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Genotoxicity of Organic Extracts of Particulate Emissions from Conventional Gasoline and Alternative Fuels

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Introduction: Modern gasoline engines represent an important source of potentially harmful emissions composed of various pollutants including particulate matter (PM). The type of fuel affects both the amount and the toxicity of the emissions. Alternative fuels containing bio-additives have recently become popular as alternatives to non-renewable fossil fuels. However, little is known about the genotoxicity of their emissions. The aim of this study was to compare genotoxic potencies and mechanisms of the potential genotoxicity of organic extracts from gasoline particulate emissions produced by neat gasoline fuel (E0) and its blends with 15% ethanol (E15), 25% n-butanol (n-But25), and 25% isobutanol (i-But25). **Methods:** Human bronchial epithelial cells (BEAS-2B) were exposed to the organic extracts from PM in non-cytotoxic concentrations (1-50 μg/mL) determined by the WST-1 test. The biomarkers of genotoxicity, such as DNA damage evaluated by the comet assay, the micronuclei formation, levels of phosphorylated histone H2AX (γH2AX), and the expression of genes relevant to the DNA damage response, were determined. **Results:** Chemical analysis revealed that despite the lowest PM mass, n-But25 extract contained the highest concentrations of polycyclic aromatic hydrocarbons (PAHs), as well as oxy-, nitro-, and dinitro-PAHs derivates, while in i-But25 extract these concentrations were the lowest. The Comet assay showed that E0 extract generated a significant dose-dependent increase of DNA strand breaks and oxidative DNA lesions. A lower, yet considerable, level of DNA damage was elicited by E15 extract. n-But25 and i-But25 extracts were the least genotoxic; only a mild increase of oxidative DNA damage was observed. The level of γH2AX, indicating DNA double-strand breaks, was not significantly elevated in any sample. The frequency of micronuclei, a marker of genotoxicity and genomic instability, was not affected by any of the tested PM extracts either; the highest doses of all extracts rather decreased the cytokinesis-blocked proliferative index indicating the increasing cytotoxicity or cell cycle delay. Gene expression analysis revealed mild activation of genes related to DNA damage response and strongly increased expression of genes indicating the activation of aryl hydrocarbon receptor (AhR), a nuclear receptor known to mediate toxic effects of PAHs. **Conclusion**: Taken together, the data suggest that PM extracts from diverse conventional and alternative gasoline fuels differ in the qualitative and quantitative chemical composition and that the genotoxic properties in BEAS-2B cells are most likely influenced by the relative proportion of individual PAHs rather than their overall content. AhR activation may play an important role in the toxicity of gasoline PM emissions.

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