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

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Abstract:

Background and Purpose: Ethyl tertiary-butyl ether (ETBE) is a component of fuel and tert-butyl alcohol (TBA) is used as a solvent or manufacture agent for flotation agents, and perfumes. TBA is also a primary metabolite of ETBE and methyl tert-butyl ether (MTBE). Exposure to these compounds occurs through inhalation, ingestion of contaminated water or food, and dermal contact, particularly in occupational settings. The objective of this assessment was to evaluate the human carcinogenic hazard of ETBE and TBA by assessing 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 ETBE- and TBA-exposed humans and experimental animals. Mechanistic data were integrated and synthesized using key characteristics of carcinogens (KCCs). Relevant animal cancer bioassays were evaluated using SciRAP and relevant mechanistic studies using Klimisch scores. Available data from 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 ETBE and TBA in humans.

Results: ETBE was evaluated for its ability to cause cancer in two reliable standard cancer studies conducted in male and female F344 rats, either via drinking water (up to 10,000 ppm) or via inhalation exposure (up to 5,000 ppm). ETBE exposure via drinking water did not result in an increase in tumors. However, inhalation exposure resulted in an increased incidence in hepatocellular adenomas in male, but not female, rats (5,000 ppm ETBE). No exposure-related histopathological changes were observed in any other tissues in either sex. No mechanistic data were available to evaluate if ETBE was an electrophile directly or after metabolic activation, or if it induced chronic inflammation or immortalization. The mutagenicity of ETBE was evaluated in multiple strains of *Salmonella typhimurium*, and all were negative with and without metabolic activation. This lack of activity was confirmed in an *in vivo* Big Blue mutagenicity assay where rats were exposed to concentrations of ETBE up to 5000 ppm for 28 days with no increase in mutation frequency in the liver or bone marrow. Genotoxic endpoints measured in animal models exposed to ETBE resulted in consistent negative findings (micronucleus formation) in a guideline rat study and inconsistent findings in studies that evaluated DNA damage. Minimal/weak data were available for altered DNA repair or genomic instability, induction of epigenetic alterations, and oxidative stress. In experimental animal studies, ETBE exposure resulted in no change in white blood cell or differential leukocyte counts, with a decrease in T-cells and inconsistent changes in IgM antibody-forming cells evaluated across several studies in the evaluation of immunosuppression. Receptor-mediated effects in the liver of ETBE-exposed animals were evaluated and findings included induction of CAR, PXR, CYP450 enzymes, along with PPARα and PPARγ. There were consistent increases in liver cell proliferation in two rat studies, either via ETBE inhalation exposure (1, 4, or 13 weeks, up to 5000 ppm) or following oral

gavage (5-6 weeks, up to 1000 mg/kg-bw/day). Overall, there were weak or limited mechanistic data across all the KCCs besides genotoxicity and increased liver cell proliferation. Together these data support a non-genotoxic mechanism of liver cancer that only occurs at the highest ETBE exposure concentration. TBA was evaluated in two reliable cancer studies conducted in rats and mice exposed via drinking water. This exposure resulted in an increased incidence of renal adenoma and carcinomas in male rats exposed to 2.5 mg/L TBA, but not in those in the high dose (5 mg/mL) group. There was decreased survival in both male and female rats exposed to 5 mg/L TBA, but the number of survivors was considered adequate for detecting carcinogenic effects. In mice, the incidence of thyroid follicular cell adenoma was significantly increased in 20 mg/mL females, and a follicular cell carcinoma was observed in one 20 mg/mL male. For TBA, no data were available for three KCCs (alter DNA repair or cause genomic instability, epigenetic alterations, and immortalization), and limited data were available to evaluate four KCCs (acts as an electrophile directly or after metabolic activation, induce chronic inflammation, immunosuppressant, and modulate receptor-mediated effects). For genotoxicity, there was consistent negative mutagenicity activity reported in two Ames studies in multiple strains of *Salmonella typhimurium* at concentrations ranging from 100-5,000 µg TBA/plate with and without metabolic activation. This lack of mutagenicity was supported by the ETBE Big Blue mutagenicity study as ETBE is metabolized to TBA. Consistent activity was reported in three studies using mammalian cell lines and primary cells measuring DNA strand breaks, mutagenicity (without S9 activation), and chromosomal aberrations with and without S9. In experimental animal models, one OECD-compliant study reported no DNA damage in the thyroid of mice exposed to 1500 mg/kg-day TBA via oral gavage. A second OECD-compliant study reported no increase in micronucleated erythrocytes in peripheral blood from male and female mice exposed to TBA in drinking water (up to 40 mg/mL) for 90 days and in bone marrow of male mice administered (i.p.) up to 1250 mg/kg-bw/day TBA for 3 days. For cell proliferation, cell death, and nutrient supply, data were inconsistent in human and mammalian cell lines exposed to TBA across three studies showing either decreased or no change in cell proliferation, and inconsistent changes in markers of cell cycle arrest. In experimental animals, consistent activity was reported in several studies that evaluated cell proliferation or hyperplasia in the kidney and urinary bladder following exposure to TBA. The studies in kidney were conducted to investigate the mechanism of TBA-induced male rat kidney tumors through exacerbation of α2u-globulin nephropathy, a mechanism that does not operate in humans.

Conclusions: There was a low incidence of all tumors reported following exposure to ETBE (liver tumors) or TBA (kidney and thyroid tumors) at high dose exposure concentrations to ETBE or TBA, respectively, across reliable cancer bioassays. ETBE and TBA were also shown to lack mutagenic and genotoxic activity. Mechanistic evidence within the other KCCs, besides increased cell proliferation, were limited either due to a lack of data, or inconsistencies in activity across studies. This assessment supports an overall low concern for carcinogenic hazard of both ETBE and TBA in humans.