Comments on ETBE and *tert*-Butanol (TBA) Preliminary Materials for IRIS Toxicological Review

Genotoxicity, Mutagenicity, and Cancer Endpoints

On Behalf of Lyondell Chemical Company

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ToxStrategies

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Topics for Comments on IRIS Materials for ETBE & TBA

ETBE (Ethyl *tert*-butyl Ether)

- Mutation Assays
- Genetic Toxicology Assays
- Cancer Endpoints

TBA (*tert*-Butanol)

- Mutation Assays
- Genetic Toxicology Assays
- Cancer Endpoints

ETBE: Mutation Assays with *Salmonella typhimurium*

No significant effects on mutant numbers in any study

Strains Used	Activation	Highest Ineffective Dose	Reference
TA1535, TA1537, TA1538, TA98	+/- liver S9 (Rat)	500 µg/plate	Institut Pasteur de Lille (1992) (Not included)
TA1535, TA97, TA98, TA100	+/- liver S9 (Rat & Syrian Hamster)	10,000 µg/plate	Zeiger et al. (1992)
TA1535, TA1537, TA1538, TA98, TA100	+/- liver S9 (Rat)	5,000 µg/plate	Pharmakon Europe (1994) (Not included)

ETBE: Mutation Assays with Mammalian Cells

No significant effects on mutation frequency in either study

In vitro Assay	Activation	Highest Dose	Reference
Gene mutation, CHO - at *hprt* locus	+/- liver S9 (Rat) Two acceptable independent assays	5,000 µg/mL	BBRC (1995c); Vergnes & Kubena (1995a)
Chromosomal aberrations - CHO	+/- liver S9 (Rat) Duplicate cultures in a non-independent assay	5,000 µg/mL	BBRC (1995b); Vergnes (1995)

ETBE: *In Vivo* Micronucleus Assays with Rodent Bone-Marrow Cells

No significant increases in micronucleated cells; total of 6 studies

Species	Route	Highest Dose	Reference
Male and female OF-1 mouse	Oral gavage	5,000 mg/kg-bw x 1	Institut Pasteur de Lille (1992c) (Not included)
Male and female CD-1 mouse	Inhalation	5,000 ppm 6h/d x 5 days	BBRC (1995a); Vergnes & Kubena (1995b)
Male and female Fischer 344/DuCrj rat	i.p.	1,000 mg/kg-bw x 2	Japan Bioassay Research Center (2007b)
Male and female Fischer 344/DuCrj rat	Oral gavage	2,000 mg/kg-bw x 2	Japan Bioassay Research Center (2007a)
Male and female Fischer 344/DuCrj rat	Drinking-water	10,000 ppm (w/w), 13 wks	Japan Bioassay Research Center (2007c)
Male and female Fischer 344/DuCrj rat	Inhalation	5,000 ppm 6h/d, 5d/wk, 13 wks	Japan Bioassay Research Center (2007d)

ETBE: DNA Damage to Liver Cells of C57BL/6 Wild-type and *Aldh2* -/- Mice

Weng et al., 2012 (Study to add to Section 2.8, Table 2-11)

Suggested Klimisch Score - 2 (reliable with restrictions)

Fold-increases over the control proportions of comets

Assay	Dose (ppm)	Statistically significant – fold increase in comets			
		Male		Female	
		+/+	-/-	+/+	-/-
Comet assay in liver cells	500		1.76		
	1750		1.83		
	5000	1.93	1.99		1.84
net hOGG1 Comet assay in liver cells	500		2.71		
	1750		2.51		
	5000	2.99	3.44		3.15
8-OH-dG residues in liver DNA	500		1.81		
	1750		1.74		
	5000	1.31	1.93		1.61

ETBE: Comet Assay on Peripheral Leucocytes of C57BL/6 Wild-type and *Aldh2* -/- Mice

Weng et al., 2011; 2013 (Studies to add to Section 2.8, Table 2-11)

Statistically significant fold-increases in comets (over control) in peripheral leukocytes

Assay	Klimisch Score	Dose (ppm)	Statistically significant fold increase in comets			
			Male		Female	
			+/+	-/-	+/+	-/-
Comet assay (Weng et al. 2011)	2 (reliable with restrictions)	500		1.4		
		1750		1.6		
		5000	1.6	1.7		1.6
net hOGG1 Comet assay (Weng et al., 2013)	3 (not reliable)	500		1.35		
		1750		1.61		
		5000	1.61	1.74		1.56

