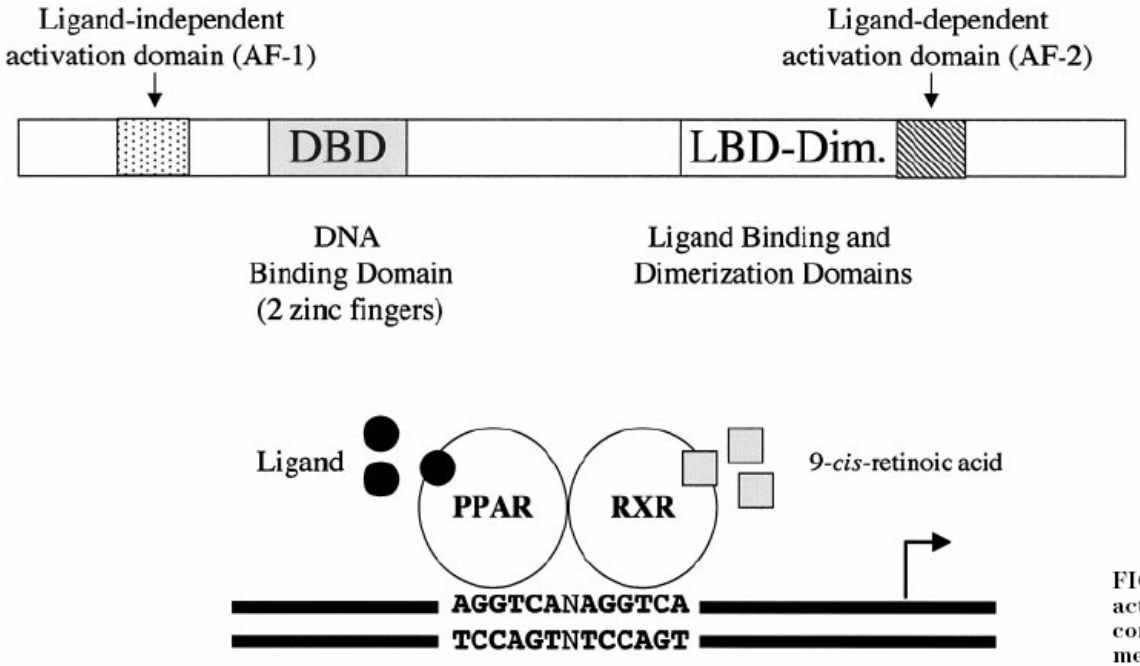
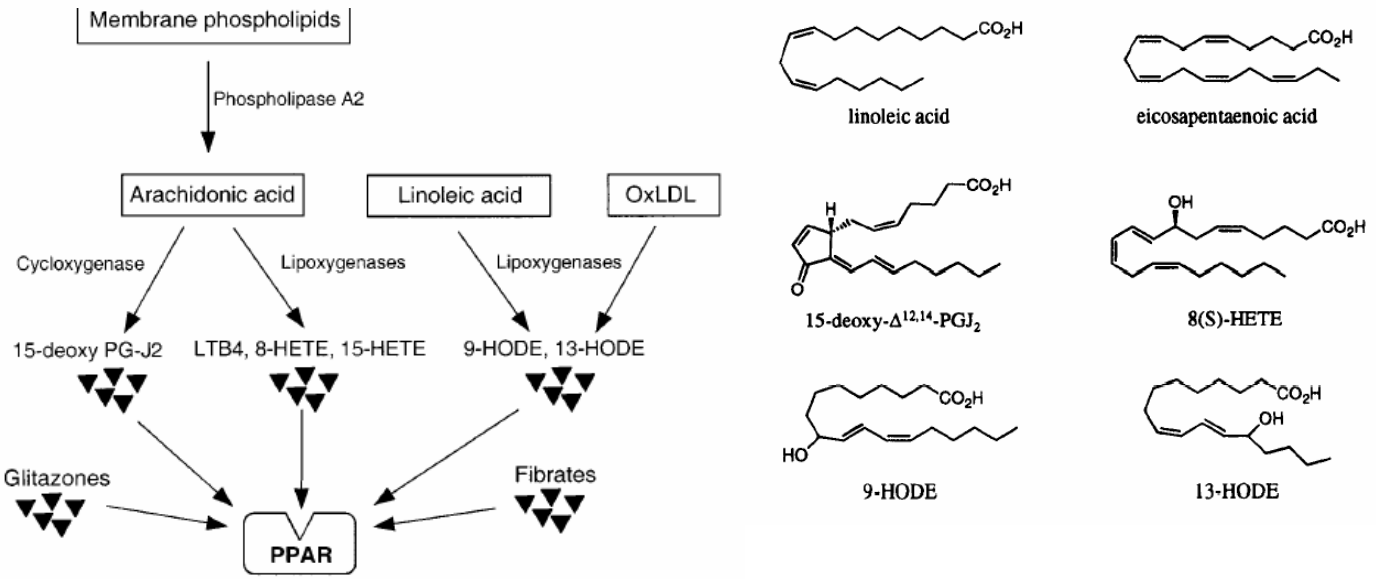


FIG. 1. General structure and mechanism of action of PPARs. PPAR isoforms share a common domain structure and molecular mechanism of action.



