Docket ID NO. EPA-HQ-ORD-2009-0229

JPEC COMMENTS ON PRELIMINARY MATERIALS FOR THE INTEGRATED RISK INFORMATION SYSTEM (IRIS) TOXICOLOGICAL REVIEW OF ETHYL *TERT*-BUTYL ETHER (ETBE)

EPA IRIS Bimonthly Meeting
EPA Conference Center, Arlington, Virginia
December 12, 2013

Fukumi Nishimaki, Ph.D.
Senior Researcher
Auto Oil and New Fuels Dept.
Japan Petroleum Energy Center (JPEC)

JPEC Comments

ETBE use as an oxygenated gasoline additive in Japan to reduce CO_2 emissions and support the objectives of the Kyoto Protocol is increasing.

JPEC conducted many studies to evaluate the toxicity and potential health risks of ETBE between 2006 and 2013, commissioned by and under the guidance of the Japan Ministry of Economy, Trade and Industry (METI).

The resulting risk assessment, performed under the guidance of an independent committee of academic and government experts, concluded that the use of 7% ETBE-blended gasoline does not pose a human health risk by either the oral or inhalation route of exposure.

JPEC Comments

EPA is an important authoritative body and its IRIS Toxicological Review of ETBE is expected to have significant regulatory impact globally.

JPEC is not aware of any significant use of ETBE as a gasoline additive within the U.S. during the past 15 years.

The U.S. Geological Survey (2006; USGS Circular 1292) reported that ETBE was "detected infrequently" in domestic and public wells. It was found in less than 0.5% of public wells at concentrations of 0.2 μg/L or greater. Detection in domestic wells was less frequent.

JPEC Comments

We request that EPA define and communicate the problem and scope that the ETBE IRIS toxicological assessment intends to address before proceeding with the ETBE IRIS assessment.

Considering that the ETBE IRIS toxicological assessment is likely to have greater regulatory impact outside the U.S. than within the U.S., we also request that EPA consider the following comments as it proceeds with the ETBE toxicological review.

JPEC Comments

We appreciate that most of the original papers and study reports on ETBE conducted by JPEC are included in the preliminary draft literature search and evidence tables for ETBE.

The preliminary draft literature search and evidence tables for ETBE, however, are insufficient for evaluating the human relevance of rodent tumorigenicity data without the inclusion of key literature regarding the mode of action (MOA) of ETBE.

We request that EPA include three original publications and two study reports that provide key information on the MOA for ETBE hepatotumorigenicity in rats in both the literature search and evidence tables.

JPEC Comments

We submitted our comments with these three key papers and two key study reports:

① **The original paper entitled: Anna Kakehashi et al**. "Mode of action of ethyl *tertiary*-butyl ether hepatotumorigenicity in the rat: Evidence for a role of oxidative stress via activation of CAR, PXR and PPARs signaling pathways." Published on December 1, 2013 in Toxicology and Applied Pharmacology. Volume 273, Issue 2, p.390-400, 2013.

② **The original paper entitled: Arata Saito et al.** "Hepatotumorigenicity of ethyl *tertiary*-butyl ether with 2-year inhalation exposure in F344 rats." Published on February 7, 2013 in Archives of Toxicology, Volume 87, Number 5, p.905-914, 2013.

③ **Accepted manuscript of the original paper entitled: Tadashi Noguchi et al.** "Lack of micronucleus induction activity of ethyl *tertiary*-butyl ether in the bone marrow of F344 rats by sub-chronic drinking-water treatment, inhalation exposure, or acute intraperitoneal injection" (in press) The Journal of Toxicological Sciences. Accepted on September 9, 2013. Manuscript No.:JTS-13141.

④ **The full study report entitled: "Investigation of the Mechanisms of Ethyl *tertiary*-Butyl Ether (ETBE) Carcinogenicity in the Liver of F344 Rats. " [Study No.: 1132]**
Conducted by DIMS Institute of Medical Science, Inc. and Osaka City University Graduate School of Medicine, Department of Pathology. (October 2012) Sponsored by JPEC.

