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Toxicological Review of Ethyl Tertiary Butyl Ether

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Ethyl *tertiary* -butyl ether (ETBE) does not occur naturally; it is a man-made ether oxygenate used primarily as a gasoline additive. It was used until 2006 in the United States and is still used in Japan and the European Union. ETBE is released into the environment through gasoline leaks, evaporation, and spills. Exposure to ETBE can occur by drinking contaminated groundwater or by inhaling off-gases containing ETBE. Dermal exposure is possible in occupational settings where ETBE is manufactured. The magnitude of human exposure to ETBE depends on factors such as the distribution of ETBE in groundwater and the extent of the contamination.

Animal studies demonstrate that exposure to ETBE is associated with noncancer kidney effects following oral and inhalation exposure. Evidence is suggestive of carcinogenic potential for ETBE based on liver tumors in rats following inhalation exposure.

EFFECTS OTHER THAN CANCER OBSERVED FOLLOWING ORAL EXPOSURE

Kidney effects are identified in this assessment as a potential human hazard of ETBE exposure. Although no human studies are available to evaluate the effects of ETBE, oral exposure studies in animals have consistently reported increased kidney weight in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia of the renal pelvis (in males), and increased blood concentrations of total cholesterol, blood urea nitrogen (BUN), and creatinine. Overall, there was consistency across multiple measures of potential kidney toxicity, including organ-weight increases, exacerbated CPN, urothelial hyperplasia of the renal pelvis, and increases in serum markers of kidney function. Additionally, effects were also observed across routes of exposure, and sex (with the exception of urothelial hyperplasia of the renal pelvis which was observed only in male rats).

The relevance of the kidney findings to humans was evaluated with respect to alpha 2u-globulin nephropathy, a disease process that occurs exclusively in the male rat kidney (Capen et al., 1999; U.S. EPA, 1991a). While ETBE binds to alpha 2u-globulin and meets some criteria of the alpha 2u-globulin U.S. Environmental Protection Agency (EPA) and International Agency for Research on Cancer (IARC) frameworks (Capen et al., 1999; U.S. EPA, 1991a), it does not meet all. With respect to male rats, U.S. EPA (1991a) noted that "[i]f a compound induces α2u-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occurring in humans." However, as alpha 2u-globulin nephropathy is strictly a male rat phenomenon, the dose-related kidney effects in female rats are not confounded by alpha 2u-globulin nephropathy.

It has been observed that chemicals that bind to alpha 2u-globulin also exacerbate the incidence and/or severity of background CPN in male rats (Frazier et al., 2012; Travlos et al., 2011; U.S. EPA, 1991a). While the etiology of CPN is unknown (NIEHS, 2019; Hard and Khan, 2004; Peter et al., 1986) and it has no known analogue in the aging human kidney (NIEHS, 2019; Hard et al., 2009), it cannot be ruled out that a chemical that exacerbates CPN in rats could also exacerbate disease processes in the human kidney [e.g., chronic kidney disease, diabetic nephropathy, glomerulonephritis, interstitial nephritis, etc.; NIEHS (2019)]. Therefore, increased incidence of kidney effects with ETBE exposure in the female rat (but not the male rat) are considered appropriate for identifying a hazard to the kidney.

Evidence is suggestive that liver toxicity follows oral ETBE exposure. The strongest supporting evidence is the increased liver weights and centrilobular hypertrophy in exposed male and female rats consistently reported across oral-exposure studies. No additional histopathological findings were observed, however, and only one serum marker potentially indicative of liver toxicity (γ-glutamyl transferase [GGT]) was elevated, while other markers (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and alkaline phosphatase [ALP]) were unchanged. The magnitude of change for these noncancer effects was minimal and, except for organ-weight data, no consistent dose-response relationships were observed. Mechanistic data suggest that ETBE exposure leads to activation of several nuclear receptors, but there is inadequate evidence to establish a relationship between receptor activation and liver toxicity resulting from ETBE exposure. In addition, mechanistic data suggest possibly greater susceptibility of toxic effects related to reduced clearance of acetaldehyde, a metabolite of ETBE. Thus, even with the consistently observed increases in rat liver weight and centrilobular hypertrophy, the evidence remains suggestive that liver toxicity follows ETBE exposure because of the relatively small magnitude of effects and inconsistent dose-response relationships.