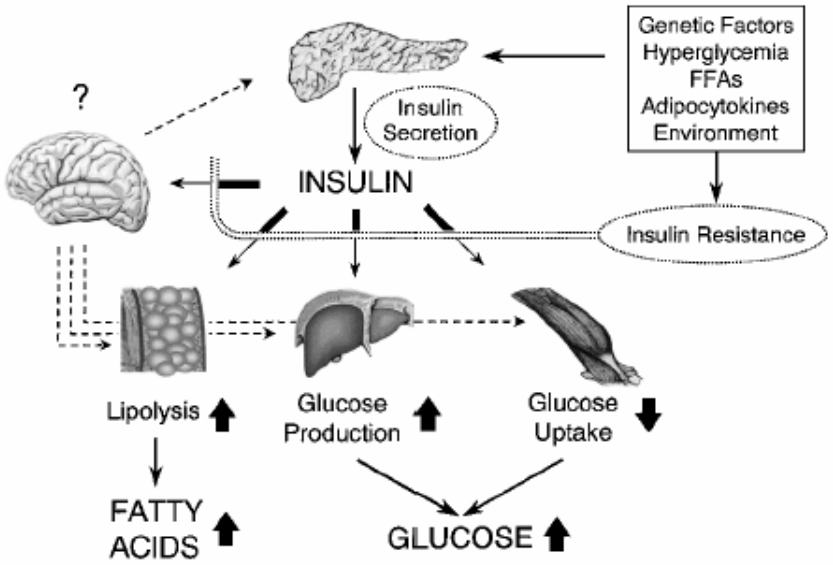


Figure 1. Insulin secretion and resistance. Organ manifestations of insulin resistance and metabolic consequences: adipose tissue — increased lipolysis and excessive FFA release; liver (and kidney) — increased glucose production; muscle — decreased glucose uptake. The role of insulin resistance in brain is theoretical at present since evidence is available only from a brain-specific insulin receptor knockout mouse (4).

Key messages

- With the thiazolidinediones rosiglitazone and pioglitazone, a novel treatment modality for type 2 diabetes has become available.
- The mechanism of action of these compounds involves binding to the peroxisome proliferator-activated receptor (PPAR) gamma , a transcription factor that regulates the expression of specific genes especially in fat cells but also in other tissues.
- As monotherapy, glycosylated hemoglobin (HbA1c) on average can be improved by almost 1%.
- Thiazolidinediones reduce insulin resistance not only in type 2 diabetes but also in non-diabetic conditions associated with insulin resistance such as obesity.