⑤ **The full study report entitled: "Investigation of the Mechanisms of Ethyl *tertiary*-Butyl Ether (ETBE) Carcinogenicity in the Liver of F344 Rats – Transmission Electron Microscopic Examination. [StudyNo.: 12138]**
Conducted by DIMS Institute of Medical Science, Inc. and Osaka City University Graduate School of Medicine, Department of Pathology. (October 2012) Sponsored by JPEC.

ETBE Toxicological Effects and Carcinogenicity
Results of a 2-Year Inhalation Carcinogenicity Study in F344 Rats

☐ Toxicological changes

	Organ	Observation	Dose	NOAEL	Human Relevance
Male	Liver	Eosinophilic/ Basophilic cell foci	5000 ppm	1500 ppm	
	Kidney	Chronic progressive nephropathy (CPN)	5000 ppm	1500 ppm	**Not relevant to humans** （Increase in age-related lesion specific to rats）
	Kidney	Mineral deposition in the renal papilla area	5000 ppm	1500 ppm	**Not relevant to humans** （Involvement of α2u globulin; observed only in males）
	Kidney	Urothelial hyperplasia in the renal pelvis	>1500 ppm	500 ppm	**Not relevant to humans** （Involvement of α2u globulin; observed only in males）
Female	Kidney	Chronic progressive nephropathy (CPN)	5000 ppm	1500 ppm	**Not relevant to humans** （Increase in age-related lesion specific to rats）

- **JPEC results and conclusions on ETBE kidney effects in rats were confirmed by a Pathological Working Group Review conducted in 2011.**
  - **Cited as “Cohen, SM; Hard, GC, Regan, KS, Seely, JC, Bruner, RH (2011) Pathology working group review of selected histological changes in the kidneys of assigned to toxicological and carcinogenicity studies of ethyl tertiary butyl ether (ETBE): Japan Bioassay Research Center studies no.: 0665 and 0691” in “Preliminary Materials for IRIS Toxicological Review of ETBE”.**

ETBE Toxicological Effects and Carcinogenicity

Results of a 2-Year Inhalation Carcinogenicity Study in F344 Rats; JPEC (2007)

- **Incidence of liver tumors**

	Male				Female			
Concentration of ETBE (ppm)	0	500	1500	5000	0	500	1500	5000
No. of examined animals	50	50	49	50	50	50	50	50
Hepatocellular adenoma	0	2	1	9**	1	0	1	1
Hepatocellular carcinoma	0	0	0	1	0	0	0	0

**: $p \leq 0.01$ by Fisher exact test

JPEC implemented two studies on Mode of Action (MOA) of ETBE hepatotumorigenicity in rats.

ETBE Hepatotumorigenicity MOA Studies in Rats

[Study No. 1132]

Investigation of the Mechanisms of Ethyl tertiary-Butyl Ether (ETBE) Carcinogenicity in the Liver of F344 Rats. Opened at JPEC English home page: http://www.pecj.or.jp/english/news/pdf/C-1-1.pdf

- Main study of ETBE hepatotumorigenicity MOA in rats.
- Conducted according to GLP.
- Results indicated:
  - activation of CAR and PXR nuclear receptors predominantly
  - induction of oxidative stress, DNA damage
  - subsequent cell cycle arrest and apoptosis, suggesting regenerative cell proliferation
- ETBE hepatotumorigenicity MOA is similar to that of Phenobarbital.
- Concluded that ETBE hepatotumorigenicity in rats is not relevant to humans.

ETBE Hepatotumorigenicity MOA Studies in Rats

[Study No. 12138]

Investigation of the Mechanisms of Ethyl *tertiary*-Butyl Ether (ETBE) Carcinogenicity in the Liver of F344 Rats – Transmission Electron Microscopic Examination.

