



# Draft IRIS Assessment of Ethyl Tertiary Butyl Ether (ETBE)

Presentation for the  
ETBE and tBA Chemical Assessment Advisory Committee of  
the Science Advisory Board  
August 15, 2017

Keith Salazar, Ph.D.  
National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency



## Outline of Presentation

This presentation will cover:

- Summary of draft IRIS assessment for ETBE
- Address major public questions raised during the July 11, 2017 teleconference
  - The discussion of tert-butanol as it relates to ETBE
  - Human relevance of ETBE-induced kidney effects
  - Interpretation of liver cancer evidence
    - PPAR/CAR/PXR
    - Centrilobular hypertrophy
    - 2-stage initiation-promotion studies



# Authors and Contributors

## Assessment Team

Keith Salazar, Ph.D. (Assessment Manager)

Christopher Brinkerhoff, Ph.D. (currently EPA/OCSP)

## Contributors

Andrew Hotchkiss,  
Ph.D.

Channa Keshava,  
Ph.D.

Jason Fritz, Ph.D.

Janice Lee, Ph.D.

Christine Cai, M.S.

Alan Sasso, Ph.D.

Paul Schlosser, Ph.D.

Karen Hogan, M.S.

Vincent Cogliano,  
Ph.D.

Susan Makris, Ph.D.

Brandy Beverly, Ph.D.

Erin Yost, Ph.D.



## Summary of Draft ETBE assessment

- Ethyl Tertiary Butyl Ether
  - Gasoline oxygenate to improve efficiency
  - 1990-2006 ETBE added to gasoline at levels up to 20%
  - 2006 use of ETBE in gasoline ceased in U.S.
  - U.S. produces 25% of world's ETBE in 2012
  - General population exposures may occur if individuals are in or around facilities where ETBE is produced, or in drinking water that is contaminated with ETBE.
- Hazard Identification- Noncancer
  - Kidney effects (urothelial hyperplasia and increased organ weight)
- Hazard Identification- Cancer
  - *Suggestive evidence of carcinogenic potential* for ETBE. This is based on induction of tumors in the liver of rats following 2-year inhalation exposure and tumors at multiple sites following an oral 2-stage initiation-promotion protocol.



# RfD Derivation

Effect	Point of Departure (mg/kg-d)	UF	Chronic RfD (mg/kg-d)	Confidence
<b>Kidney:</b> Urothelial hyperplasia in male rats Suzuki et al. (2012) 2-year drinking water study in F344 rats	BMDL <sub>HED</sub> : 14.5	Total UF = 30 UF <sub>A</sub> = 3 UF <sub>H</sub> = 10	$5 \times 10^{-1}$	High
<b>Overall Reference Dose (RfD) – Kidney</b>			$5 \times 10^{-1}$	High



## RfC Derivation

Effect	Point of Departure (mg/m <sup>3</sup> )	UF	Chronic RfC (mg/m <sup>3</sup> )	Confidence
<b>Kidney:</b> Urothelial hyperplasia in male rats Saito et al. (2013) 2-year inhalation study in F344 rats	BMCL <sub>HEC</sub> : 265	Total UF = 30 UF <sub>A</sub> = 3 UF <sub>H</sub> = 10	9 x 10 <sup>0</sup>	High
<b>Overall Reference Dose (RfC) – Kidney</b>			<b>9 x 10<sup>0</sup></b>	<b>High</b>



## Summary of the Dose Response Analysis for Oral Cancer Data

Principal Study	Elevated tumor types	Extrapolation Method	Oral Slope factor <sub>HED</sub> (mg/kg-d) <sup>-1</sup>
Saito et al. (2013) 2-year inhalation study in F344 rats	Incidences of combined hepatocellular adenomas or carcinomas	Linear extrapolation from the POD (BMDL <sub>10-HED</sub> )* derived from multistage modeling of data	1 x 10 <sup>-3</sup>