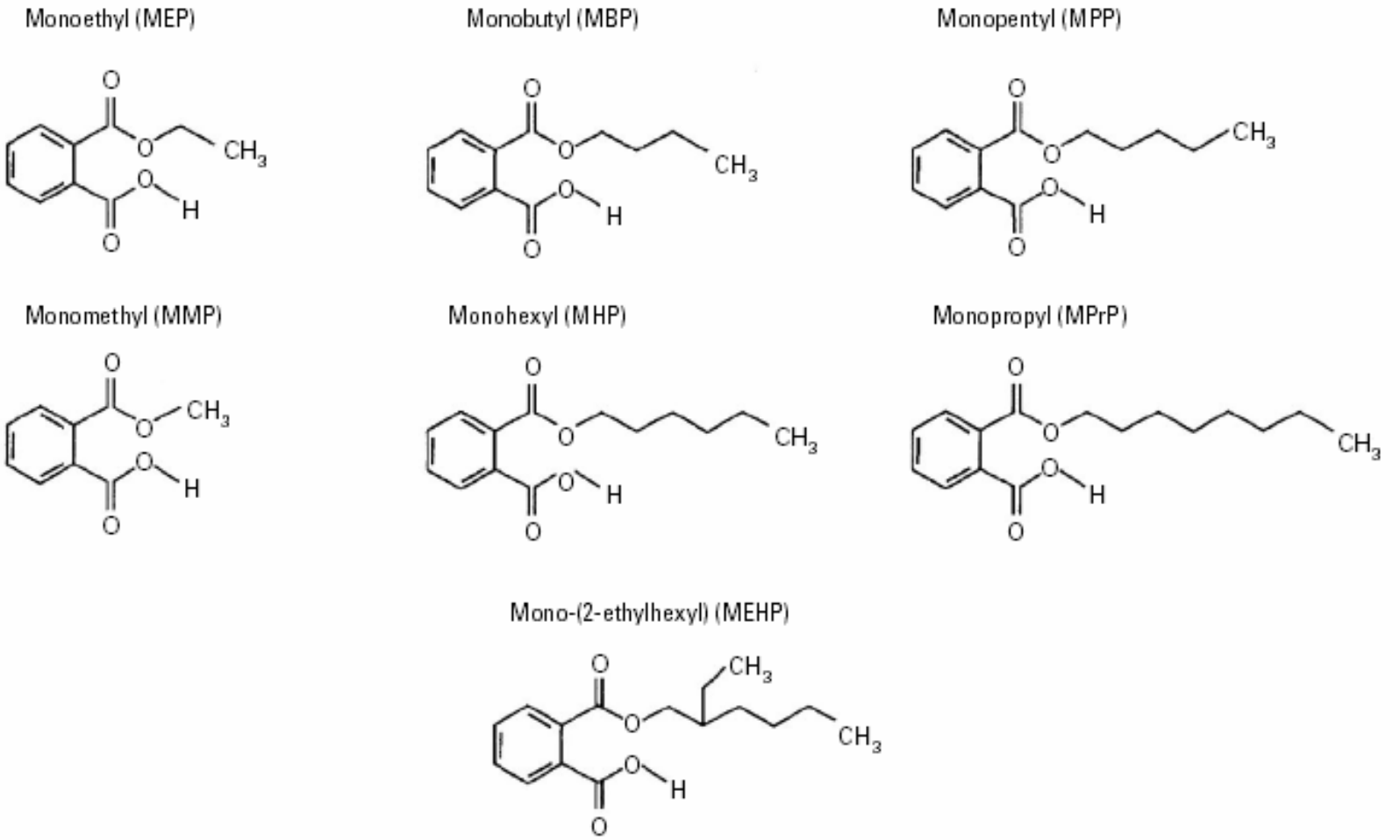


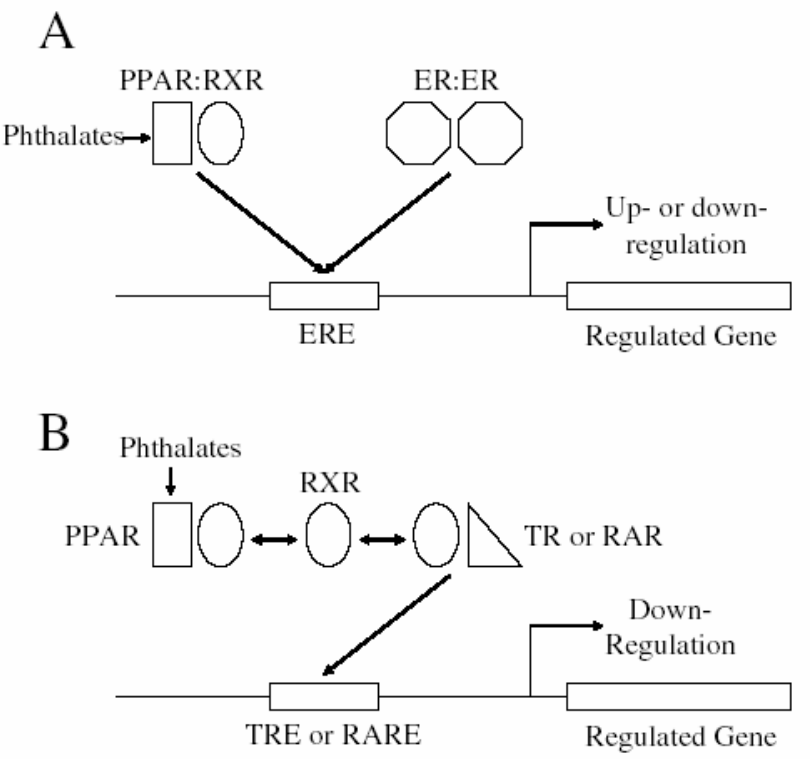
Figure 1. Structurally related phthalate monoesters. Diesters of *o*-phthalic acid are quickly metabolized *in vivo* to their active metabolites, the monesters. The length and structure of the side chain is important for toxicity.

Ftaláty jako ligandy PPAR – efekty na samčí a samičí reprodukční systém

TABLE 1

Summary of Effects of *in Utero* Exposure to Phthalates on the Developing Male Reproductive Tract

Endpoint measured[a]	DBP	DEHP	BBP	DINP
Testis				
↓ Weight	+	+	+	–
↓ Sperm number	+	+	+	
Degeneration/atrophy of seminiferous tubules	+	+	+	
Leydig cell hyperplasia/aggregates	+	+	+	
Leydig cell adenoma	+	+		
Cryptorchidism	+	+	+	
Sex organs				
Epididymis: ↓ wt, agenesis/malformed	+	+	+	–
Penis: delayed/incomplete preputial separation, hypospadias, ↓ wt of glans	+	+	+	–
Prostate: ↓ wt, agenesis	+	+	+	–
Seminal vesicle: ↓ wt	+	+	+	–
Vas deferens: ↓ wt, malformed/agenesis	+			
Miscellaneous				
Anogenital distance (↓)	+	+	+	+
Nipple retention	+	+	+	