ETBE: Micronucleus Assay on Circulating Reticulocytes in C57BL/6 wild-type and *Aldh2* -/- mice

ETBE Inhalation (6hday, 5 days/wk, 13 weeks)

Statistically significant fold-increases in micronucleated cells

Assay	Dose (ppm)	Statistically significant fold increase in micronucleated cells			
		Male		Female	
		+/+	-/-	+/+	-/-
Micronucleus assay in C57BL/6 mice (Weng et al., 2013*)	500				
	1750		1.18		
	5000	1.42	1.24		1.25**

***New reference with suggested Klimisch Score of 3 (not reliable)**

** Borderline significant (p=0.052)

ETBE Metabolites in Mouse Blood (Weng et al., 2013)

***Aldh2* -/- as fold-increase over *Aldh2*+/+**

	Acetaldehyde				TBA				2-Methyl-1,2-Propanediol			
	Male		Female		Male		Female		Male		Female	
	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
Cmax (μM)	81	495	107	677	270	1426	777	2206	72	108	67	356
Fold increase in AUC (*Aldh2* -/- over +/+)	2.69-fold		2.52-fold		5.78-fold		3.01-fold		1.76-fold		4.95-fold	

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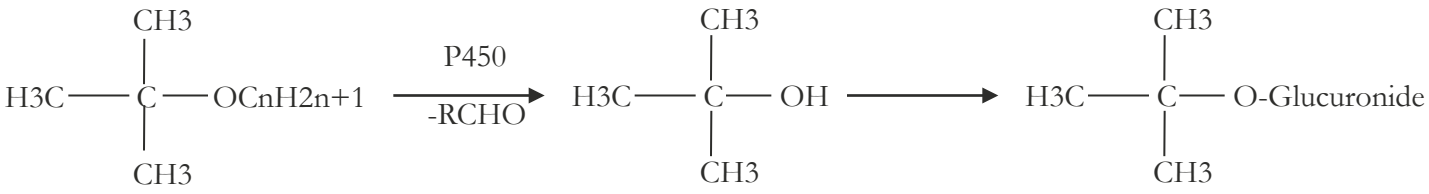
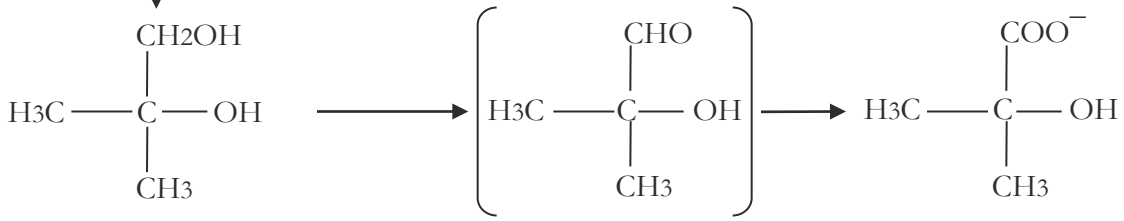
Metabolic Degradation of Alkyl Ethers to TBA



MTBE or ETBE

TBA

TBA - Glucuronide



2*-Methyl -*1,2-propanediol

2-Hydroxyisobutyrate

Acetone

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F344/DuCrlCrl Rats Exposed to ETBE Vapour for 104 Weeks (JBRC, 2010b)

Calculation of ETBE Uptake by Rats (Amberg et al., 2000 & Nihlén et al., 1998)

Parameter	Default Value	Reference
A: Alveolar ventilation rate (minute volume)	0.17 L/min	Amberg et al. (2000)
B: Exposure time	360 min/day, 5 days/wk	JBRC (2010b)
C: ETBE concentration	0, 2.12, 6.36, or 21.20 mg/L	JBRC (2010b)
D: Net respiratory uptake of ETBE	0.26 rounded to 0.3	Nihlén et al. (1998)
E: Body weight (0-104 wks)	Males: 0.41, 0.40, or 0.35 Kg Females: 0.22, 0.22, or 0.20 Kg	JBRC (2010b)
Formula	**A × B × 5/7 × C × D ÷ E = mg/Kg bw/7-day week**	
Calculated net ETBE uptake rate	**Males: 0, 97, 292 or 973 mg/Kg bw/day** **Females: 0, 126, 379 or 1390 mg/Kg bw/day**	

