

**[S-6]**

**Effects of Pahs and Pcb's and Their Toxic Metabolites on Inhibition of Gjic and Cell Proliferation in Rat Liver Epithelial Wb-F344 Cells**

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The liver progenitor cells could form a potential target cell population for both tumor-initiating and -promoting chemicals. Induction of drug-metabolizing and antioxidant enzymes, including AhR-dependent CYP1A1, NQO-1 and AKR1C9, was detected in the rat liver epithelial WB-F344 „stem-like“ cells. Additionally, WB-F344 cells express a functional, wild-type form of p53 protein, a biomarker of genotoxic events, and connexin 43, a basic structural unit of gap junctions forming an important type of intercellular communication.

In this cellular model, two complementary assays have been established for detection of the modes of action associated with tumor promotion: inhibition of gap junctional intercellular communication (GJIC) and proliferative activity in confluent cells. We found that the PAHs and PCBs, which are AhR agonists, released WB-F344 cells from contact inhibition, increasing both DNA synthesis and cell numbers. Genotoxic effects of some PAHs that lead to apoptosis and cell cycle delay might interfere with the proliferative activity of PAHs. Contrary to that, the nongenotoxic low-molecular-weight PAHs and non-dioxin-like PCB congeners, abundant in the environment, did not significantly affect cell cycle and cell proliferation; however both groups of compounds inhibited GJIC in WB-F344 cells.

The release from contact inhibition by a mechanism that possibly involves the AhR activation, inhibition of GJIC and genotoxic events induced by environmental contaminants are three important modes of action that could play an important role in carcinogenic effects

of toxic compounds. The relative potencies to inhibit GJIC, to induce AhR-mediated activity, and to release cells from contact inhibition were determined for a large series of PAHs and PCBs and their metabolites. In vitro bioassays based on detection of events on cellular level (deregulation of GJIC and/or proliferation) or determination of receptor-mediated activities in both „stem-like“ and hepatocyte-like liver cellular models are valuable tools for detection of modes of action of polyaromatic hydrocarbons. They may serve, together with concentration data, as a first step in their risk assessment.

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### EFFECTS OF PAHs AND PCBs AND THEIR TOXIC METABOLITES ON INHIBITION OF GJIC AND CELL PROLIFERATION IN RAT LIVER EPITHELIAL WB-F344 CELLS

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KSOT, Seoul, 2004

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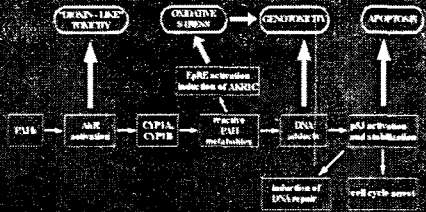
### IN VITRO BIOMARKERS OF EXPOSURE AND EFFECT CAN BE DETERMINED AT CELLULAR AND SUBCELLULAR LEVELS:

- EVENTS ON CELLULAR LEVELS:**  
 CYTOTOXICITY / GENOTOXICITY / CELL CYCLE ARREST / APOPTOSIS  
 MODULATION OF CELL CYCLE / CELL PROLIFERATION  
 INHIBITION OF GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC)
- INTRACELLULAR EVENTS:**  
 INDUCTION OF GENE EXPRESSION
- DNA MICROARRAYS – modulations of gene expression pattern
  - RT-PCR – expression of selected genes (CYP1A, CYP1B, VTG)
  - REPORTER GENE ASSAYS – expression of reporter gene(s) under control of a specific transcription factor (DR-CALLUX, ER-CALLUX)
  - POST-TRANSLATIONAL MODIFICATIONS OF TRANSCRIPTION FACTORS, PROTEIN KINASES, PHOSPHOLIPASES AND OTHER COMPONENTS OF SIGNAL TRANSDUCTION PATHWAYS
  - functional proteomics (Western blot, MALDI-TOF), determination of enzymatic activities
  - second messengers (release of Ca, production of AA, DAG, ceramide, ROS, cytokines)
- METABOLISM XENOBIOTICS, HORMONES, LIPIDS / DNA DAMAGE**

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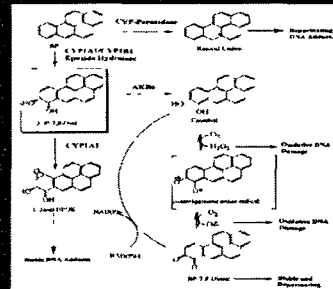
### Metabolic activation of PAHs



