Comments to "Toxicological Review of Ethyl Tertiary Butyl Ether (External Review Draft), June 2017"

1. Executive Summary, xxiv, line 4-9, Evidence of Human Carcinogenicity

Comment 1: Please consider the following description, because 2-year carcinogenicity study is essential evidence to evaluate carcinogenicity of chemicals, including negative data.

No carcinogenicity was indicated by a 2-year drinking-water study of ETBE in rats at the dosage up to technically maximum and maximum tolerated dose.

Basically, positive results of 2-stage carcinogenesis bioassay indicate that chemicals exert either promoting or carcinogenic activity. With ETBE, positive results were obtained for liver, thyroid, colon, urinary bladder, and kidneys in a 2-stage carcinogenesis bioassay by oral administration. However, in a 2-year carcinogenicity test with oral administration, ETBE was negative even in those organs. The results of these two studies indicate that orally administered ETBE exhibits promoting, but not the carcinogenic activity in liver, thyroid, colon, urinary bladder, and kidneys."

2. 1-55, line1-19, Overall Conclusions on MOA for Liver Effects

1) line 9

Comment 2: "only following oral exposure in male rats" should be corrected as "only following inhalation exposure in male rats".

2) lines 4-6, "The database is inadequate to determine if nuclear receptor-mediated pathways (i.e., PPAR and CAR/PXR) contribute to the tumorigenesis observed in ETBE-treated male rats."

Comment 3: Similarities (CAR/PXR) as well as differences (PPAR) of the effects of ETBE and Phenobarbital in the rat liver obtained by the proteome and Ingenuity Pathway analyses results were further confirmed by the real-time quantitative RT-PCR, immunohistochemistry and transmission electron microscopy. Therefore, we believe that Kakehashi et al studies (2013, 2015) adequately demonstrate a contribution of CAR/PXR and PPAR to the liver tumorigenesis of ETBE in Fisher 344 male rats. This mechanism is similar to that of Phenobarbital activity in rat liver tumorigenesis, which is known to be not human- relevant. Furthermore, there is no evidence that PPAR proliferators cause elevated risk of cancer or any other neoplasms in humans thus indicating a species difference in the carcinogenic response between rodents and human. Therefore, both CAR/PXR and PPAR-mediated liver

tumorigenesis mechanisms found in experimental animals are concluded to be not relevant to human. From these statements, we considered ETBE not to be human-relevant.

3) **lines 6-10**, "Furthermore, centrilobular hypertrophy was observed at the same concentrations that induced liver weight changes in rats of both sexes after 13-week inhalation and 26-week oral exposure, yet liver tumors were observed only following oral exposure in male rats. This observation suggests that these transient effects are not associated with the observed rat liver tumorigenesis."

Comment 4: It is well known that induction of liver centrilobular hypertrophy is associated with liver tumorigenesis in experimental animals. Furthermore, male rats are known to be much more sensitive than females to induction of centrilobular hypetrophy and liver tumorigenesis by various chemical agents including Phenobarbital. We consider that in our studies, male rats were more sensitive as compared with females. Therefore, sex difference in liver tumorigenicity does not necessarily mean "no-association of liver weight changes", as these findings are generally observed in numerous studies.