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F344/DuCrlCrl Rats Exposed to ETBE Vapour for 104 Weeks (JBRC, 2010b)

Calculation of ETBE Uptake by Rats

Minute volume based on power law (allometric) relationship with body weight formula of Bide et al. (2000) *J. Appl. Toxicol.* **20**, 273–290

Parameter	Default Value	Reference
A: Alveolar ventilation rate (minute volume)	$V_m = 0.499 \cdot BW^{0.809}$ Males: 0.25; 0.24; 0.21 L/min Females: 0.15, 0.15, 0.14 L/min	Bide et al (2000)
E: Body weight (0-104 wks)	Males: 0.41, 0.40, or 0.35 Kg Females: 0.22, 0.22, or 0.20 Kg	JBRC (2010b)
Formula	**A × B × 5/7 × C × D ÷ E = mg/Kg bw/7-day week**	
Calculated net ETBE uptake rate	**Males: 981 mg/Kg bw/day** **Females: 1145 mg/Kg bw/day**	

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ETBE: Chronic Inhalation Bioassay (104 weeks) Female F344/DuCrlCrl Rats

Japanese Bioassay Research Center (2010b)

Number of female rats with specified tumors

Exposure concentration (ppm)		0	500	1500	5000	Stats. Analysis	
No. animals/group		50	50	50	50	Peto	Coch/Ar
Organ	**Tumour diagnosis**						
Pituitary	Adenoma	18	14	11	13		
Thyroid	C-cell adenoma	7	5	**1***	3		
Mammary gl.	Fibroadenoma	8	10	4	**2***		p <
Uterus	Endometrial stromal polyp	8	15	9	8		0.05
	Endometrial stromal sarcoma	2	2	3	2		
Spleen	Adenocarcinoma	2	3	1	4		
	Mononuclear cell leukaemia	8	7	6	4		

Fisher's exact test * $p < 0.05$ ** $p < 0.01$

ETBE: Chronic Inhalation Bioassay (104 weeks) Male F344/DuCrlCrl Rats

Japanese Bioassay Research Center (2010b)

Number of male rats with specified tumors

Exposure concentration (ppm)		0	500	1500	5000	Stats. Analysis	
No. animals/group		50	50	49	50	Peto	Coch/Ar
Organ	**Tumour diagnosis**						
Subcutis	Fibroma	8	7	3	**2***		p <
Liver	Hepatocellular adenoma	0	2	1	**9****	p<0.01	0.05
	Hepatocellular carcinoma	0	0	0	1		p <
Pancreas	Islet cell adenoma	7	7	6	2		0.01
Pituitary	Adenoma	13	10	18	6		
Thyroid	C-cell adenoma	7	4	9	5		
	C-cell carcinoma	3	4	1	2		
Adrenal	Pheochromocytoma	9	8	3	4		
Testis	Leydig cell tumour	42	44	45	46		
Preputial gl.	Adenoma	1	1	1	3		
Lung	Bronchiolar-alveolar carcinoma	1	3	1	0		
Spleen	Mononuclear cell leukaemia	6	8	11	8		
Peritoneum	Mesothelioma	0	2	3	0		

Fisher's exact test * $p < 0.05$ ** $p < 0.01$

ETBE: Incidences and Grades of Selected Liver Pathology 2-year Inhalation with F344 Rats

Japanese Bioassay Research Center (2010b)

Number of rats with specified liver lesions

Exposure concentration (ppm)		0	500	1500	5000
No. animals/group		50	50	49	50
Sex	**Liver lesion**				
Male	Clear foci	5 (1)	0	0	4 (1)
	Acidophil foci	31 (1)	28 (1)	36 (1)	30 (1) 9 (2)
	Basophil foci	18 (1)	10 (1)	13 (1)	31 (1) 2 (2)
Female	Clear foci	2 (1)	0	0	3 (1)
	Acidophil foci	2 (1)	1 (1)	4 (1)	2 (1)
	Basophil foci	32 (1) 4 (2)	24 (1) 7 (2)	27 (1) 5 (2)	23 (1) 5 (2)

Grades: 1, Slight; 2, Moderate; 3, Marked; 4, Severe

ETBE: Chronic Oral Gavage Bioassay, Female Sprague-Dawley Rats (2-year dose administration; held till death)