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### METABOLIC PATHWAYS OF PAHs ACTIVATION



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### Genotoxic and nongenotoxic (epigenetic) mechanisms are involved in chemical carcinogenesis after exposure to PAHs

- INITIATION**
- AhR-dependent induction of CYP1A, CYP1B; metabolic activation of promutagens → DNA adducts
  - oxidative stress → oxidative DNA damage
- TUMOR PROMOTION**
- Activation of AhR a other transcription factors → modulation of cell cycle, increased DNA synthesis and cell proliferation
  - Oxidative stress, modulation of intracellular signal transduction pathways, protein kinases, phospholipases
  - Inhibition of GJIC → release from homeostatic control
- CYTOTOXICITY (APOPTOSIS)**
- Release of arachidonic acid (lymphocytes)
  - Activation of transcription factors (p53, p21) → cell cycle arrest (block in S-phase), DNA repair or cascade of apoptotic signals)

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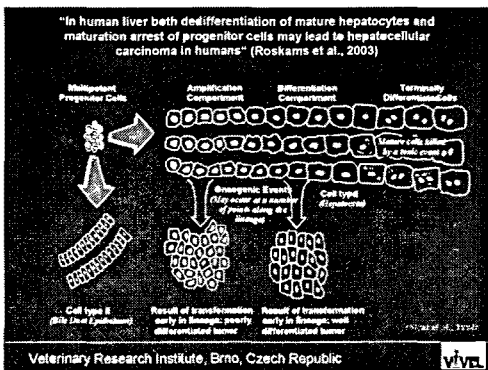


### Two cell types used in the study:

- poorly differentiated "stem-like" (progenitor) cells (model: non-transformed liver epithelial WB-F344 cells)
- primary hepatocytes / hepatoma cell lines (e.g., rat hepatoma H4IIE cells)

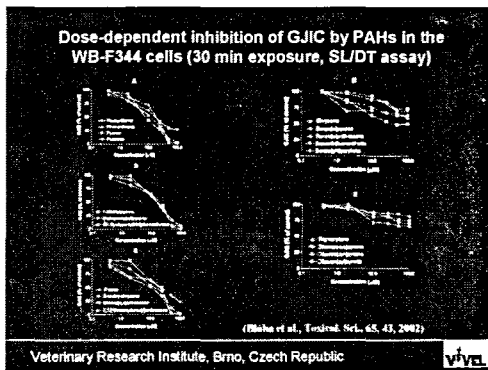
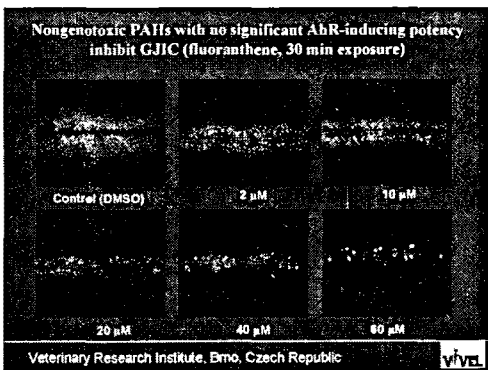
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Low-molecular-weight PAHs inhibit GJIC in rat liver "stem-like" WB-F344 cells

