Environmental Mutagens & Carcinogens 23-2 : 56-62 (2003)

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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(Received June 7, 2003 / Accepted June 16, 2003)

The liver progenitor cells could form a potential target cell population fore both tumor-initiating and -promoting chemicals. Induction of drug-metabolizing and anti-oxidant 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 inhibiton 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.

supported by the Czech Ministry of Agriculture grant No. MZE00002716201.

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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, CYP19, VTG)
- REPORTER GENE ASSAYS – expression of reporter gene(s) under control of a specific transcription factor (DR-CALUX, ER-CALUX)

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 activites
- second messengers (release of Ca, production of AA, DAG, ceramide, ROS, cytokines)

METABOLISM XENOBIOTICS, HORMONES, LIPIDS / DNA DAMAGE

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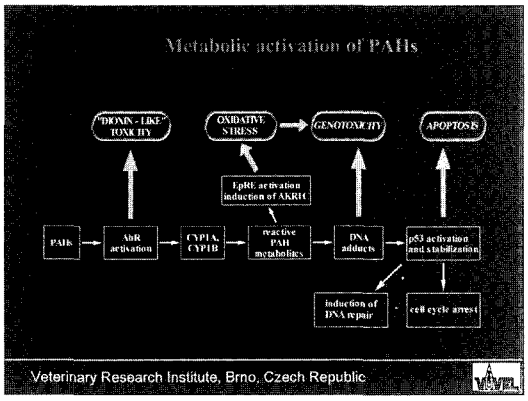

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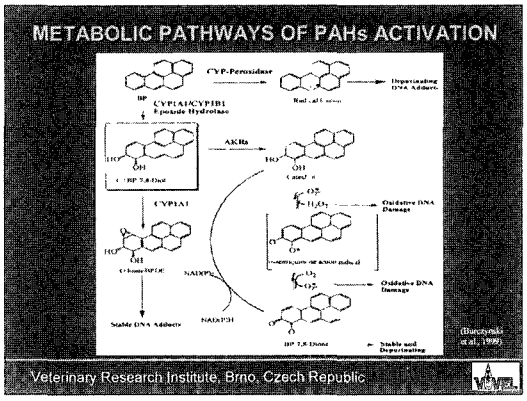

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Genotoxic and nongenotoxic (epigenetic) mechanisms are involved in chemical carcinogenesis after exposure to PAHs

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:

1) poorly differentiated "stem-like" (progenitor) cells (model: non-transformed liver epithelial WB-F344 cells)

2) primary hepatocytes / hepatoma cell lines (e.g., rat hepatoma H4IIE cells)

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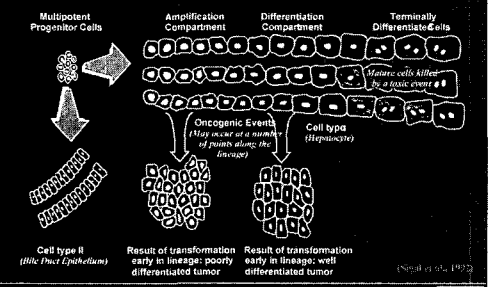
"In human liver both dedifferentiation of mature hepatocytes and maturation arrest of progenitor cells may lead to hepatocellular carcinoma in humans" (Roskams et al., 2003)



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

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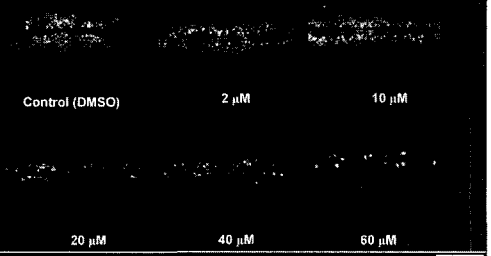
Nongenotoxic PAHs with no significant AhR-inducing potency inhibit GJIC (fluoranthene, 30 min exposure)



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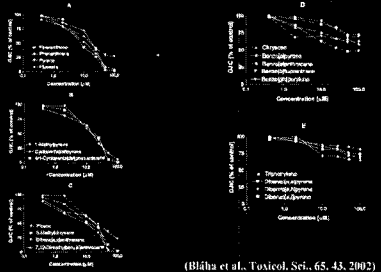
Dose-dependent inhibition of GJIC by PAHs in the WB-F344 cells (30 min exposure, SL/DT assay)



(Bláha et al., Toxicol. Sci., 65, 43, 2002)