Ramazzini Foundation; Maltoni et al. (1999)

Number of female rats with specified tumors

Dose (mg/Kg bw/day, 4 d/wk)		0	250	1000
Dose (mg/Kg bw/day, 7 d/wk basis)		0	143	571
No. animals/group		60	60	60
Organ	**Tumour diagnosis**			
Mammary gl.	Fibroma and fibroadenoma	33	30	22
Pancreas	Islet cell adenoma	0	1	1
Pituitary	Adenoma	24	24	22
Thyroid	C-cell adenoma	2	6	3
Adrenal	Pheochromocytoma	29	27	26
Spleen	Mononuclear cell leukaemia	3	6	5

ETBE: Chronic Oral Gavage Bioassay, Male Sprague-Dawley Rats (2 year dose administration; held till death)

Ramazzini Foundation; Maltoni et al. (1999)

Number of rats with specified tumors

Dose (mg/Kg bw/day, 4 d/wk)		0	250	1000
Dose (mg/Kg bw/day, 7 d/wk basis)		0	143	571
No. animals/group		60	60	60
Organ	**Tumour diagnosis**			
Subcutis	Fibroma	0	0	1
Pancreas	Islet cell adenoma	1	3	2
Pituitary	Adenoma	28	29	23
Thyroid	C-cell adenoma	4	1	1
Adrenal	Pheochromocytoma	15	15	6
Testis	Leydig cell tumour	0	1	0
Spleen	Mononuclear cell leukaemia	3	8	6

Incidences of Uterine Proliferating Lesions Comparison of RI Re-Evaluation and PWG Consensus

Maltoni et al., 1999 vs. Malarkey et al., 2011*

Dose (mg/Kg bw/day, 4 d/wk)	0	250	1000
Dose (mg/Kg bw/day, 7 d/wk basis)	0	143	571
No. animals/group	60	60	60
Counts reported in ETBE evidence Table 2-10 (derived from Maltoni et al., 1999)			
Polyp	14	11	14
Malignancies	2	10*	2
Counts by Ramazzini Institute (SD) & NIEHS Pathology Working Group (PWG)*			

	SD	PWG	SD	PWG	SD	PWG
Metaplasia, Squamous	3	3	3	6	4	5
Stromal Polyp	21	21	11	12	14	14
Carcinoma	1	0	1	1	0	0
Leiomyoma	0	0	0	0	2	3
Leiomyosarcoma	1	1	2	0	0	0
Schwannoma, Malignant	0	0	6	7	2	2
Histiocytic Sarcoma	1	1	0	0	1	1

ETBE: Chronic Drinking Water Bioassay (104 weeks) Male F344/DuCrlCrl Rats

Japanese Bioassay Research Center (2010a); Suzuki et al (2012)

Number of male rats with specified tumors

Exposure concentration (ppm)		0	625	2500	10000
Calculated average dose (mg/kg bw/day)		0	28	121	542
No. animals/group		50	50	50	50
Organ	**Tumour diagnosis**				
Subcutis	Fibroma	6	2	2	2
Pancreas	Islet cell adenoma	6	7	4	2
Pituitary	Adenoma	25	**13***	**15*[a]**	16
Thyroid	C-cell adenoma	13	**5***	11	9
Adrenal	Pheochromocytoma	11	6	11	10
Testis	Leydig cell tumour	29	32	37	34
Spleen	Mononuclear cell leukaemia	3	4	7	7

Fisher's exact test *$p < 0.05$ in two-tailed test, *[a] NS in two-tailed test

ETBE: Chronic Drinking Water Bioassay (104 weeks) Female F344/DuCrlCrl Rats

Japanese Bioassay Research Center (2010a); Suzuki et al (2012)

Number of female rats with specified tumors

Exposure concentration (ppm)		0	625	2500	10000
Calculated average dose (mg/kg bw/day)		0	46	171	560
No. animals/group		50	50	50	50
Organ	**Tumour diagnosis**				
Pituitary	Adenoma	15	14	13	15
Thyroid	C-cell adenoma	8	8	6	13
Adrenal	Pheochromocytoma	1	0	3	2
Uterus	Endometrial stromal polyp	6	9	3	7
Mammary	Fibroadenoma	6	4	12	7
Spleen	Mononuclear cell leukaemia	6	7	9	2