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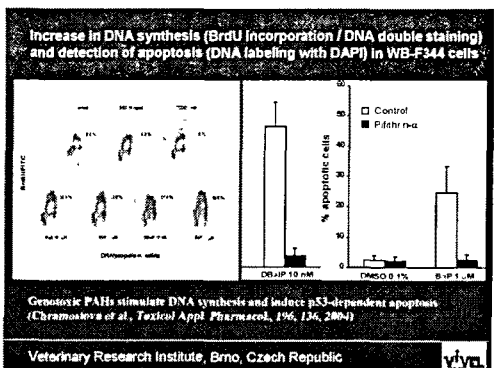
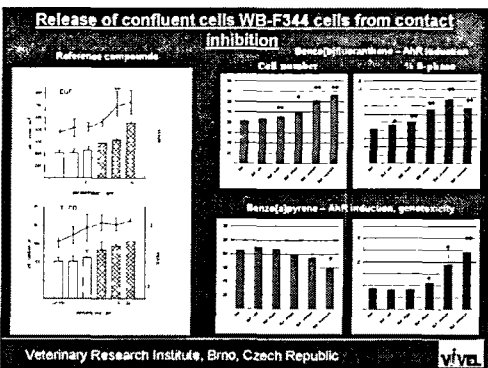
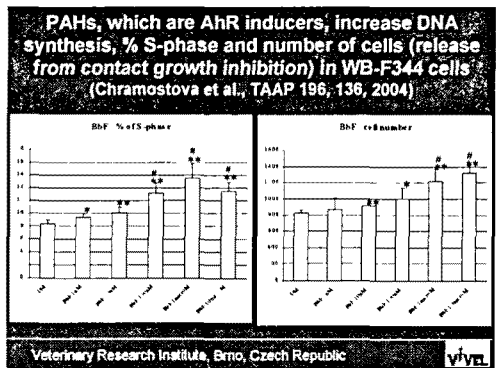
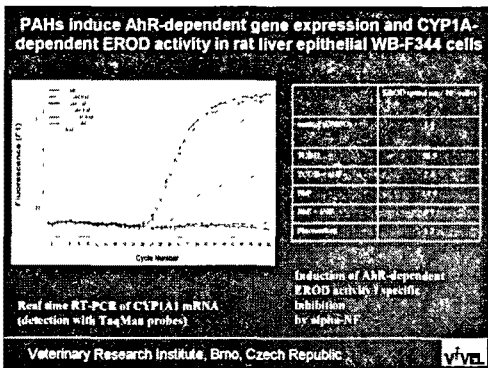
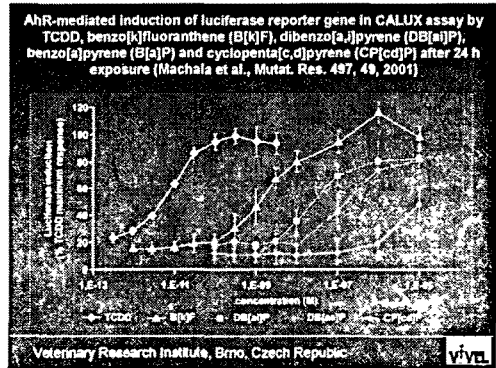
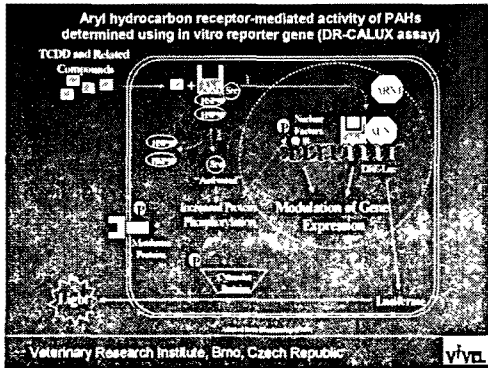
**INHIBITION OF GJIC: CONCLUSIONS**

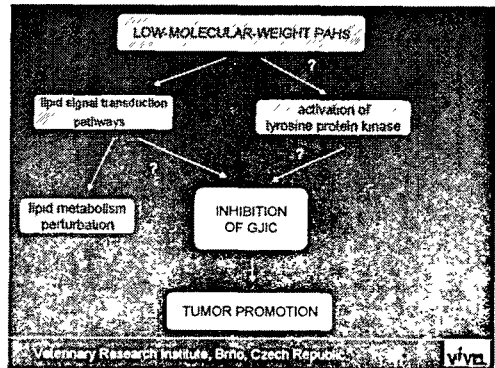
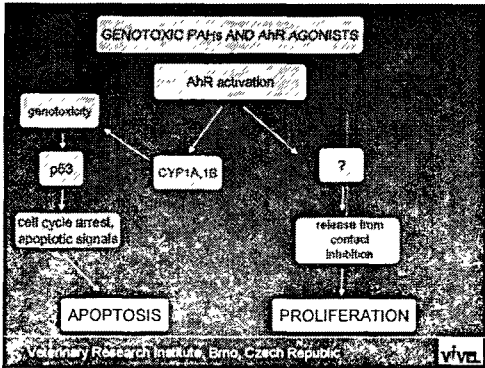
- ◆ Low-molecular-weight PAHs incl. fluoranthene, pyrene, and phenanthrene inhibited significantly GJIC; most of high-molecular-weight PAHs with known strong carcinogenic properties possessed only weak (DBPyrènes) or no inhibition potency (DBaKf, DBaJf, N[23a]P).
- ◆ Using of selective inhibitors of protein kinases suggested mechanism of inhibition of GJIC after exposure to PAHs different from effects of EGF or TPA; possible roles of src kinase, PC-PLC and DAG-lipase in this process.
- ◆ Receptor tyrosine kinases EGFR, ErbB-2, and NGFR, as well as mitogen-activated protein kinases ERK1/2, p38, PKC, Akt, phospholipases PI-PLC and PLA2 are probably not involved in the GJIC inhibition by PAHs.