Opened at JPEC English home page: http://www.pecj.or.jp/english/news/pdf/C-2-1.pdf

- Additional testing for Study No.1132.
  - Electron microscopic examination of liver cells in rats.
- Conducted according to GLP.
- Suggested very slight activation of PPARs nuclear receptors in liver cells in ETBE treated rats.

Anna Kakehashi et al.

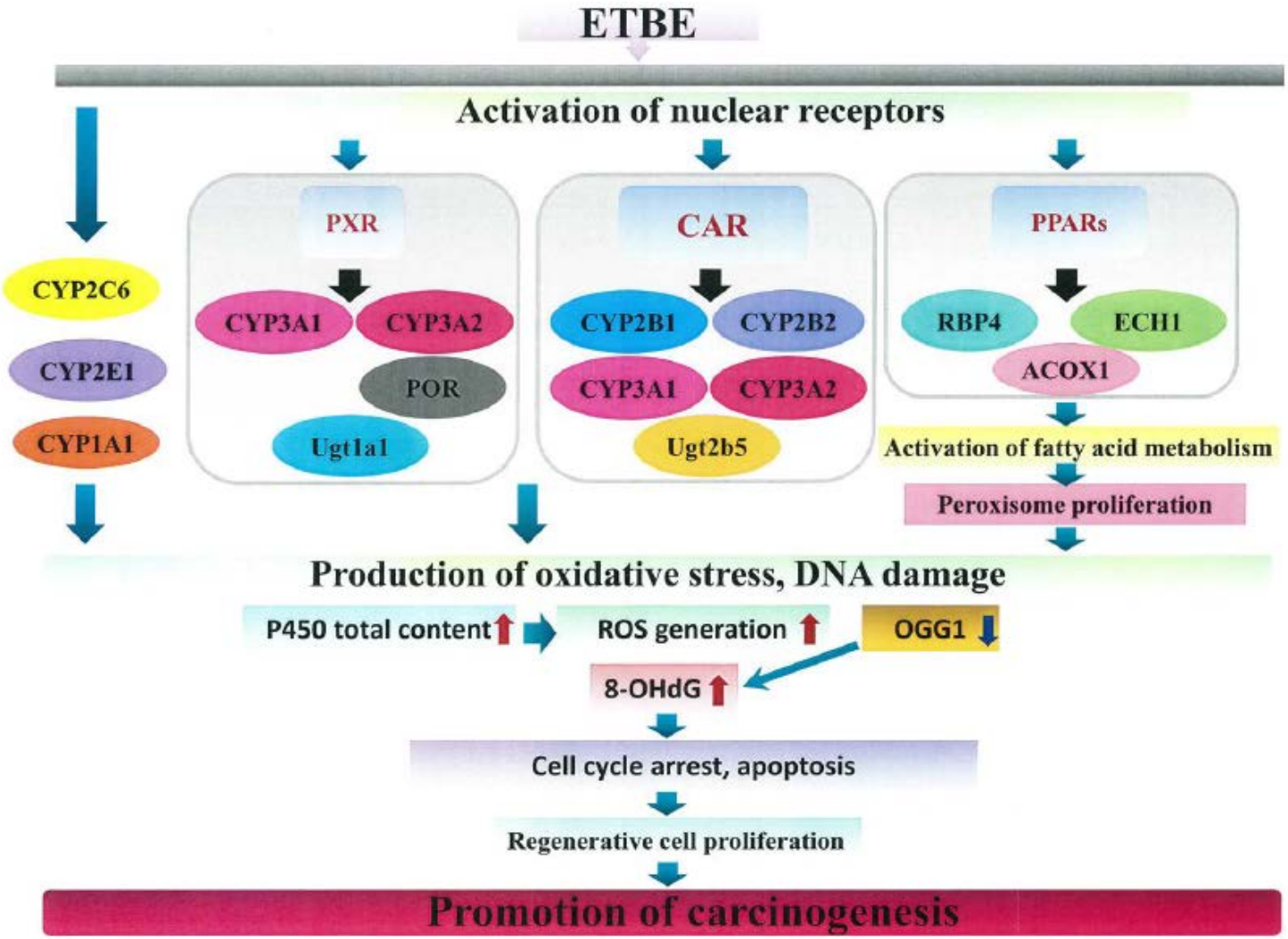
"Mode of action of ethyl tertiary-butyl ether hepatotumorigenicity in the rat: Evidence for a role of oxidative stress via activation of CAR, PXR and PPARs signaling pathways"