ETBE: Initiation – Promotion Study in Rats
Hagiwara et al. (2011) & JPEC (2008d)

- The carcinogenic promoting ability of ETBE (4-wk DMBDD initiation and 23-wk ETBE promotion) was evaluated in male rats***; ETBE was shown to promote thyroid, colon, and liver tumors in DMBDD initiated rats.***
- IARC (1999); consensus statement on the use of multiple- and single- organ models***; "When data are available only on promoting activity, the evidence is suggestive of carcinogenicity in rodents, but the information should be evaluated in conjunction with other data on biological effects of the agent, such as genotoxicity, initiating activity, and cell proliferation."***
- Two-stage models of carcinogenesis in rats demonstrated promoting activity of ETBE in liver only; ***These models have utility in the absence of full carcinogenicity tests, and can provide support for results from two-year bioassays; (DIMS, 2008a; DIMS, 2008b; DIMS, 2010a; DIMS, 2010b).***

Therefore, the use of these data on the Exposure-Response Array is questioned.

ETBE: MOA for Hepatotumorigenicity in Rats

- Recent publication to be included:

  Kakehashi et al., Mode of action of ethyl tertiary-buty ether hepatotumoigenicity in the rat: Evidence for a role of oxidative stess via activation of CAR, PXR and PPAR signaling pathways. Toxicol. Applied Pharmacol. 273 (2013) 390-400.

- Oral gavage of ETBE (0, 150 or 1000 mg/kg) for one or two-weeks compared to phenobarbital (500 ppm in diet).
- Conclusion: ETBE MOA of hepatotumorigenicity is similar to that of PB except for activation of PPARs. The proposed mechanism is related to activation of CAR and PXR through oxidative stress, which leads to a sequence of events resulting in regenerative cell proliferation. It is unlikely that this MOA has human relevance.

Topics for Comments on IRIS Materials for TBA

TBA (*tert*-Butanol)

- Mutation Assays
- Genetic Toxicology Assays
- Cancer Endpoints

TBA: Mutation Assays with *Salmonella typhimurium*

Strains used	Activation	Dose	Reference
S. typhimurium TA 1535, TA98, TA100	+/- liver S9 (Rat)	7800 µg/plate No response	ARCO (1994) Not included by IRIS [or same as EG&G Mason, 1981? Included]
S. typhimurium TA1535, TA1537, TA98, TA100	+/- liver S9 (Rat and Syrian Hamster)	10000 µg/plate No response	Zeiger et al. (1987) Included
S. typhimurium TA102	+ liver S9 (Rat)	2250 µg/plate Significant	Williams-Hill et al. (1999) Included. Single test
S. typhimurium TA102 and	+/- liver S9 (Rat)	5000 µg/plate No response	McGregor et al. (2005) Requires editing Two laboratories. Water and DMSO solvents. Independent repetition

TBA: *In vitro* Genotoxicity and Mutation Assays with Eukaryotic Cells

Target cells	Activation	Dose/Response	Reference
Neurospora crassa ade-3A locus, reverse mutation	None, but cells competent	84% mortality No response	Dickey et al. (1949)
Saccharomyces cerevisiae, "petite" mitochondrial mutations	None, but cells competent	4% TBA, 3 days Significant	Jimenez et al. (1988)
Human leukaemia (HL-60) Comet assay	None, but cells competent	1 mM, 1hr Significant	Tang et al. (1997)
Rat-1 fibroblasts Comet assay	None	0.44 mM, 0.5-12hr Significant	Sgambato et al. (2009) Oxidative damage not listed in IRIS [8OH-dGua]

TBA: *In Vitro* Genotoxicity and Mutation Assays with Mammalian Cells

Target cells	Activation	Dose/Response	Reference
Mouse lymphoma L5178Y cell *tk* locus assay	+/- liver S9 (Rat)	5000 µg/mL +S9: 2 NR -S9: 1 NR 1 wk (Sig)	McGregor et al. (1988)
Mouse lymphoma L5178Y cell *tk* locus assay	+/- liver S9 (Rat)	17000 µg/mL NR	EG&G Mason (1981); ARCO (1994)
Sister-chromatid exchange, CHO cells	+/- liver S9 (Rat)	+S9: 15600 µg/mL (Sig) -S9: 7800 µg/mL (Sig)	ARCO (1994b)
Sister-chromatid exchange, CHO cells	+/- liver S9 (Rat)	5000 µg/mL NR	NTP (1995)
Chromosomal aberrations, CHO cells	+/- liver S9 (Rat)	5000 µg/mL +S9: 1 NR, 1 Equiv. -S9: 2 NR tests	NTP (1995)