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PAHs induce AhR-dependent ("dioxin-like") activity in hepatoma and non-transformed liver epithelial cells

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### CLASSIFICATION OF PAHS ACCORDING TO MAJOR MECHANISMS OF ACTION

- Low-molecular-weight PAHs (fluoranthene): tumor promotion (inhibition of GJIC), acetic acid release
- Genotoxic PAHs (BaP, DBaP) - DNA adducts, oxidative damage, p53 activation and apoptosis, however, they increase S-phase percentage and DNA synthesis of small portion of survived cells
- AHR ligands (benzo[a]anthracene, BaA, chrysene)
  - induction of CYP1A, CYP1B; increase in metabolic activation of aromatic amines
  - tumor-promoting activity; proliferation of liver epithelial cells

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### Classification of PCB congeners

- Coplanar PCBs
- Mono-ortho-substituted PCBs (156, 114, 118, 105, 74, >> 105...)
- Prevalent di-ortho-substituted PCBs (138, 153, 170, 100, 107, >>...)
- Episodic PCBs (18, 49, 149...)
- OH-PCBs, MeSO<sub>2</sub>-PCBs

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### Multiple modes of action of PCBs

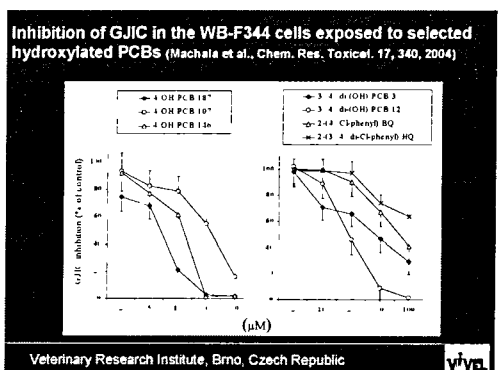
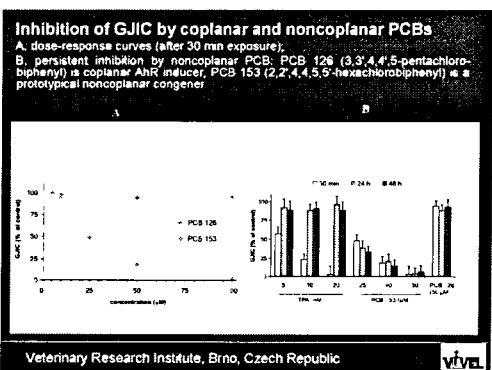
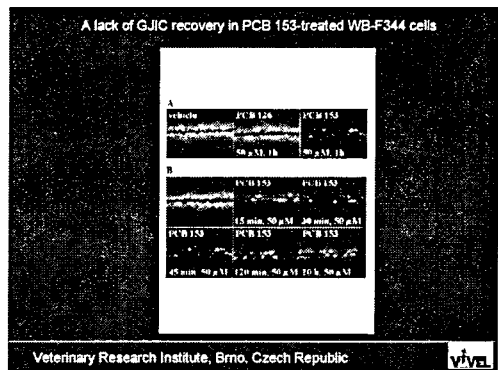
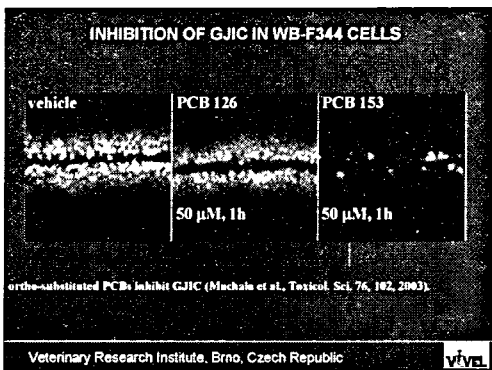
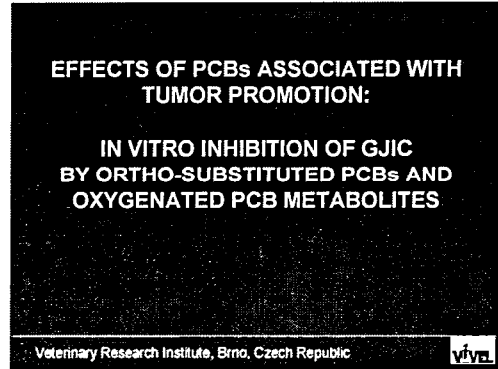
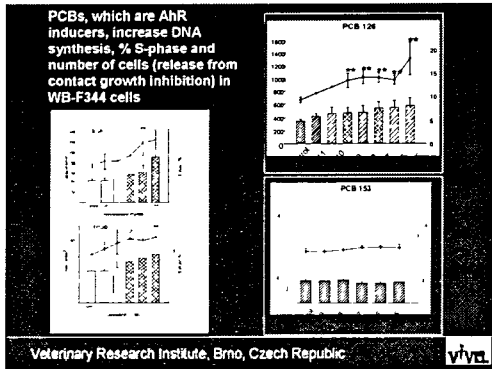
- ENDOCRINE DISRUPTION
  - AHR-dependent ("dioxin-like") activity; Modulation of biosynthesis and metabolism of steroid hormones (CYP19, CYP17A...)
  - Suppression of thyroid hormone signaling (binding to TTR, THR-transactivation...)
  - Weak estrogenicity of lower-molecular-weight PCBs, antiestrogenicity of prevalent di-ortho-substituted PCBs;
  - Antagonistic effects on estrogen receptor activity
- TUMOR PROMOTION
  - Increased proliferation / release from contact inhibition (AHR-dependent activity); modulation of cell cycle, increased DNA synthesis and cell proliferation
  - Inhibition of apoptosis (both AHR-dependent and AHR-independent mechanisms)
  - Additional modulation of intracellular signal transduction pathways, protein kinases, phospholipases
  - Inhibition of GJIC - release from homeostatic control
- EFFECTS ASSOCIATED WITH NEUROTOXICITY
  - Dopamine depletion
  - Disruption of calcium homeostasis and release (RyR, Ca-ATPase, Ca uptake)

