

Comments for Toxicological Review of Ethyl Tertiary Butyl Ether (External Review Draft)-1

**The Public Meeting of the Science Advisory Board
Chemical Assessment Advisory Committee
Augmented for the Review of EPA's Draft Ethyl
Tertiary Butyl Ether(ETBE) and tert-Butyl
Alcohol(tert-butanol;TBA) Assessments**

August 15-17, 2017
The Residence Inn Arlington Crystal View
Arlington VA, USA

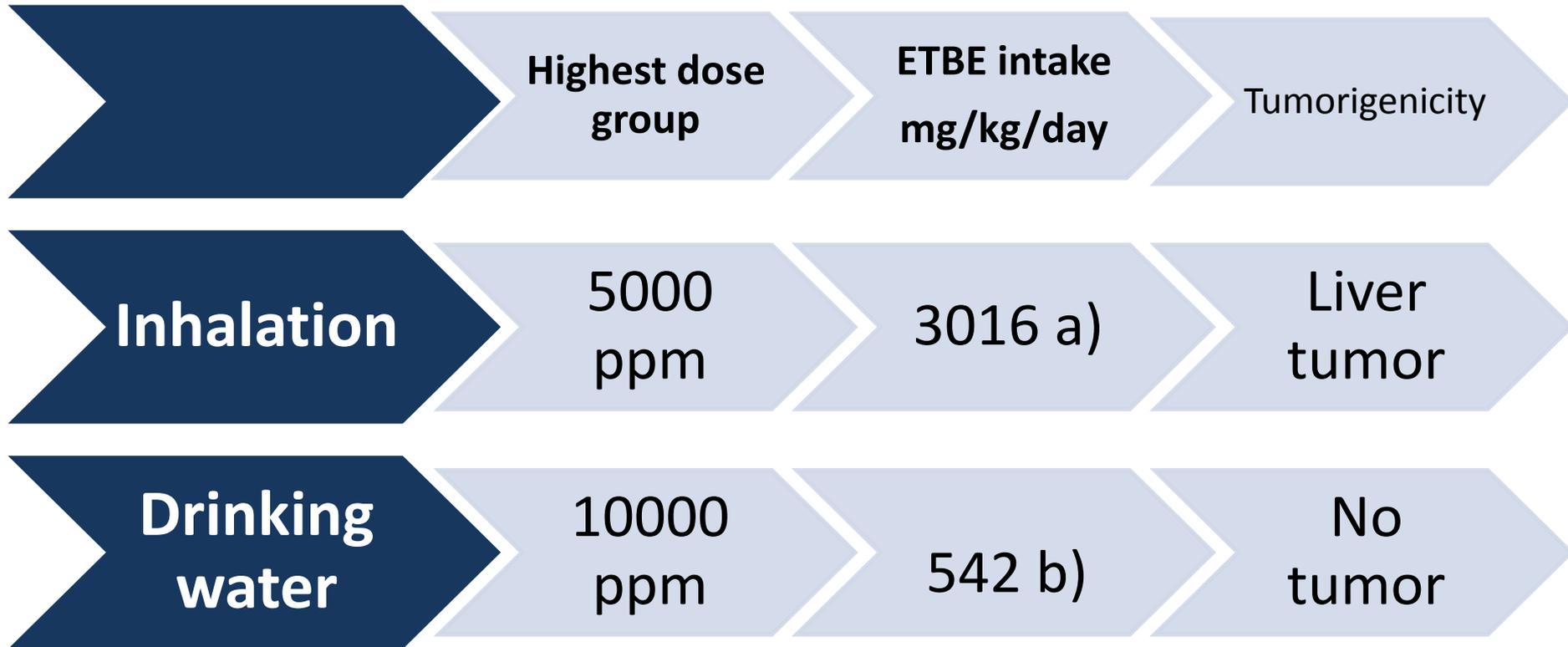
1. Executive Summary_xxiv, line4-9, Evidence of Human Carcinogenicity

- Please add the following description. Because 2-year carcinogenicity studies are essential evidence to evaluate carcinogenicity of chemicals, including negative data.
 - “In a 2-year carcinogenicity test with oral administration of ETBE in rats, carcinogenicity was negative in any organs.”