NR = No response; Sig = statistically significant; Equiv. = Equivocal

TBA: Micronucleus Induction Assay in Peripheral Blood Cells

Study	NTP (1995)
Species	Mice (male and female), 10/sex/group
Route	Drinking water
Doses	3,000-40,000 ppm (Highest exposure likely 4,000-6,000 mg/kg bw/day)
Duration	13 weeks
Results	Micronucleated normocytes <u>Males</u> Control proportion: 0.09 High dose proportion: 0.06 <u>Females</u> Control proportion: 0.06 High dose proportion: 0.07
Conclusions	No response No bone marrow toxicity

TBA: *In vivo* "Adducts" of TBA in Mouse DNA; Study Reliability Questioned

Yuan et al. (2007); if EPA-IRIS agrees, then this study should not be included in the review.

Reasons for judging the study not reliable

- Study is one of a series from the same laboratory in which Accelerator Mass Spectrometry (AMS) is used on DNA from mice dosed with:
  - *tertiary*-single ^{14}C-labelled TBA or
  - methoxy-^{14}C-labelled MTBE (Yuan et al., 2007);
  - dual *tertiary*- and methoxy-^{14}C-labelled MTBE (Du et al., 2005); and
  - ^{14}C-labelled formic acid (Wang et al., 2004).
- DNA is reduced to elemental carbon, after which the radioactivity is measured, but it not known where the radioactivity originated without prior analytical steps – which were not investigated.
- Without further analysis, ^{14}C measured could be:
  - an adduct;
  - metabolically incorporated (e.g., from acetone); or
  - a contaminant.

“Adducts” of TBA with Mouse DNA *in vivo*

Uncertainties identified in other studies using AMS to measure DNA adducts.

Mauthie et al. (1999), p 541

“AMS provides a measure of total radiocarbon present in the samples which may come from MeIQx metabolites, DNA adduct or contaminant. Since AMS measures isotopes only and does not differentiate between these sources, data are reported as MeIQx equivalents/sample.”

Phillips et al. (2000), p 224

“AMS is a technique that measures isotope ratios only. It provides no information on the nature or chemical form of the isotope and thus has the potential to provide false positives in DNA binding studies where genomic DNA isolates are measured.”

Himmelstein et al. (2009), p 682

“As all information of the chemical structure of the analyte is consequently lost, comparison of the chromatographic properties of the analyte with synthetic adducts is essential to ensure that the binding being detected is due to DNA adduct formation. In addition, great care has to be taken to avoid contamination and to properly subtract background levels of radioactivity.”

TBA: Thyroid Follicular Cell Adenomas and Hyperplasia US NTP (1995)

Two-Year TBA Drinking-Water Study (NTP, 1995)

Male mice

TBA Dose (mg/Kg bw/day)*	0	535	1035	2065
Adenoma incidence	1/60	28/50	4/59	1/57
Hyperplasia incidence (severe grade)	5/60 (1.2)	18/59* (1.6)	15/59* (1.4)	18/57* (2.1)

Female mice

TBA Dose (mg/Kg bw/day)	0	510	1015	2105
Adenoma incidence	2/58	3/60	2/59	9/59
Hyperplasia incidence (severe grade)	19/58 (1.8)	28/60 (1.9)	33/59* (1.7)	47/59* (2.2)

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TBA: Thyroid Follicular Cell Adenomas and Hyperplasia in CD-1 Mice

Inhalation Exposure to MTBE; Doses expressed as TBA derived via Metabolism (Burleigh-Flayer et al., 1992)

Male mice

TBA Dose (mg/Kg bw/day)	0	117	879	2269
Adenoma incidence	0/49	0/11	0/19	0/48
Hyperplasia incidence	0/49	0/11	0/19	0/48

Female mice

TBA Dose (mg/Kg bw/day)	0	120	898	2328
Adenoma incidence	0/50	0/11	0/14	0/49
Hyperplasia incidence	1/35	0/11	0/14	0/29