TDS = testicular dysgenesis syndrome

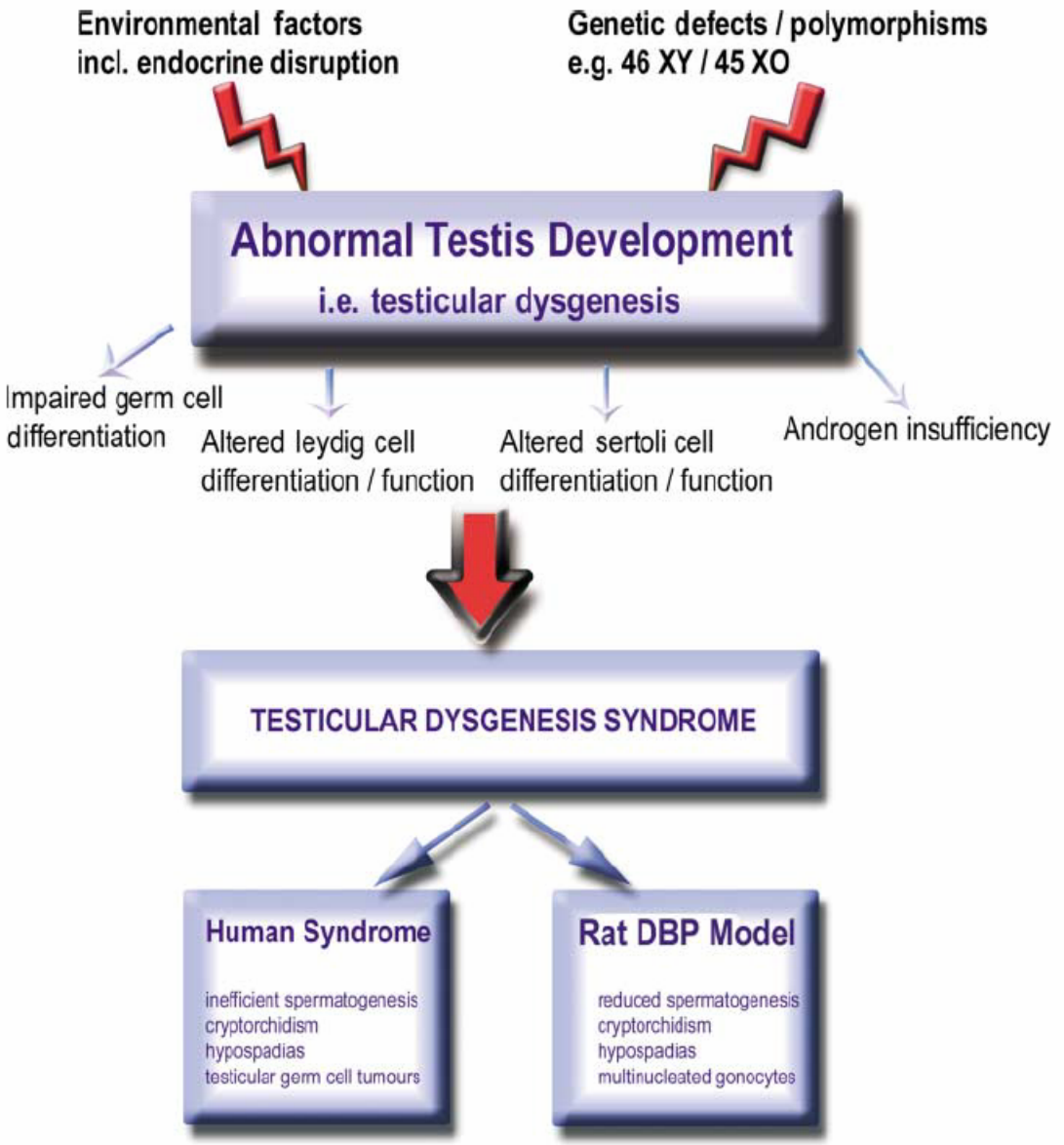
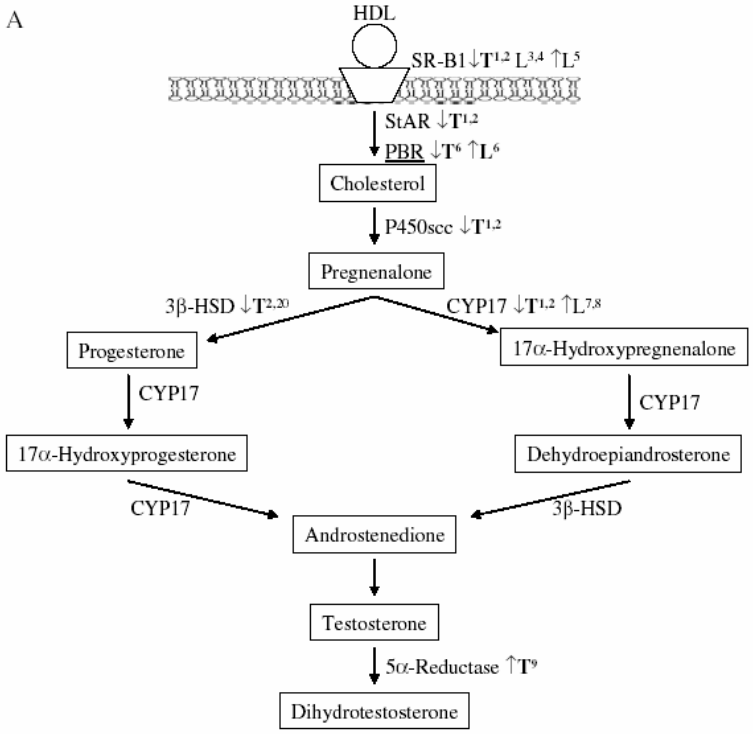
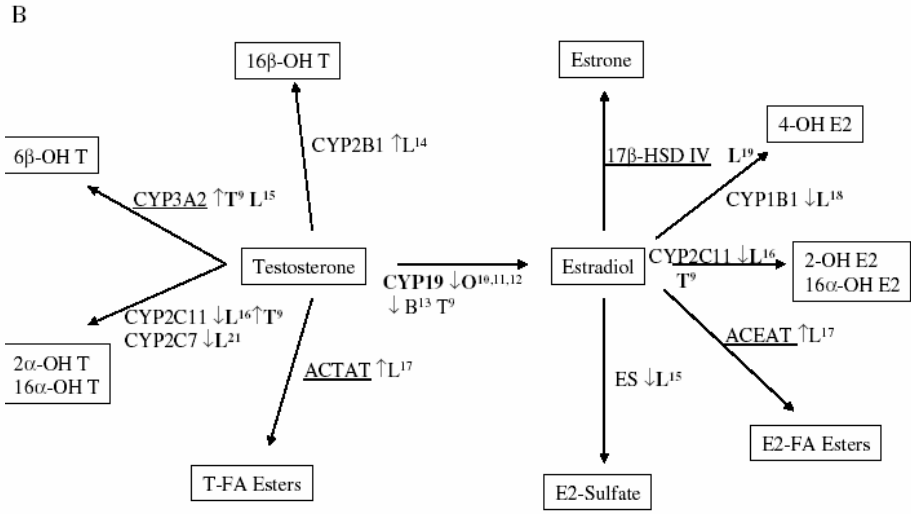