2. Draft_p1-55, line9, Overall Conclusions on MOA for Liver Effects

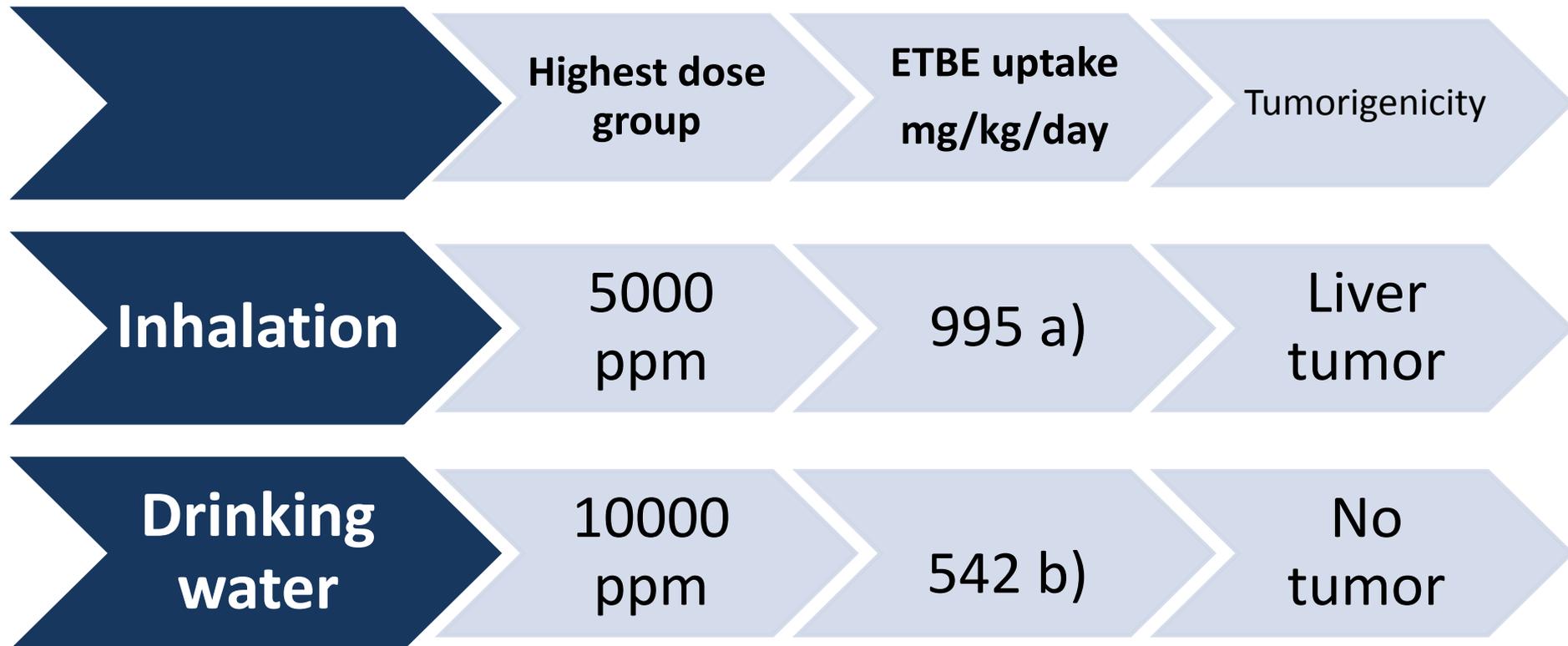
- The following description should be corrected:
 - “only following oral exposure in male rats” should be corrected as “only following inhalation exposure in male rats”.

3.1 ETBE intake in the 2-year inhalation and drinking water studies at highest dose group



- a) Estimated from minute volume of rats (561 ml/min, kg body weight, Mauderly et al, 1979), and converted 5 inhalation days/week into 7 inhalation days
- b) Calculated from the water consumption of male rats in the 2-year drinking water study (Suzuki et al, 2012)

3.2 ETBE uptake in the 2-year inhalation and drinking water studies at highest dose group



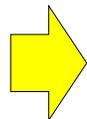
- a) Estimated from minute volume of rats (561 ml/min, kg body weight, Mauderly et al, 1979) and absorption rate (33%, Nihlén et al, 1998), and converted 5 inhalation days/week into 7 inhalation days
- b) Calculated from the water consumption of male rats in the 2-year drinking water study (Suzuki et al, 2012) (absorption rate was assumed as 100%)

4. Draft_p1-55, line4-6; "the data base is inadequate to determine if nuclear receptor mediated pathways(i.e., PPAR and CAR/PXR) contribute to the tumorigenicities observed in ETBE treated male rats"

- 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 the tumorigenicity of ETBE in male rats not to be human- relevant.

5. Summary of Genotoxicity studies by JPEC

Test	Result
Ames Tests using Salmonella and E. coli	Negative
Chromosome aberration test using Chinese hamster lung cell	Negative
in vivo Micronucleus tests using bone marrow in rats as follows;	Negative
● Using bone marrow in 13-week repeated oral test: Top dose : 10000 ppm (Actual averaged intake : 629 mg/kg/day)	Negative
● Using bone marrow in 13-week repeated inhalation test: Top dose : 5000 ppm	Negative
● Intraperitoneal administration: ● Top dose : 1000mg/kg/day (All animals had been dead at 2000mg/kg/day)	Negative
● Gavage: Top dose : 2000mg/kg/day	Negative



ETBE is non-genotoxic.

6. ETBE Exposure Level simulated in Human and Health Risk level

ETBE Exposure level and Health Risks	7% ETBE blended gasoline
<p>Exposure level: High concentration meshes(100mx100m) by simulated calculation</p> <ul style="list-style-type: none"> ● Cair=Maximum atmospheric ETBE concentration 	<p>Cair=38 $\mu\text{g}/\text{m}^3$ (\approx 0.009ppm)</p>
<ul style="list-style-type: none"> ● NOAEL in rats <ul style="list-style-type: none"> ▪ 2-year inhalation carcinogenicity (liver adenoma in male rat): <p>NOAEL 1500ppm</p>	<p>NOAEL= 6,270,000$\mu\text{g}/\text{m}^3$ (1500ppm)</p>
<ul style="list-style-type: none"> ● MOE(Margin of exposure) = NOAEL/Cair 	<p>MOE=165,000</p>
<ul style="list-style-type: none"> ● Risk level; MOE is adequately large compared to USFs. USFs=100 (species10 * private10) 	<p>MOE > USFs 165,000 >>> 100</p>
<p>CONCLUSION : Inhalation exposure to ETBE does not pose health risk to humans.</p>	

Thank very much for your attention

2. Aspects on positive results from 2-Stage Carcinogenicity Bioassay

- 福島先生ご担当

5. Mode of Action

- Anna & Fukushima先生ご担当