TBA: Thyroid Follicular Cell Adenomas and Hyperplasia US NTP (1995)

- Table 2-3, Including only one category for “Follicular cell adenoma and carcinoma” is misleading; ***The only difference between “Follicular cell adenoma” and “Follicular cell adenoma and carcinoma” is the presence of one carcinoma in male mice at the highest dose level. Diagnoses should be recorded separately as has been done for rat data.***

- Figure 2-3, The legend is divided into non-cancer and cancer; ***Cancer is a malignant condition, so not clear why adenoma is listed in this space. Recommend providing descriptions for both hyperplasia and adenoma.***

TBA: Kidney Histopathology in Male F344 Rats US NTP (1995)

Two-Year TBA Drinking-Water Study (NTP, 1995)

	Incidence in Male Rats (Female Rats)			
Dose (mg/Kg bw/day)	**0**	**90**	**200**	**420**
Mineralization	26/50	28/50	35/50	48/50
Linear Mineralization (Inflammation, suppurative)	0/50 (2/50)	5/50* (3/50)	24/50* (13/50*)	46/50* (15/50*)
Renal tubule hyperplasia (extended evaluation)	12/50	16/50	14/50	23/50*
Transitional epithelium hyperplasia	25/50 (0/50)	32/50 (0/50)	36/50* (3/50)	40/50* (17/50*)
Nephropathy severity	3 (1.6)	3.1 (1.9*)	3.1(2.3*)	3.3*(2.9*)

TBA: Renal Cell Adenomas or Carcinomas in Male F344 Rats

Two-Year TBA Drinking-Water Study (NTP, 1995)

Dose (mg/Kg bw/day)	0	90	200	420
Renal Tubule Adenoma[a]	7/50	7/50	10/50	10/50
Renal Tubule Adenoma (multiple)[d]	1/50	4/50	9/50*	3/50
Renal Tubule Adenoma or Carcinoma[d]	8/50	13/50	19/50*	13/50

Re-evaluations (Hard et al., 2011)

Dose (mg/Kg bw/day)	0	90	200	420
Renal Tubule Adenoma[a]	3/50	9/50	9/50	9/50
Renal Tubule Adenoma (multiple)[d]	1/50	3/50	9/50	3/50
Renal Tubule Adenoma or Carcinoma[d]	4/50	13/50*	18/50*	12/50*

MOA Endpoints for Male Rat Specific Renal Tumors; Information to Include and Clarify in Materials

- Recommend extracting data from Williams and Borghoff *(2001) into the Histopathology section;* ***The study provides exposure response induction of measures of α2u-globulin nephropathy which include histology changes.***
- Recommend extracting data from Hard et al., 2011 into the Histopathology section;
  - Dose-related increase in hyaline droplet formation in male rats
  - Confirmed the crystal-like form of the hyaline droplets that is characteristic of α2u-globulin nephropathy
  - Identified the presence of precursors of granular casts at the "corticomedullary" junction
- Recommend extracting additional data from Cirvello et al. (1995); Lindamood et al. (1992); and NTP (1995);
  - Quantitation of severity of the crystal-like nature of hyaline droplets
  - Quantitative cell proliferation

Incorporation of Mechanistic Data

- **EPA's consideration and application of mechanistic data is unclear and inconsistent.**
  - EPA provides inconsistent criteria for mechanistic data, noting that such studies were not extracted for evidence tables if "the data in the study only included endpoints related to possible mechanisms of toxicity."
  - Whereas EPA then states "Supporting data that provide mechanistic information for each selected endpoint was also included."
- **Generally mechanistic data on key health endpoints must be included as pertinent to assessment of these endpoints. For example:**
  - ETBE induced liver tumors; induction of liver weights, enzymes, etc.
  - TBA induced renal tumors; mode of action associated with measures of a2u-globulin nephropathy; histopathology, cell proliferation, chemical binding.

In Summary

On behalf of Lyondell Chemical Company

- We appreciate the opportunity to provide both written and oral comments on EPA's Preliminary Materials for IRIS Toxicological review for ETBE and TBA.
- Based on the comments presented today and the detailed comments submitted by Lyondell Chemical Company, we feel that there are significant deficiencies and inconsistencies in these materials and encourage EPA to address these deficiencies before proceeding with preparation of the draft assessment.