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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 (DBPyrenes) 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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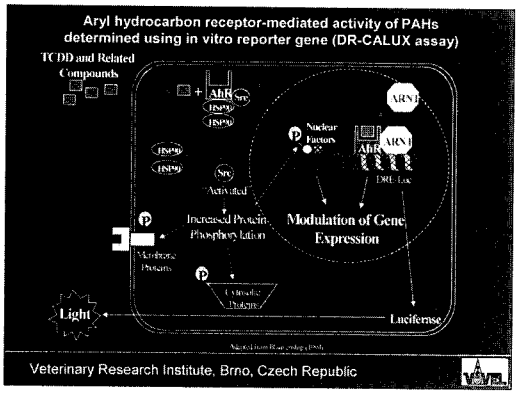
Aryl hydrocarbon receptor-mediated activity of PAHs determined using in vitro reporter gene (DR-CALUX assay)
TCDD and Related Compounds
AhR
ARNT
Nuclear Factors
Modulation of Gene Expression
Increased Protein Phosphorylation
Light
Luciferase
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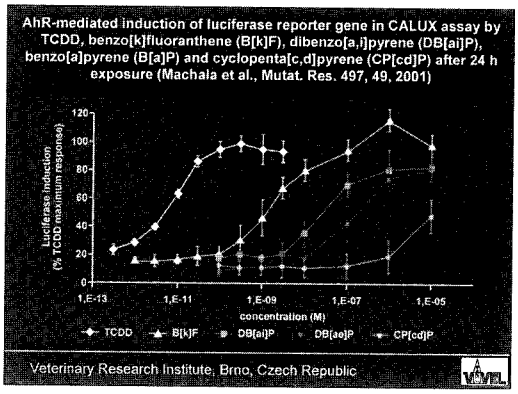
AhR-mediated induction of luciferase reporter gene in CALUX assay by TCDD, benzo[k]fluoranthene (B[k]F), dibenzo[a,i]pyrene (DB[ai]P), benzo[a]pyrene (B[a]P) and cyclopenta[c,d]pyrene (CP[cd]P) after 24 h exposure (Machala et al., Mutat. Res. 497, 49, 2001)
Luciferase induction (% TCDD maximum response)
concentration (M)
TCDD
B[k]F
DB[ai]P
DB[ae]P
CP[cd]P
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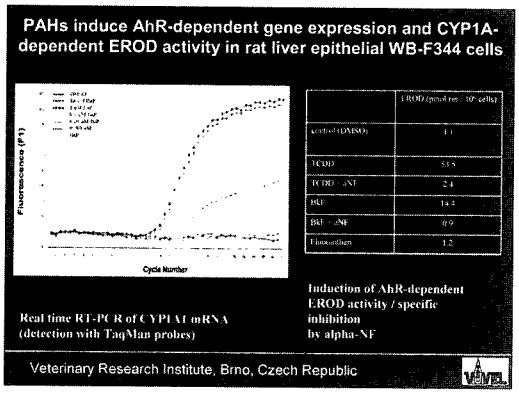
PAHs induce AhR-dependent gene expression and CYP1A-dependent EROD activity in rat liver epithelial WB-F344 cells
Fluorescence (F1)
Cycle Number
Real time RT-PCR of CYP1A1 mRNA (detection with TaqMan probes)
Induction of AhR-dependent EROD activity / specific inhibition by alpha-NF
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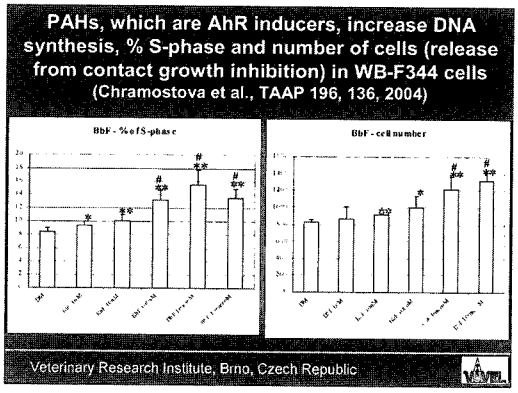
PAHs, which are AhR inducers, increase DNA synthesis, % S-phase and number of cells (release from contact growth inhibition) in WB-F344 cells (Chramostova et al., TAAP 196, 136, 2004)
BbF - % of S-phase
BbF - cell number