Figure 1 Schematic representation of the potential pathogenic links between testis development and the clinical manifestations of testicular dysgenesis syndrome (TDS). The similarities in the pathologies induced by *in utero* dibutyl phthalate (DBP) administration and human TDS are compared.

Ftaláty modulují expresi enzymů kontrolujících syntézu a odpourávání steroidních hormonů





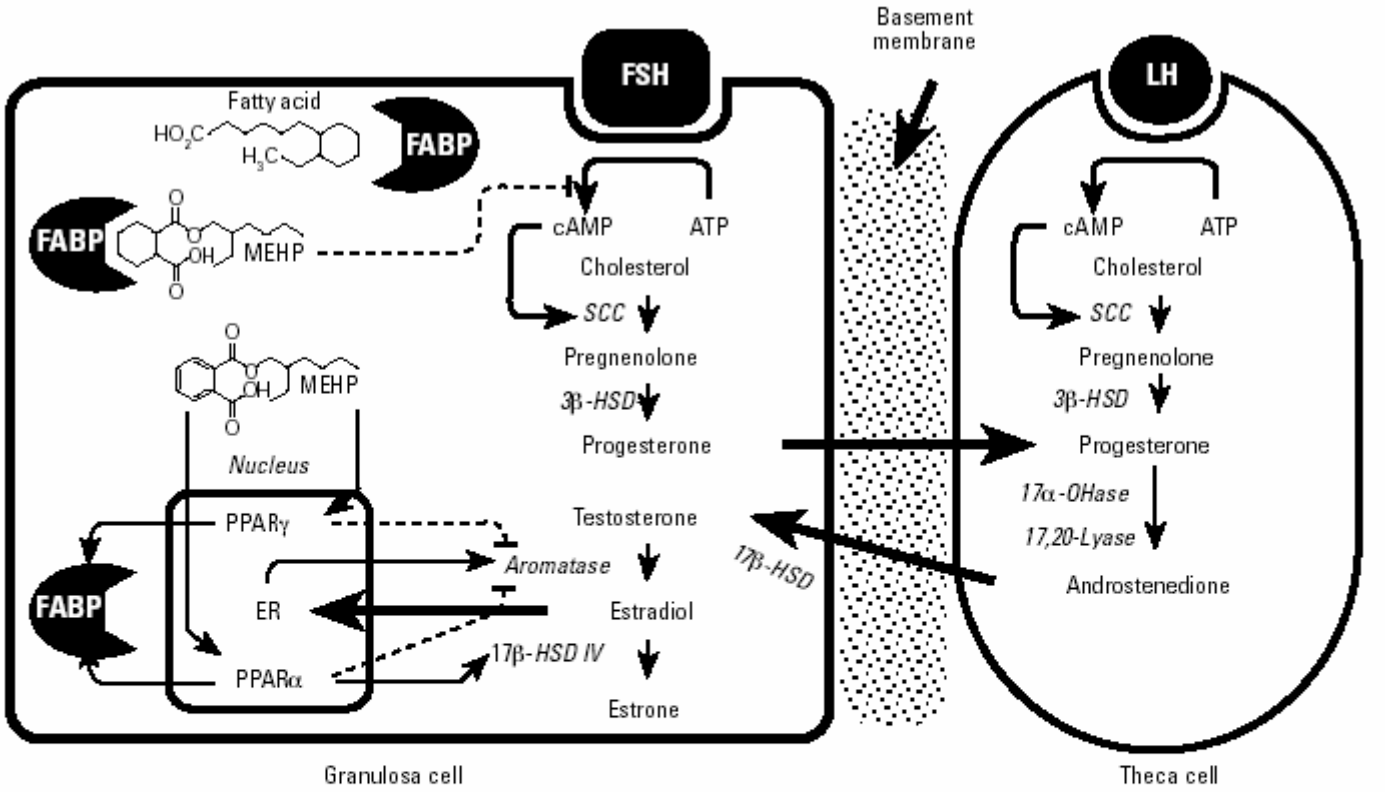


Figure 4. Proposed model of MEHP action in the granulosa cell. MEHP interferes with two points of the steroid hormone pathway. Abbreviations: ER, estrogen receptor; 17α-OHase, 17α-hydroxylase; SCC, P450 side chain cleavage enzyme. First, MEHP suppresses FSH-stimulated cAMP, possibly by inhibiting binding of FSH to its receptor or altering activation of adenylate cyclase (Grasso et al. 1993). MEHP also activates PPARs, possibly by release of fatty acids, endogenous activators of PPAR. The activation of either PPARα or PPARγ decreases aromatase mRNA. PPARα activation also causes an increase in the transcript level of 17β-HSD IV, which metabolizes estradiol to estrone. Finally, both PPARα and γ increase levels of FABP in the cell, which is able to transport MEHP and fatty acids through the cell, delivering these ligands to the PPAR receptors.