Inadequate information exists to draw conclusions regarding reproductive, developmental, or immune system effects. The ETBE database does include developmental, reproductive, and multigenerational studies, which are generally null and do not appear to indicate an area of concern (see hazard discussions in Sections 1.2.3 and 1.2.4). However, this body of evidence is not sufficiently robust to conclude that ETBE is not likely to be a reproductive or developmental hazard. Regarding immune system effects, the ETBE database contains no evidence of altered immune function that correlate with modest T cell population reductions and altered splenic organ weights (see Appendix B of the Supplemental Information); thus, the available immune data are inadequate to draw conclusions as a human hazard of ETBE exposure.

ORAL REFERENCE DOSE (RFD) FOR EFFECTS OTHER THAN CANCER

Kidney toxicity, represented by increased absolute kidney weight in female rats, was chosen as the basis for the overall RfD (see Table ES-1). The chronic study by JPEC (2010a) [with selected data published as Suzuki et al. (2012)] and the observed kidney effects were used to derive the RfD. The endpoint of increased kidney weight was selected as the critical effect because it is a specific

and sensitive indicator of kidney toxicity and was induced in a dose-responsive manner. The increases in kidney weight seen in male and female rats are likely a result of the increases in the severity of CPN seen with ETBE exposure. A characteristic feature of CPN in the rat is increasing kidney size and weight (Hard et al., 2013). Benchmark dose (BMD) modeling was used to derive the benchmark dose lower confidence limit corresponding to 10% extra risk ($BMDL_{10}$) of 120 mg/kg-day. The BMDL was converted to a human equivalent dose (HED) of 28.8 mg/kg-day using body weight$^{3/4}$ scaling (U.S. EPA, 2011), and this value was used as the point of departure (POD) for RfD derivation.

The overall RfD was calculated by dividing the POD for increased absolute kidney weight by a composite uncertainty factor (UF_C) of 30 to account for extrapolation from animals to humans (3) and interindividual differences in human susceptibility (10).

Table ES-1. Organ/system-specific RfDs and overall RfD for ETBE

Hazard	Basis	Point of departure[a] (mg/kg-d)	UF_C	Chronic RfD (mg/kg-d)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight	28.8	30	1×10^0	Chronic	High
Overall RfD	**Kidney**	**28.8**	**30**	**1×10^0**	**Chronic**	**High**

[a]Human equivalent dose PODs were calculated using body weight scaling to the 3/4 power ($BW^{3/4}$) (U.S. EPA, 2011).

EFFECTS OTHER THAN CANCER OBSERVED FOLLOWING INHALATION EXPOSURE

Kidney effects are a potential human hazard of inhalation exposure to ETBE. Although no human studies are available to evaluate the effects of exposure, studies in animals have observed increases in kidney weight, altered kidney histopathology, as well as alterations in clinical chemistry including serum cholesterol, BUN, and creatinine. While the histological lesion of urothelial hyperplasia of the renal pelvis was a sensitive endpoint in male rats, it was not observed in female rats or mice of either sex, whereas increases in kidney weight were observed in multiple studies in rats of both sexes and in mice. Changes in kidney weight in female rats were dose-dependent, consistent across multiple studies and are not confounded by alpha 2u-globulin nephropathy, and therefore considered appropriate for identifying a hazard to the kidney.

INHALATION REFERENCE CONCENTRATION (RFC) FOR EFFECTS OTHER THAN CANCER

Kidney toxicity, represented by increased absolute kidney weight, was chosen as the basis for the overall RfC (see Table ES-2). The chronic study by JPEC (2010b) [selected data published as Saito et al. (2013)] and the observed kidney effects were used to derive the RfC. The endpoint, increased absolute kidney weight, was selected as the critical effect because it is a specific and

sensitive indicator of kidney toxicity and was induced in a dose-responsive manner. The increases in kidney weight are likely a result of the increases in the severity of CPN seen with ETBE exposure, as CPN is characterized by cell proliferation and chronic inflammation that results in increased kidney weight (Melnick et al., 2012; Travlos et al., 2011).