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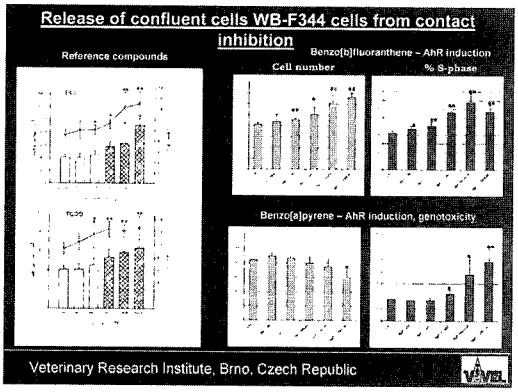
Release of confluent cells WB-F344 cells from contact inhibition
Reference compounds
Benzo[b]fluoranthene – AhR induction
Cell number
% S-phase
Benzo[a]pyrene – AhR induction, genotoxicity
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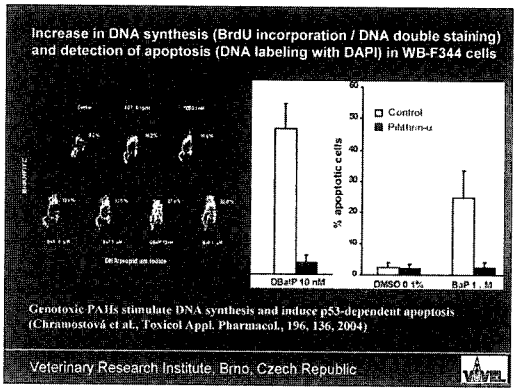
Increase in DNA synthesis (BrdU incorporation / DNA double staining) and detection of apoptosis (DNA labeling with DAPI) in WB-F344 cells
Control
% apoptotic cells
DBaiP 10 nM
DMSO 0.1%
Genotoxic PAHs stimulate DNA synthesis and induce p53-dependent apoptosis (Chramostová et al., Toxicol Appl. Pharmacol., 196, 136, 2004)
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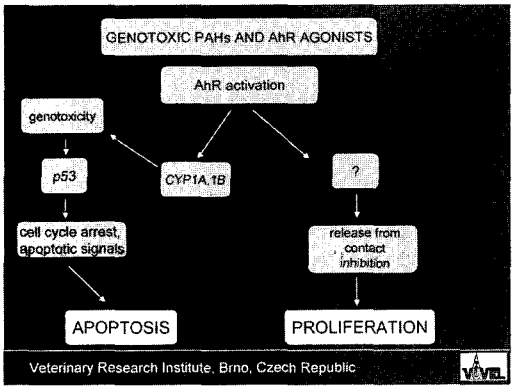
GENOTOXIC PAHs AND AhR AGONISTS
AhR activation
genotoxicity
p53
CYP1A,1B
?
cell cycle arrest, apoptotic signals
release from contact inhibition
APOPTOSIS
PROLIFERATION
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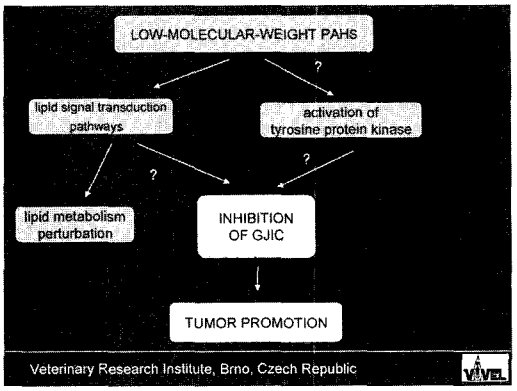
LOW-MOLECULAR-WEIGHT PAHS
?
lipid signal transduction pathways
activation of tyrosine protein kinase
?
?
lipid metabolism perturbation
INHIBITION OF GJIC
TUMOR PROMOTION
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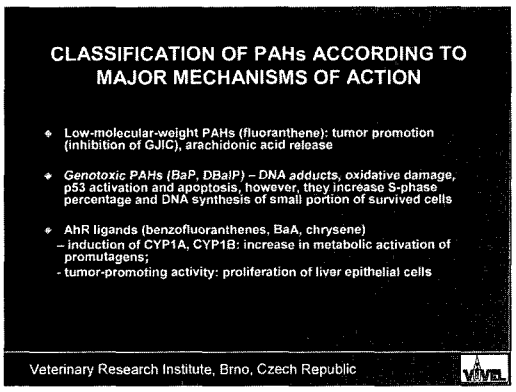
CLASSIFICATION OF PAHs ACCORDING TO MAJOR MECHANISMS OF ACTION
Low-molecular-weight PAHs (fluoranthene): tumor promotion (inhibition of GJIC), arachidonic acid release
Genotoxic PAHs (BaP, DBalP) – DNA adducts, oxidative damage, p53 activation and apoptosis, however, they increase S-phase percentage and DNA synthesis of small portion of survived cells
AhR ligands (benzofluoranthenes, BaA, chrysene)
