# Systematic Evaluation of the Evidence Base on Methyl tert-Butyl Ether for Carcinogenic Potential in Humans; Low Concern Based on Animal Cancer Studies and Mechanistic Data

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Abstract:

**Background and Purpose:** Methyl tert-butyl ether (MTBE) is a high-octane fuel component that helps gasoline burn cleaner and reduces automobile emissions. In 1999, the International Agency for Research on Cancer (IARC) categorized MTBE as "not classifiable" regarding human carcinogenicity (Group 3). Since then, further studies, including a 90-day drinking water study and a cancer bioassay, have been conducted, as well as *in vivo* and *in vitro* mechanistic studies/assays to examine MTBE's carcinogenic potential. This evaluation aims to assess the carcinogenic potential of MTBE by evaluating available epidemiological and animal cancer studies, as well as mechanistic data.

**Methods:** A systematic literature search and review was conducted to identify mechanistic data, as well as studies investigating cancer in MTBE-exposed humans and experimental animals. Mechanistic data was integrated and synthesized using key characteristics of carcinogens (KCCs). Critical appraisal was performed for relevant animal cancer bioassays using SciRAP and relevant mechanistic studies using Klimisch scores. Available data from epidemiological, experimental animal, and mechanistic studies were integrated using a weight of evidence approach incorporating study reliability to evaluate the strength of the evidence of carcinogenic potential of MTBE in humans.

**Results:** In two reliable cancer studies, chronic inhalation exposure to MTBE increased the incidence of hepatocellular adenomas (HCA) (8,000 ppm) in B6C3F1 female mice, and the incidence of renal adenomas/carcinomas (3,000 ppm) and interstitial cell adenomas (3,000 ppm and 8,000 ppm) in male F344 rats. In a reliable two-year drinking water study, the only tumor response was a low incidence of brain astrocytomas observed in male Wistar Han rats exposed to 7.5 mg/mL MTBE. The observed tumor responses are unlikely to be relevant to humans as they either occurred at doses that exceeded the maximum tolerated dose (HCA), developed through a mode of action (MoA) not relevant to humans (renal adenomas/carcinomas), were common rat age-related findings (interstitial cell adenomas), or occurred at incidences that were only marginally statistically significant (P=0.032) (brain astrocytomas). Inconsistent results across different species, strains, and genders further reduces the confidence that these tumor findings were directly attributable to MTBE exposure or through a MoA that does not operate in humans (male rat kidney tumors). Robust mechanistic data was available for MTBE that allowed evaluation of nine out of the 10 KCCs. Reliability exclusions included any assay that did not include an assessment of corresponding measures of cytotoxicity or assays known to use unreliable methods as corroborated by existing literature. Overall, the data for six KCCs were found to be inconsistent or minimal/weak (i.e., one study), or were consistently negative across model systems and endpoints. There were three KCCs (electrophilic or can be metabolically activated to an electrophile (KCC1), oxidative stressor (KCC5) and cell proliferation, cell death and nutrient supply (KCC10) that had consistent activity in at least one model system (i.e., primary human cells, experimental animal, human

cell lines, or mammalian primary cells/cell lines). Several studies reported the formation of DNA-adducts and crosslinks. MTBE is metabolized to equimolar levels of tert-butyl alcohol and formaldehyde; formation of formaldehyde provides evidence of metabolic activation to an electrophile. MTBE lacked genotoxic activity, and in studies where rodents were exposed to MTBE at concentrations used in MTBE cancer bioassays, there was no mutagenic or genotoxic activity including measures of mutant frequency, micronucleus formation, and chromosomal aberrations. Other than genotoxicity, the most data rich KCCs were induction of oxidative stress (KCC5), modulation of receptor-mediated effects (KCC8), and alteration of cell proliferation, cell death, and nutrient supply (KCC10). Overall, conclusions on data for oxidative stress, receptor-mediated effects and alteration of cell proliferation, cell death, and nutrient supply were limited in their strength for considering carcinogenic activity in humans. For oxidative stress, minimal data were available for primary human cells, and there was inconsistent activity across animal studies. For receptor-mediated effects, measures of activity in estrogen pathway, aromatase activity, and thyroid pathway were overall weak or negative. No activity was reported for effects on androgen pathway in human or mammalian cell lines. However, in animal studies, although there was adequate data, the results were inconsistent across similar studies and endpoints. For KCC10 (cell proliferation, cell death and nutrient supply), animal models provided strong evidence that MTBE caused increased cell proliferation in both liver and kidney, tissues in which tumors were identified in mice and rats, respectively, but not in other tissues (e.g., ovary, uterus, or pituitary gland) in which no tumors were identified. The MoAs for these liver and kidney tumors involved increased mitogenicity or cytotoxicity driving tumor development. Inconsistent data were reported for *in vitro* mammalian or *in vitro* human cell lines; no data were available in human primary cells.

Conclusions: There was a low incidence of selected tumors (liver, kidney, brain) at high exposure concentrations across the three reliable MTBE cancer bioassays with evidence supporting that MTBE lacks mutagenic and genotoxic activity. Evidence streams of mechanistic activity within each KCC, besides increased cell proliferation, were at best limited due to the unreliable, inconsistent, or minimal data available. Therefore, this assessment supports an overall low concern for carcinogenic hazard of MTBE in humans.