Benchmark concentration modeling of increased kidney weight (in female rats) was attempted, but an adequate fit was not achieved. Therefore, a no-observed-adverse-effect level (NOAEL) was used to derive the POD of 6,270 mg/m^3. The NOAEL was adjusted for continuous exposure and converted to a human equivalent concentration (HEC) of 1,110 mg/m^3.

The overall RfC was calculated by dividing the POD for increased absolute kidney weight by a UF of 30 to account for toxicodynamic differences between animals and humans (3) and interindividual differences in human susceptibility (10).

Table ES-2. Organ/system-specific RfCs and overall RfC for ETBE

Hazard	Basis	Point of departure[a] (mg/m^3)	UF	Chronic RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight	1,110	30	4×10^1	Chronic	Medium
Overall RfC	**Kidney**	**1,110**	**30**	**4×10^1**	**Chronic**	**Medium**

[a]Continuous inhalation HEC was adjusted for continuous daily exposure and calculated by adjusting the duration-adjusted POD (POD_{ADJ}) by the dosimetric adjustment factor (DAF = 0.992) for a Category 3 gas.

EVIDENCE OF HUMAN CARCINOGENICITY

Under EPA Cancer Guidelines (U.S. EPA, 2005a), the evidence of carcinogenic potential for ETBE is *suggestive* for inhalation exposure but *inadequate* for oral exposure. ETBE induced liver tumors in male (but not female) rats in a 2-year inhalation exposure study (Saito et al., 2013; JPEC, 2010b). No significant effects were observed in two chronic oral studies in male and female rats [one of high quality; see Section 1.2.5; JPEC (2010a); Maltoni et al. (1999)]. Data on tumorigenicity in mice following ETBE exposure were not available. However, supplementary evidence from two-stage initiation-promotion oral carcinogenesis bioassays indicate increased mutagen-initiated liver tumors, as well as increased tumor incidence in the thyroid, colon, and urinary bladder.

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

A quantitative estimate of carcinogenic potential from oral exposure to ETBE was not derived because an increase in tumors was not observed in the two available chronic oral cancer bioassays (JPEC, 2010a; Maltoni et al., 1999). A route-to-route extrapolation of cancer risk from the inhalation-to-oral route was not carried out because there was no consistent dose-response

relationship observed for liver tumors when compared across oral and inhalation studies on the basis of physiologically based pharmacokinetic (PBPK) modeled internal dose.

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

Although ETBE was considered to have *suggestive evidence of carcinogenic potential*, the main study (Saito et al., 2013; JPEC, 2010b) was conducted according to well-established guidelines for examining potential carcinogenicity and was suitable for quantitative analyses. Thus, while recognizing the uncertainty in the data and the suggestive nature of the weight of evidence, the analysis may be useful for some purposes, such as providing a sense of the magnitude of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a).

A quantitative estimate of carcinogenic potential from inhalation exposure to ETBE was based on the increased incidence of hepatocellular adenomas and carcinomas in male F344 rats following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b). The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated the animals for up to 2 years, and included detailed reporting of methods and results.

An inhalation unit risk was derived for liver tumors in male F344 rats. The modeled ETBE POD was scaled to an HEC according to EPA guidance based on inhalation dosimetry for a Category 3 gas (U.S. EPA, 1994). Using linear extrapolation from the benchmark concentration lower confidence level corresponding to 10% extra risk ($BMCL_{10}$), a human equivalent inhalation unit risk was derived using inhalation unit risk = $0.1/BMCL_{10}$ and calculated to be **8×10^{-5} per mg/m^3**.