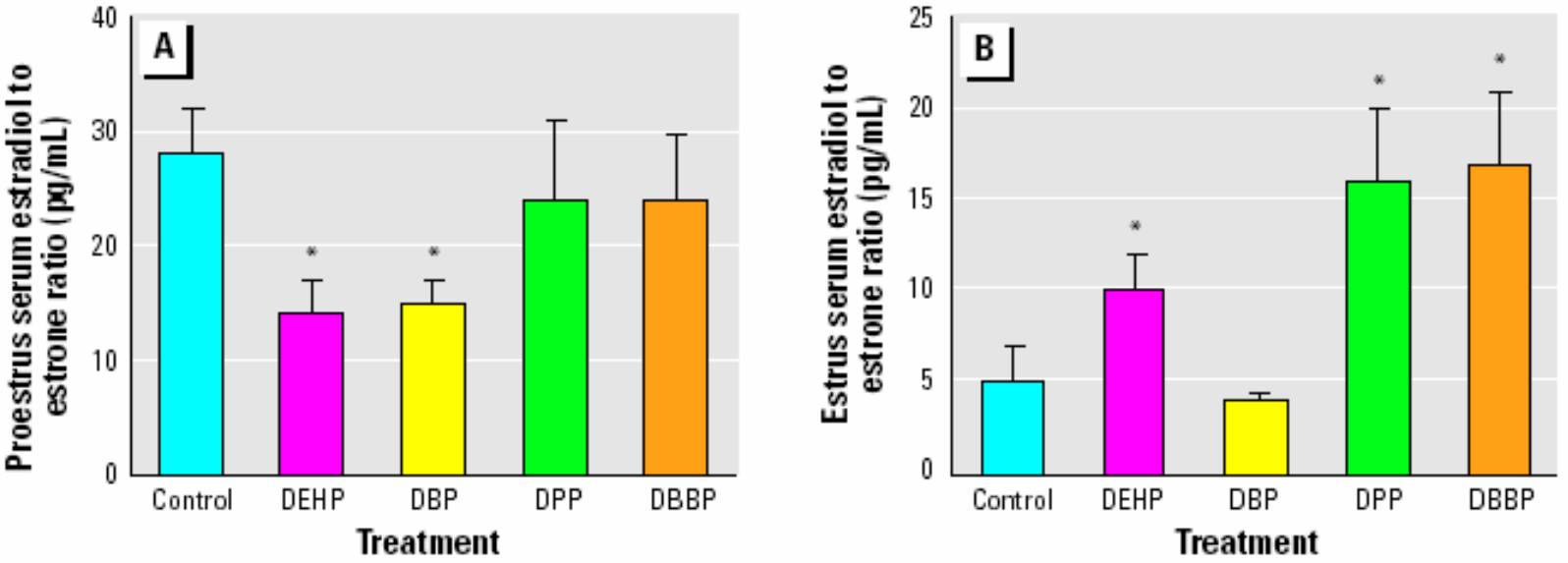
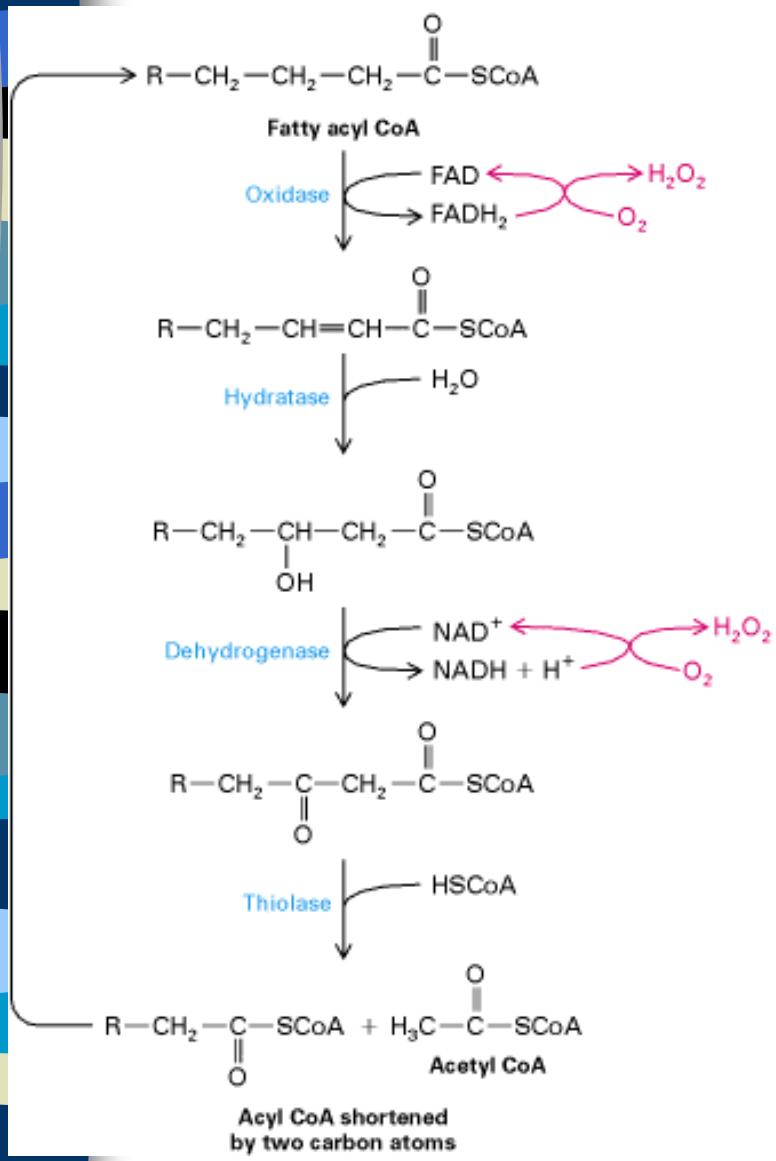