– induction of CYP1A, CYP1B: increase in metabolic activation of promutagens;
- tumor-promoting activity: proliferation of liver epithelial cells
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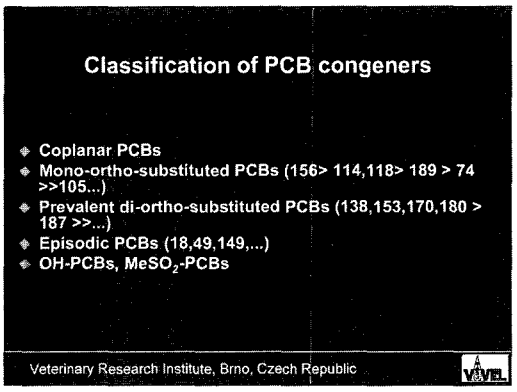
Classification of PCB congeners
Coplanar PCBs
Mono-ortho-substituted PCBs (156> 114,118> 189 > 74 >>105...)
Prevalent di-ortho-substituted PCBs (138,153,170,180 > 187 >>...)
Episodic PCBs (18,49,149,...)
OH-PCBs, MeSO2-PCBs
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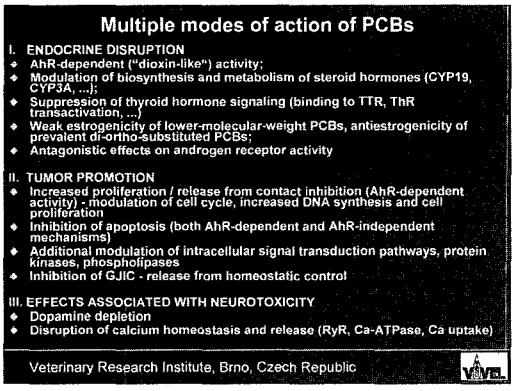
Multiple modes of action of PCBs
I. ENDOCRINE DISRUPTION
AhR-dependent ("dioxin-like") activity;
Modulation of biosynthesis and metabolism of steroid hormones (CYP19, CYP3A, ...);
Suppression of thyroid hormone signaling (binding to TTR, ThR transactivation, ...)
Weak estrogenicity of lower-molecular-weight PCBs, antiestrogenicity of prevalent di-ortho-substituted PCBs;
Antagonistic effects on androgen receptor activity
II. 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
III. EFFECTS ASSOCIATED WITH NEUROTOXICITY
Dopamine depletion
Disruption of calcium homeostasis and release (RyR, Ca-ATPase, Ca uptake)
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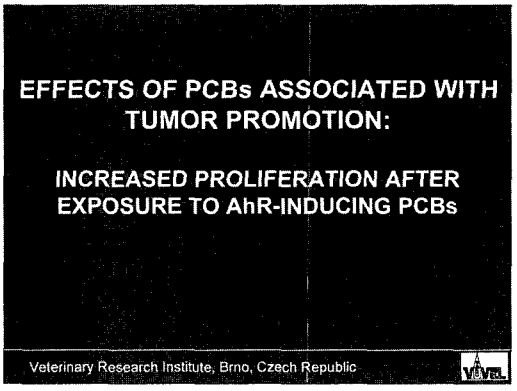
EFFECTS OF PCBs ASSOCIATED WITH TUMOR PROMOTION:
INCREASED PROLIFERATION AFTER EXPOSURE TO AhR-INDUCING PCBs
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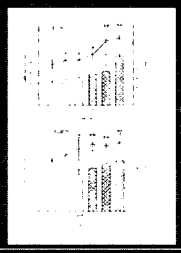
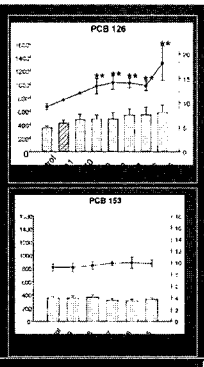
PCBs, which are AhR inducers, increase DNA synthesis, % S-phase and number of cells (release from contact growth inhibition) in WB-F344 cells





