Table 4 Histopathological changes of the kidney in F344 rats exposed to ETBE by inhalation for 2 years

Group name (ppm)	Male				Female			
No. of animals examined	Control 50	500 50	1,500 49	5,000 50	Control 50	500 50	1,500 50	5,000 50
Chronic progressive nephropathy	49	50	49	50**	32	38	41	40*
<+>	⟨1⟩	⟨2⟩			⟨19⟩	⟨13⟩	⟨19⟩	⟨10⟩
<2+>	⟨24⟩	⟨20⟩	⟨17⟩	⟨12⟩	⟨11⟩	⟨24⟩	⟨19⟩	⟨20⟩
<3+>	⟨23⟩	⟨24⟩	⟨31⟩	⟨19⟩	⟨2⟩	⟨1⟩	⟨3⟩	⟨9⟩
<4+>	⟨1⟩	⟨4⟩	⟨1⟩	⟨19⟩				⟨1⟩
Mineral deposition: renal papilla	0	0	1	6*	0	0	0	0
<+>			⟨1⟩	⟨6⟩				
Urothelial hyperplasia: pelvis	2	5	16**	41**	0	0	0	0
<+>	⟨2⟩	⟨5⟩	⟨16⟩	⟨41⟩				

Values indicate number of animals bearing lesions

The values in angle bracket indicate the severity grade of lesions: +, slight; 2+, moderate; 3+, marked; 4+, severe

Significant difference: *, $p < 0.05$; **, $p < 0.01$ by Chi-square test

increased hepatocyte proliferation in male and female mice exposed to ETBE at the concentration of 5,000 ppm. In addition, Bird et al. (1997) reported an increased incidence of hepatocellular adenomas in female mice exposed to MTBE, a related chemical, by inhalation for 18 months, and also showed an elevation of cell proliferation in female mouse liver in the follow-up study. Therefore, although causative mechanisms of the enhancement of liver tumors observed in male rats exposed to ETBE are inconclusive at present, a non-genotoxic mode of action (MoA) can be presumed, including CAR-responsive elements. Further study is needed for MoA in ETBE hepatotumorigenicity.

Concerning ETBE carcinogenicity, increases in oncological lesions of the mouth epithelium and forestomach, malignant tumors of the uterus and hemolymphoreticular neoplasias have been previously reported from one oral carcinogenicity study in which ETBE was administered to Sprague–Dawley rats by gavage at daily doses of 250 or 1,000 mg/kg body weight (Maltoni et al. 1999). However, as noted by the authors of the study, a clear dose–response relationship was not found. In addition, McGregor (2007) pointed out several other aspects of the study by Maltoni et al. which precluded drawing definite conclusions as to the carcinogenicity of ETBE: Survival rates at 96 weeks were relatively poor, no information was provided on time of emergence of the tumor types, there was no mention of pre-neoplastic lesions, there was no treatment-related induction of forestomach dysplasias, and there was no evidence that ETBE induced lymphoid neoplasms. Recently, in one medium-term multi-organ carcinogenesis study featuring initiation with strong carcinogens, using male F344 rats, tumor-promoting potential of ETBE in the liver was demonstrated with intragastric administration at 1,000 mg/kg body weight (Hagiwara et al. 2011). However, we found no rat carcinogenicity, particularly for the liver, when animals were treated with ETBE at doses of 625–10,000 ppm in drinking water for 2 years. Average daily ETBE intake throughout the treatment period in the 10,000 ppm groups was 542 mg/kg body weight for males (unpublished data). However, in the present rat 2-year inhalation study, an increased incidence of neoplastic lesion was found in the livers of rats exposed to ETBE at 5,000 ppm. Variation in results may depend on differences in ETBE blood dynamics. It can be estimated that 6-hr inhalation exposure to 5,000 ppm corresponds to daily ETBE uptake of 4,222 mg/kg body weight in males, assuming the minute volume as 561 ml/min (Mauderly et al. 1979) in rats and the lung absorption ratio of ETBE as 100 %.

Besides liver and kidney lesions, no treatment-related histopathological lesions were here observed in any other organs or tissues of ETBE-exposed rats of either sex. While statistically significant changes were found in hematology, blood biochemistry and urinalysis, they could be attributed to the kidney damage, reduced food consumption or other causes not associated with histopathological change.

The significant increase in severity of CPN at the concentration of 5,000 ppm in both sexes deserves comment. CPN is a commonly encountered lesion in aging laboratory rats and is regarded as having no relevance for extrapolation to human risk assessment, since CPN is a rodent-specific disease with no apparent equivalent human kidney disease condition (Barthold 1979; Hard and Khan 2004; Seely and Hard 2008). In the 2-year inhalation study for MTBE, enhancement of CNP was indicated in F344 male rats exposed to MTBE at 400 ppm and above and in female rats at 3,000 ppm and above. In addition, renal tubular cell tumors were induced in male rats at 3,000 ppm and above

(Bird et al. 1997). Therefore, the influence of ETBE on the kidney can be considered to be weaker than that of MTBE.

In the present study, increased incidences of renal papilla mineral deposition and urothelial hyperplasia of the pelvis were apparent in males, but not in females. From the dose and sex dependence, those lesions were not likely associated with hyaline droplet nephropathy resulting from excessive cytoplasmic accumulation of α2u-globulin in renal tubular epithelium as a chronic change (Alden and Frith 1991). An increase in α2u-globulin-containing protein droplets was earlier reported in F344 rats exposed to ETBE vapor by inhalation for 13 weeks (Medinsky et al. 1999). Induction of α2u-globulin nephropathy in male rats has been reported for many chemicals including *d*-limonene and other components of unleaded gasoline (Alden and Frith 1991). The U.S. EPA recommends that such nephropathy in male rats is not an appropriate endpoint to determine non-cancer effects in humans, because there is no evidence that α2u-globulin accumulation occurs in humans (U.S. EPA 1991).

Whether the hepatotumorigenicity of ETBE observed in the present study can be extrapolated to humans is inconclusive at present since ETBE has no genotoxicity. There is a threshold in carcinogenic potential of non-genotoxic carcinogen, and the no-observed-effect level (NOEL) for hepatotumorigenicity of ETBE is considered as 1,500 ppm in rats. Since induction of tumor is hepatocellular adenoma (benign tumor) at high dose, male only, low incidence and proliferative lesion at high dose, the reference value for general population for tumorigenic effect of ETBE by inhalation exposure is calculated as 15 ppm (an uncertainly factor supposed to be in the order of 100 times). The maximum atmospheric concentration of ETBE in the general environment is estimated at 0.038 mg/m^3 (approximately 0.0091 ppm) (JPEC 2008). Therefore, the reference value is very high compared to the maximum atmospheric concentration in point of perspective of tumorigenicity risk in human. In addition, Eitaki et al. (2011) reported that the highest TWA-8 h ETBE for a gas station worker was 0.28 ppm, and that for tank truck drivers was 0.21 ppm based on the exposure monitoring results. The reference concentration for worker of 150 ppm (an uncertainly factor supposed to be in the order of 10 times) is thus very much greater than usual human exposure level of ETBE by the inhalation route even in such highly exposed occupation.

Conclusions

The present 2-year inhalation study demonstrated hepatotumorigenicity of ETBE in male but not female rats. The NOEL for hepatotumorigenicity of ETBE by the inhalation route was 1,500 ppm based on the incidence of liver tumors.

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