Figure 2. Phthalate effects on serum estradiol and estrone levels at (*A*) proestrus and (*B*) estrus. Adult 90-day-old female Sprague-Dawley rats (n = 12 per group) were treated with corn oil vehicle or 1,000 mg/kg of DEHP, DBP, DPP, or DBBP in corn oil given daily by gavage beginning at vaginal metestrus. Rats were killed at vaginal proestrus (n = 6 per treatment) or estrus (n = 6 per treatment) 8 or 9 days after dosing began, following methodology described by Davis et al. (1994a).

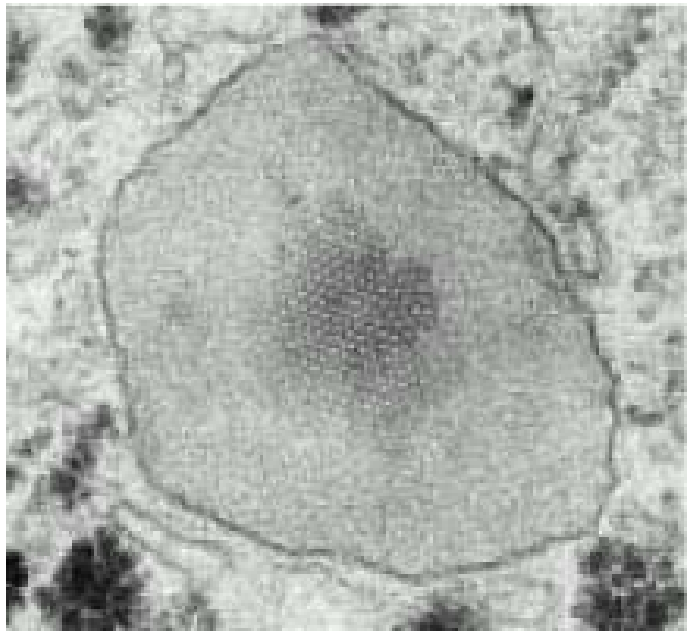
*Significantly different compared to control, $p < 0.05$.

Peroxisomes

- Peroxisomes: 0.5μM diameter, contribute to 20% cytoplasmic volume.
- Enzymes for oxidation of fatty acids
- Oxidation produces H_2O_2
- Increase in peroxisomal number and volume induced by peroxisome proliferators

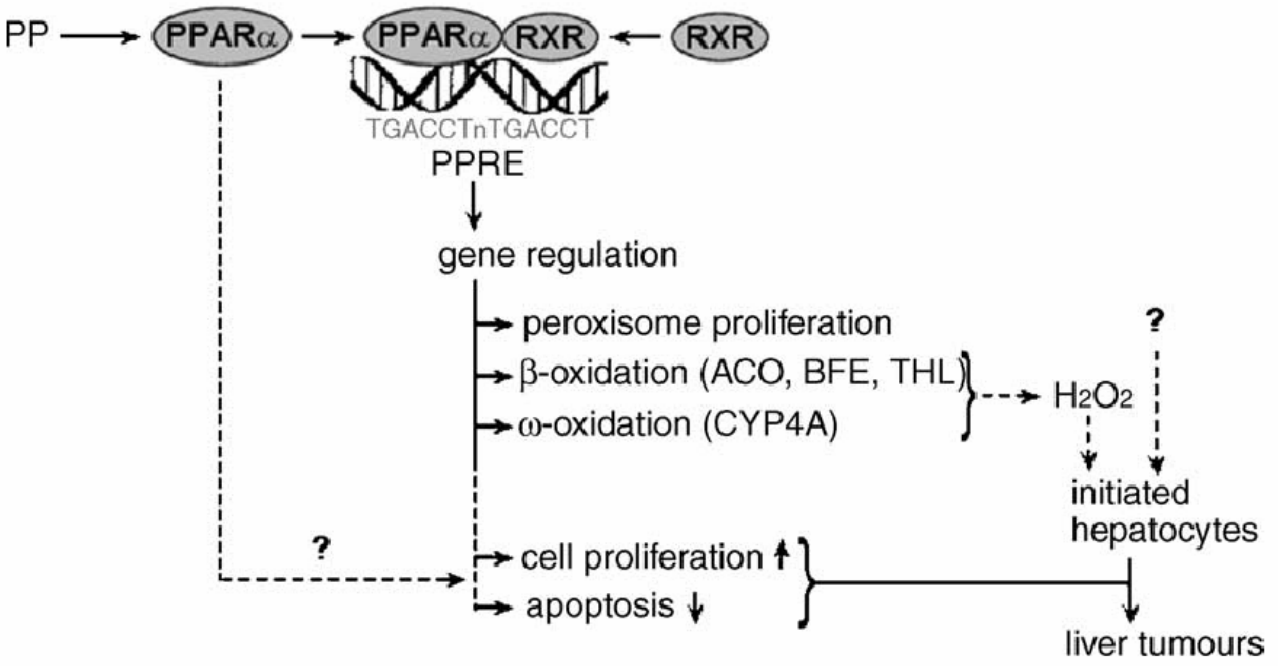


Oxidation of fatty acids by peroxisomes. Peroxisomes degrade fatty acids with more than 12 carbon atoms by a series of reactions similar to those used by liver mitochondria. In peroxisomes, however, the electrons and protons transferred to FAD and NAD^+ during the oxidation reactions ar subsequently transferred to oxygen, forming H_2O_2