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EFFECTS OF PCBs ASSOCIATED WITH TUMOR PROMOTION:

IN VITRO INHIBITION OF GJIC BY ORTHO-SUBSTITUTED PCBs AND OXYGENATED PCB METABOLITES

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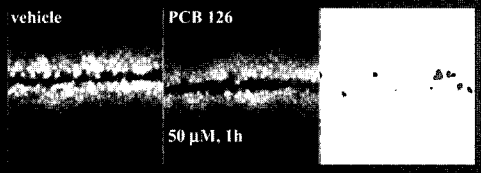
INHIBITION OF GJIC IN WB-F344 CELLS



ortho-substituted PCBs inhibit GJIC (Machala et al., Toxicol. Sci. 76, 102, 2003).

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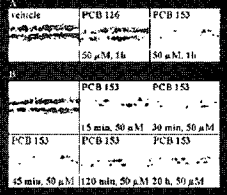
A lack of GJIC recovery in PCB 153-treated WB-F344 cells



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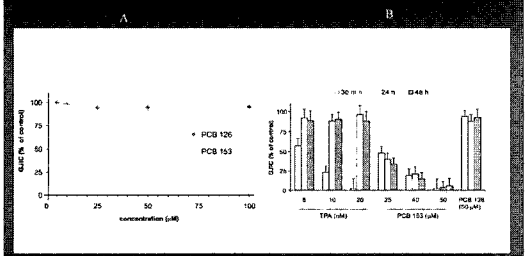
Inhibition of GJIC by coplanar and noncoplanar PCBs

A, dose-response curves (after 30 min exposure);
B, persistent inhibition by noncoplanar PCB: PCB 126 (3,3',4,4',5-pentachlorobiphenyl) is coplanar AhR inducer, PCB 153 (2,2',4,4,5,5'-hexachlorobiphenyl) is a prototypical noncoplanar congener



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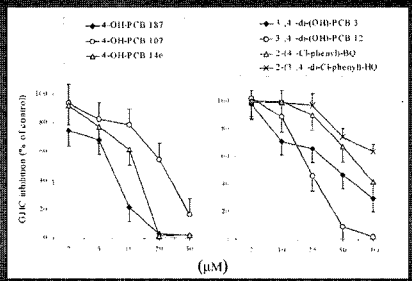
Inhibition of GJIC in the WB-F344 cells exposed to selected hydroxylated PCBs (Machala et al., Chem. Res. Toxicol. 17, 340, 2004)



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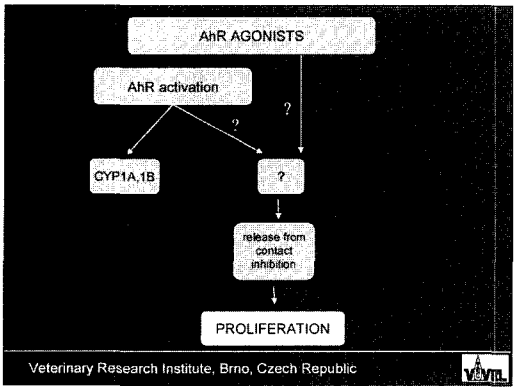
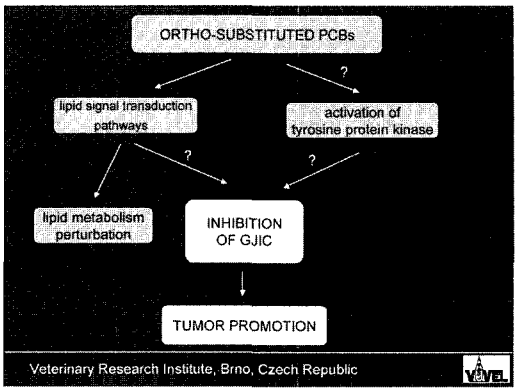

AhR AGONISTS
AhR activation
?
?
CYP1A,1B
?
release from contact inhibition
PROLIFERATION
Veterinary Research Institute, Brno, Czech Republic


ORTHO-SUBSTITUTED PCBs
?
lipid signal transduction pathways
activation of tyrosine protein kinase
?
?
lipid metabolism perturbation
INHIBITION OF GJIC
TUMOR PROMOTION
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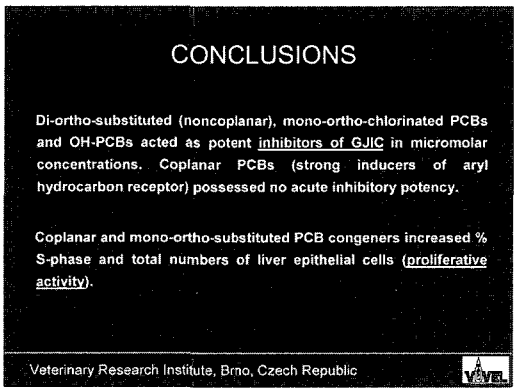
CONCLUSIONS
Di-ortho-substituted (noncoplanar), mono-ortho-chlorinated PCBs and OH-PCBs acted as potent inhibitors of GJIC in micromolar concentrations. Coplanar PCBs (strong inducers of aryl hydrocarbon receptor) possessed no acute inhibitory potency.
Coplanar and mono-ortho-substituted PCB congeners increased % S-phase and total numbers of liver epithelial cells (proliferative activity).
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