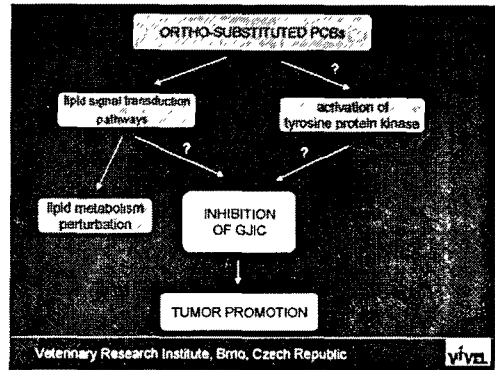
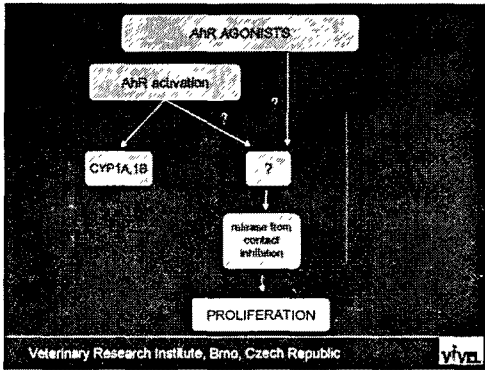
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### EFFECTS OF PCBs ASSOCIATED WITH TUMOR PROMOTION:

#### INCREASED PROLIFERATION AFTER EXPOSURE TO AHR-INDUCING PCBs

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### CONCLUSIONS

Di-ortho-substituted (noncoplanar), mono-ortho-chlorinated PCBs and OH-PCBs acted as potent inhibitors of GJIC in micromolar concentrations. Coplanar PCBs (strong inducers of aryl hydrocarbon receptor) possessed no acute inhibitory potency.

Coplanar and mono-ortho-substituted PCB congeners increased % S-phase and total numbers of liver epithelial cells (proliferative activity).

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