PPARα a karcinogeneze

S. Bosgra et al. / Toxicology 206 (2005) 309–323



Peroxisome proliferation

- Liver growth
  - hypertrophy
  - hyperplasia
- Induction of liver enzymes
  - peroxisomal enzymes (peroxisome proliferation)
  - P450 - the CYP4 genes
- Proliferation of the Endoplasmic Reticulum and peroxisomes
- Hypolipidaemia

Oxidative Theory

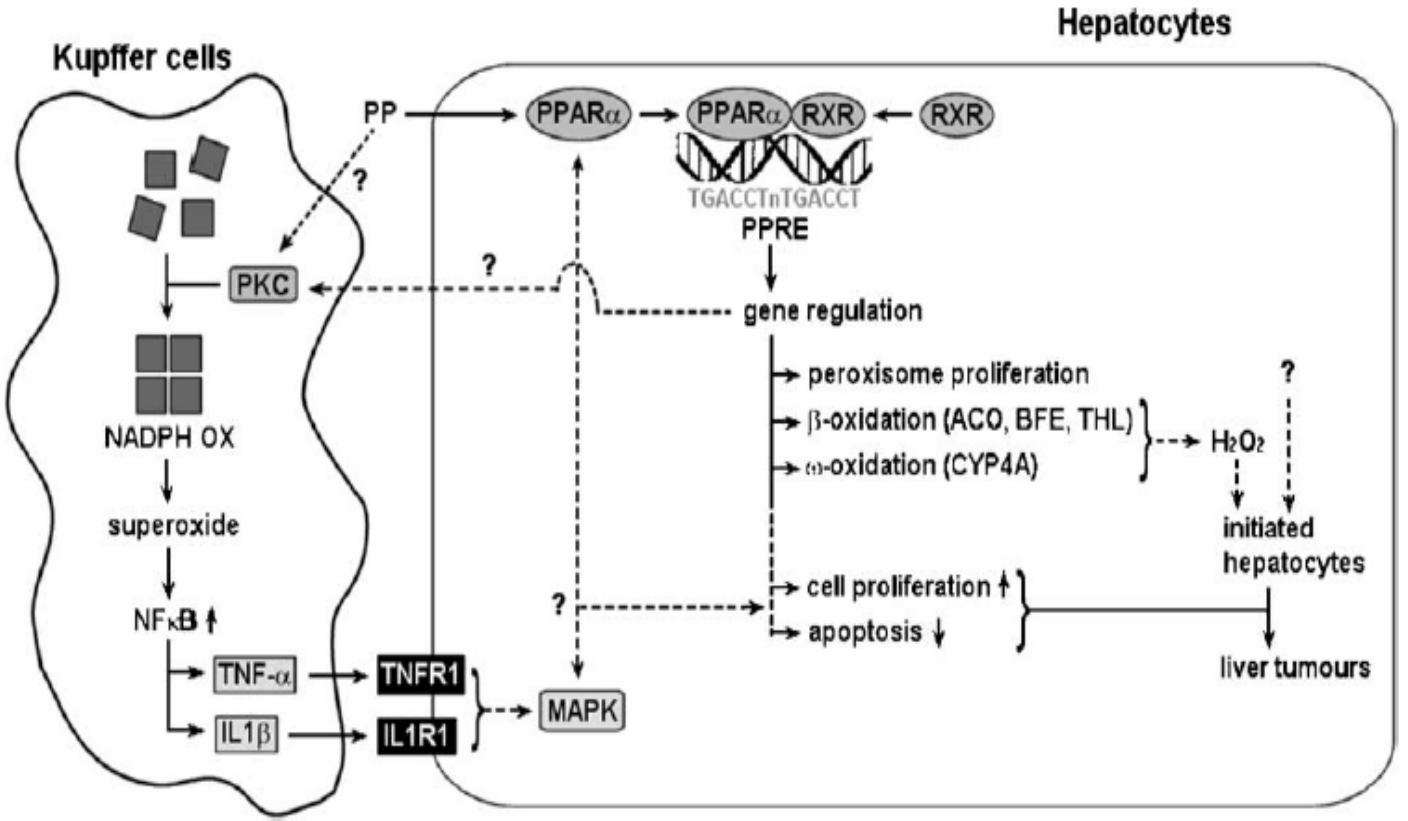
Fatty acids

increase in peroxisomal β-oxidation without catalase (<2 fold)

Increase H_2O_2 → genotoxic DNA damage

Cancer

Úloha Kupfferových buněk



Species Differences and Human Risk Assessment

- PPARα exists in mouse, rat, guinea pig and human
- Low hepatic levels in human and g-pig
- Human liver
  - No peroxisome proliferation
  - No induction of liver growth
- If PPARs cause cancer in rats, do they cause cancer in humans? Therefore, no risk of cancer???