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Lack of genotoxic mechanisms in isoeugenol-induced hepatocellular tumorigenesis in male B6C3F1 mice

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Abstract

Isoeugenol (IEG) is a natural alkenylbenzene compound which is used as a flavoring additive in foods. However, it has been shown to be a hepatocarcinogen in male B6C3F1 mice. Although there are negative results in several genotoxicity tests, the genotoxicity of IEG in the livers of male mice has not been investigated. To determine whether a genotoxic mechanism is involved in hepatocarcinogenesis, we carried out histopathological analyses, comprehensive DNA adduct analyses, *in vivo* mutation assays and global gene expression analyses in the livers of male and female B6C3F1 *gpt* delta mice treated with IEG by gavage at doses of 0, 150, 300 or 600 mg/kg bw/day for 13 weeks. IEG induced slight hepatocyte hypertrophy along with liver weight gain in male mice treated with 300 mg/kg bw/day IEG and more, but not in similarly treated female mice. Comprehensive DNA adduct analyses by LC-MS/MS showed no specific DNA adduct formation in the liver, and there were no changes in *gpt* or Spi$^-$ mutant frequencies in the livers. A pathway analysis of mRNA expression data as determined with a cDNA microarray suggested activation of pathways associated with peroxisome proliferator-activated receptor (PPAR) α and γ in the livers of male mice. Overall, our data show a lack of genotoxicity in the mechanisms leading to the hepatocarcinogenesis of IEG in mice, and they suggest the involvement of PPARα and γ pathway activation in this process.

Keywords : Alkenylbenzene, isoeugenol, mutagenicity, carcinogenicity, DNA adduct

I Introduction

Isoeugenol (4-hydroxy-3-methoxypropenyl benzene; IEG) is an alkenylbenzene compound, which is a natural constituent of several herbs, including clove, nutmeg, tobacco and mace. It has been found use as a flavoring agent in non-alcoholic drinks, baked foods, and chewing gums. Toxicology and carcinogenesis studies for IEG were conducted by the National Toxicology Program (NTP) and were reported in an NTP technical report[1)]. In carcinogenesis study in F344/N rats, no clear evidence was identified to establish carcinogenic activity of IEG. However, oral administration of 0, 75, 150 and 300 mg/kg bw IEG for 2 years increased incidences of hepatocellular adenoma and hepatocellular carcinoma in male mice, but not in female mice. Therefore, the NTP concluded that there was clear evidence of carcinogenic activity of IEG in male B6C3F1 mice. In genotoxicity tests, IEG showed negative results in reverse mutation assays using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and using *Escherichia coli* (*E. coli*) strain WP2 uvrA with or without activation with the S9 crude liver enzyme extract[1)]. Similarly, negative results were found in chromosome aberration tests using Chinese hamster ovary cells with or without metabolic activation[1)]. *In vivo* micronucleus assays in mouse bone marrow samples led to positive results in female mice, but not in male mice[1)]. Thus, it was concluded that genotoxic mechanisms did not contribute to the observed IEG-induced hepatocarcinogenesis in male mice.

Some other alkenylbenzene compounds, such as estragole, methyleugenol and safrole, have been also known to be rodent hepatocarcinogens[2-7)]. Importantly, these alkenylbenzenes are

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