Published on December 1, 2013 in Toxicology and Applied Pharmacology. Volume 237, Issue 2, p.390-400, 2013.

Table 1. Difference of the included data in the reports or original paper on ETBE hepatotumorigenicity MOA

Included Data	Study Reports		Paper
	#1132	#12138	Anna Kakehashi et al. Toxicology and Applied Pharmacology. (in press). DOI :10.1016/j.taap.2013.09.016 Accepted on Sept. 20, 2013.
● Nuclear receptor activation (CAR, PXR, PPAR etc.)	○	–	○
● P450 induction（mRNA, Protein)	○	–	○
● Oxidative stress	○	–	○
● DNA damage (8OHG)	○	–	○
● Cell cycle arrest	○	–	○
● Apoptosis	○	–	○
● Cell proliferation	–	–	○
● Peroxisome proliferation	–	○	○

Summary of the Proposed ETBE MOA for Hepatotumorigenicity in Rats



Anna Kakehashi et al. Toxicology and Applied Pharmacology, Volume 273 (2), p.390-400, 2013.

Conclusion: ETBE hepatotumorigenicity in rats lacks human relevance.

ETBE Inhalation Exposure Level and Health Risk in Humans

(based on "ETBE risk assessment report" in ETBE Risk Assessment Study by JPEC (March 2008))
Opened at JPEC English home page: http://www.pecj.or.jp/english/news/pdf/b-2-8.pdf

ETBE Exposure level and Health Risk	7% ETBE blended with gasoline
Exposure level: ETBE concentration in environment estimated by simulation ● **Cair** = Maximum atmospheric ETBE concentration	**Cair** = **38 μg/m³**
● **NOAEL in rats** ➢ Centrilobular hypertrophy of liver cells, hyaline droplet in kidney(90-day inhalation toxicity study) • **NOAEL=500ppm =2,090mg/m³** ➢ Kidney changes (2-year inhalation carcinogenicity study) • **NOAEL = 500ppm =2,090mg/m³** ➢ Liver adenoma (2-year inhalation carcinogenicity study) • **NOAEL =1500ppm =6,270mg/m³**	**NOAEL :** ① Kidney changes **=2,090,000μg/m3** ② Liver adenoma **= 6,270,000μg/m3**
● **MOE** (Margin of Exposure) = NOAEL* /Cair	① MOE = **55,000** ② MOE =**165,000**
● **Uncertainty factors (UF)** ➢ Interspecies **10** ∗ Intraspecies **10** ∗ Test period **1** (Based on 2-year carcinogenicity study)	**UF** = **100**
Risk is assessed on comparison of "MOE versus UF" • In case of ; MOE < UF → High Risk • **In case of ; MOE > UF → Low Risk**	**Extremely Low Risk** **MOE > UF** ➡ **55,000 >>100** **165,000 >>100**

CONCLUSION : Inhalation exposure to ETBE does not pose a health risk to humans.

JPEC Comments

We thank EPA for the opportunity to present these comments.

We request that EPA define and communicate the problem and scope that the ETBE IRIS toxicological assessment intends to address before proceeding with the assessment.

After EPA has defined the problem and scope of this assessment, we strongly request that EPA include our papers and our studies of ETBE toxicity, carcinogenicity, and the hepatotumorigenicity mode of action as part of a sound scientific evaluation of the toxicological effects of ETBE.

Thank you very much for your attention.