SUSCEPTIBLE POPULATIONS AND LIFESTAGES FOR CANCER AND NONCANCER OUTCOMES

ETBE is metabolized to *tert*-butanol and acetaldehyde. Evidence is suggestive that genetic polymorphism of aldehyde dehydrogenase (ALDH)—the enzyme that oxidizes acetaldehyde to acetic acid—could affect ETBE toxicity. The virtually inactive form, ALDH2*2, is found in about one-half of all East Asians [and by extension people of East Asian ancestry; Brennan et al. (2004)]. Evidence is strong in humans that this ALDH2 variant increases the internal dose of acetaldehyde and the cancer risks from acetaldehyde, especially in the development of ethanol-related cancers (Eriksson, 2015; IARC, 2010). Several in vivo and in vitro genotoxicity assays in Aldh2 knockout (KO) and heterozygous mice reported that genotoxicity was significantly increased compared with wild-type controls following ETBE exposure to similar doses associated with cancer and noncancer effects in rodents (Weng et al., 2019; Weng et al., 2014; Weng et al., 2013; Weng et al., 2012; Weng et al., 2011). Inhalation ETBE exposure increased blood concentrations of acetaldehyde in Aldh2 KO mice compared with wild type (Weng et al., 2013). Thus, exposure to ETBE in individuals with the ALDH2*2 variant would be expected to increase the internal dose of acetaldehyde and potentially increase risks associated with acetaldehyde produced by ETBE metabolism in the liver.

Collectively, these data present evidence that people with diminished ALDH2 activity could be considered a susceptible population that could be more sensitive to liver toxicity from ETBE exposure.

KEY ISSUES ADDRESSED IN ASSESSMENT

Dose-related kidney effects were observed in male and female rats. The human relevance of these effects, particularly as they relate to alpha 2u-globulin nephropathy and the exacerbation of chronic progressive nephropathy, is a key issue analyzed in this assessment. An evaluation of whether ETBE causes alpha 2u-globulin-associated nephropathy was performed using the EPA and IARC frameworks (Capen et al., 1999; U.S. EPA, 1991a). ETBE induced an increase in hyaline droplet accumulation and increased alpha 2u-globulin deposition in male rats; however, most of the subsequent steps in the pathological sequence were not observed. U.S. EPA (1991a) states that "[i]f a compound induces α2u-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occurring in humans." However, because alpha 2u-globulin nephropathy is strictly a male rat phenomenon, the dose-related kidney effects in female rats are not confounded by alpha 2u-globulin nephropathy. CPN also plays a role in exacerbating nephropathy in rats; however, the mode of action (MOA) is unknown. Given that there is no definitive pathogenesis for CPN, it cannot be fully ruled out that chemicals that exacerbate CPN in rats may have the potential to exacerbate other disease processes in the human kidney (NIEHS, 2019). Dose-related changes in several indicators of kidney toxicity were observed in male and female rats, including increased absolute kidney weight, histological changes, and increased blood biomarkers (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). These specific effects are considered relevant to humans, particularly the endpoints observed in female rats, because they are not confounded by alpha 2u-globulin related processes.

In addition, the human relevance of the observed liver tumors is discussed in the assessment (see Sections 1.2.2 and 1.3.2). Briefly, a well-conducted inhalation study demonstrated a significant, positive exposure-response for hepatocellular adenomas and carcinomas in male rats (Saito et al., 2013; JPEC, 2010b). While the majority of liver tumors occurred at the highest exposure, statistical tests conducted by the study authors found a significant dose-response trend by both the Peto (incidental tumor test) and the Cochran-Armitage tests. However, two chronic oral exposure studies (one with unrelated mortality were negative for liver tumors (JPEC, 2010a; Maltoni et al., 1999). The integration of relevant carcinogenic evidence is discussed in Section 1.3.2.

The potential MOA for the observed liver tumors was accessed in the Section 1.2.2. The available evidence base for the nuclear hormone receptor MOAs (i.e., peroxisome proliferator-activated receptor α [PPARα], pregnane X receptor [PXR], and the constitutive androstane receptor [CAR]) was inadequate to determine the role these pathways play, if any, in ETBE-induced liver carcinogenesis. Acetaldehyde-mediated genotoxicity also was evaluated as a possible MOA, and although evidence suggests that *ALDH2* deficiency enhanced ETBE-induced

genotoxicity in exposed mice, the available database was inadequate to establish acetaldehyde-mediated mutagenicity as an MOA for ETBE-induced liver tumors. No other MOAs for liver carcinogenesis were identified. Because an MOA for liver carcinogenicity could not be established, in the absence of data to indicate otherwise, the rat liver tumors observed following inhalation exposure are considered relevant to humans (U.S. EPA, 2005a).