Environmental distribution and modes of action of methylated PAHs in rat liver epithelial and hepatoma cells

Miroslav Machala[1], Katerina Pencikova[1], Lenka Svihalkova-Sindlerova[2], Sona Marvanova[1], Pavel Krcmar[1], Miroslav Ciganek[1], Jiri Neca[1], Jan Topinka[3], Jan Vondracek[2]

[1]Veterinary Research Institute

[2]Laboratory of Cytokinetics, Institute of Biophysics, Brno

[3]Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR, Prague

Combined GC/MS and HPLC/DAD analysis of a large set of polycyclic aromatic hydrocarbons (PAHs) and methylated PAH derivatives identified high levels of methylated naphthalenes, anthracenes, phenanthrenes, chrysenes, and benz[a]anthracenes in river sediments and airborne samples collected in the Czech Republic. Their levels were comparable to the most abundant PAHs found in river sediments in the areas with petrochemical industry. One of the essential steps in their risk assessment is in vitro characterization of tissue- and cell-specific mechanisms of their toxicity and determination of their relative toxic potencies. Rat liver epithelial WB-F344 cells and rat hepatoma H4IIE cells might be used as respective models for studies on effects of xenobiotics in immature progenitor liver cells and hepatocytes, two possible cellular targets of hepatocarcinogens. Both nongenotoxic and genotoxic events were detected after exposure to a series of methylated naphthalenes, anthracenes, phenanthrenes, chrysenes and benz[a]anthracenes in WB-F344 and/or H4IIE cells, including the activation of aryl hydrocarbon receptor (AhR), induction of CYP1A1, CYP1A2, CYP1B1 and AKR1C9, major enzymes of metabolic activation of PAHs, detection of cell proliferation and apoptosis, accumulation of phosphorylated p53 protein, and inhibition of gap junctional intercellular communication (GJIC).

Methylnaphthalenes did not modulate AhR-dependent gene expression, cell cycle, proliferation or apoptosis in WB cells. 1- and 9-methylanthracene, as well as methylphenanthrenes inhibited strongly GJIC, but they did not affect cell numbers and they were only weak inducers of AhR-mediated activity. In contrast, high AhR-mediated activity in both H4IIE and WB cells, associated with induction of metabolizing enzymes, S-phase increase and release from contact inhibition of WB cells, were found after the treatment with all six methyl derivatives of chrysene and majority of methylated benz[a]anthracenes. Additionally 5- and 6-methylchrysene, as well as benz[a]anthracenes methylated at 1-, 2-, 8-, 10-, 11- or 12-positions, inhibited GJIC in WB cells. Although relatively high DNA adduct formation was detected in WB cells exposed to 1 mM 5-methylchrysene, the nongenotoxic modes of action of methylchrysenes appeared to be major events that might contribute to the carcinogenic potential of these compounds. With exception of model 7,12-dimethyl derivative (DMBA) and partly 10-methylbenz[a]anthracene, these compounds elicited only nongenotoxic modes of action in the WB cells. The DMBA elicited genotoxicity led to decrease in cell number and apoptosis. In summary, nongenotoxic modes of action seemed to prevail for a majority of methyl-PAHs under study. These data allow us to conclude that methyl-PAHs could be important toxicants acting via multiple modes of action associated with carcinogenesis and tumor promotion processes. [Supported by the Czech Ministry of Agriculture, grant No. 0002716201 and by the Grant Agency of the Czech Republic, grant No. 525/03/1527.]