**\* Route-to-route extrapolation of the inhalation BMCL was performed to derive an oral POD**



## Summary of the Dose Response Analysis for Inhalation Cancer Data

Principal Study	Elevated tumor types	Extrapolation Method	Inhalation Unit Risk (mg/m <sup>3</sup> ) <sup>-1</sup>
Saito et al. (2013) 2-year inhalation study in F344 rats	Incidences of combined hepatocellular adenomas or carcinomas	Linear extrapolation from the POD (BMDL <sub>10-HEC</sub> ) derived from multistage modeling of data	8 x 10 <sup>-5</sup>



# Teleconference Questions

- When relevant to discussions concerning mechanistic questions, tert-butanol data are discussed in the ETBE assessment
- Kidney Effects (similarities in ETBE and tBA):
  - Tert-butanol and ETBE alpha<sub>2</sub>u-globulin data are compared in sections 1.2.1. and 1.3.1
- Liver effects (lack of liver effects with tBA):
  - The metabolism of ETBE to tert-butanol is discussed in sections 1.2.2 and 1.3.2 as it relates to the toxicokinetics of ETBE-induced liver toxicity and tumors



## Human relevance of kidney effects

**Comment:** CPN should not be considered relevant to humans because it is rat-specific with no known human counterpart. In addition, the selection of urothelial hyperplasia as the key endpoint reflecting a potential human kidney hazard from ETBE exposure is inappropriate because urothelial hyperplasia is associated with chronic progressive nephropathy (CPN).

- Section 1.2.1 discusses EPA's analyses and conclusion
  - Urothelial hyperplasia is weakly correlated with CPN
  - The individual lesions comprising the spectrum of lesions known as CPN could occur in a human kidney
  - EPA considers that the individual lesions comprising the condition to be relevant for human health
- A written response to this public comment is provided in Appendix D
- Pathologists from both EPA and NTP reviewed the revised document.
- Charge question 3a to the panel addresses this topic

**Comment:** Executive summary should be rephrased to emphasize that 2-year drinking water study of ETBE in rats did not observe carcinogenicity while 2-stage carcinogenesis bioassay indicates either promoting or carcinogenic activity. [written comment]

- The executive summary presents only the outcome of the evaluations. However, a detailed explanation describes how EPA reached its conclusion
- Section 1.3.2 summarizes and integrates the evidence for ETBE carcinogenicity
- A written response to this public comment is provided in Appendix D
- Charge question 4b to the panel addresses this topic

**Comment:** Similarities (CAR/PXR) as well as differences (PPAR) of the effects of ETBE and phenobarbital in the rat liver obtained by the proteome and pathway analysis, immunohistochemistry, and TEM results demonstrate that ETBE induces the CAR/PXR and PPAR pathways which contribute to liver tumorigenesis. [written comment]

- Nuclear receptor-mediated effects as well as other key characteristics of carcinogens are discussed in section 1.2.2.
- Several data gaps in the receptor-mediated effects data were explicitly noted to explain why the data were inadequate to sufficiently support the proposed mode of action.
- Charge question 4a to the panel addresses this topic

**Comment:** Centrilobular hypertrophy is associated with liver tumorigenesis in animals. Male rats are known to be more sensitive than females for this effect.  
[written comment]

- Histopathological evidence for ETBE does not suggest centrilobular hypertrophy is more severe in males than females (similar incidence and severity in both sexes)
- All centrilobular hypertrophy evidence is discussed throughout section 1.2.2.
- A focused discussion of centrilobular hypertrophy as it relates to receptor – mediated effects is provided in the Mode of Action Analysis- Liver Effects
- A written response to this public comment is provided in Appendix D
- Charge question 4a to the panel addresses this topic

**Comment:** The draft assessment inappropriately considers the ETBE two-stage tumor promotion studies. The animal experimental data indicates that ETBE might be acting as a promoter of mutagen induced liver tumors when administered at high doses of ETBE (1,000 mg/kg-day), a dose level that also exceeds metabolic saturation. This promotional activity has clear thresholds.

- The two stage carcinogenicity bioassay data provide several instances of increased tumors or preneoplastic lesions at doses below 1,000 mg/kg-day
- No MOA was identified so it is not possible to conclude that induction of tumors by ETBE at one dose is not also operative at lower doses.
- A written response to this public comment is provided in Appendix D
- Charge question 4b to the panel addresses this topic