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Re: EPA External Review Draft Toxicological Review of Ethyl Tertiary Butyl Ether (CAS No. 637-92-3) In Support of Summary Information on the Integrated Risk Information System (IRIS) June 2017. EPA/635/R-17/016a

Dear Dr. Hill-Hammond:

LyondellBasell appreciates the opportunity to provide comments to the U.S. Environmental Protection Agency (EPA) and the SAB Chemical Assessment Advisory Committee augmented for the review of the EPA draft assessment: Toxicological Review for Ethyl Tertiary Butyl Ether (External Review Draft, dated June 2017) in response to the June 16, 2017 Federal Register notice [FRL-9963-24-OA] announcing the availability of the External Review Draft for its Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE), which was prepared to support the summary information that appears on EPA's Integrated Risk Information System (IRIS). LyondellBasell, as a producer of ETBE, has a significant interest in any review of ETBE's health and environmental risks by an authoritative body. We are committed to accurate and up-to-date scientific information and assessments of our products and hence we have reviewed and provide comments on the ETBE External Review Draft assessment to assure that the latest information is available so that the best possible assessment is achieved.

The present External Review Draft of the ETBE IRIS Assessment is substantially similar to the August 2016 External Peer Review Draft, hence our numerous and significant comments submitted previously to the EPA on October 31, 2016 are still valid and should be considered for the present External Review Draft. As with the previous version, the External Review Draft's assessment of the ETBE health effects database is not supported by the available scientific information and draws some erroneous conclusions regarding ETBE's potential for human health hazards and risks.

We reiterate our substantive issues with the External Draft Review for ETBE below:

- The EPA Cancer Risk Assessment Guidelines indicate that a cancer classification of "not likely to be carcinogenic to humans" is appropriate for ETBE. ETBE, a non-genotoxic compound, is not carcinogenic by the oral route at doses up to the limit of drinking water solubility. The EPA guidance allows a classification of "not likely" when "convincing evidence [supports] that carcinogenic effects are not likely by a particular exposure route".

The External Review Draft cross-route extrapolation of an oral cancer risk from liver tumors seen only at 5000 ppm in an inhalation bioassay is not justified in that the likely absorbed ETBE dose at this exposure is approximately 850 mg/kg bw/day, well above the maximum oral dose of approximately 540 mg/kg bw/day achieved in the drinking water bioassay in which no liver tumors were observed. The guidance also allows for "not likely" when "convincing evidence [supports] that carcinogenic effects are not likely below a defined dose range." ETBE liver tumors (almost all benign and only in males) occur only at 5000 ppm, an exposure well above onset of metabolic saturation at 1800-2000 ppm. Inhalation occupational exposures (gas station workers and tanker drivers) are 0.04-0.08 ppm, approximately 25,000 less than the inflection point for onset of nonlinear toxicokinetics (62,500 less than a tumorigenic dose). No liver tumors are seen at 1500 ppm, a dose near the inflection point for ETBE metabolic saturation, or in oral doses up to 540 mg/kg bw/day.

If the ETBE inhalation bioassay had been designed in accordance with current EPA and OECD recommendations for consideration of toxicokinetic data in dose selection, the 5000 ppm ETBE high dose would not have been toxicologically justified and ETBE liver tumors would not have emerged a cancer hazard or risk. The available mode of action (MOA) investigations demonstrate that ETBE activates nuclear receptors (CAR/PXR, PPARα) and induces oxidant stress only at a dose above saturation of metabolism, offering support to the conclusion that such non-genotoxic and potentially tumorigenic mechanisms are secondary to altered high-dose specific toxicokinetics of ETBE.

Thus, integration of the evidence that liver tumors and associated MOA responses were observed only under conditions of saturated ETBE metabolism, when further coupled to robust evidence indicating ETBE is not genotoxic, indicate development of a cancer risk slope factor based on incidence of liver tumors is not justified. ETBE was also negative for liver tumors or any tumor increases in a rat chronic drinking water study, findings that provide further evidence that ETBE is not a human cancer concern.

- The External Review Draft assessment's conclusion that the ETBE genotoxicity database is inadequate or insufficient to draw any conclusions on the effect of ETBE is not scientifically justified. ETBE has been thoroughly tested for genotoxicity, finding negative results for all apical genotoxicity endpoints of regulatory interest (i.e., gene mutations, clastogenicity, and aneugenicity) using quality standards such as protocols compliant with existing guidelines and GLP standards. The available information supports the conclusion that ETBE is not genotoxic.

- The External Review Draft assessment's consideration of an acetaldehyde-mediated MOA for the rat liver tumors is not supported by the available scientific evidence or human exposure realities. The External Review Draft assessment concludes that there is an inadequate database to conclude that ETBE induces liver tumors via an acetaldehyde-mediated mutagenic MOA, however the assessment repeatedly refers to this MOA as

suggestive giving more credence to it than the data supports. The acetaldehyde-mediated genotoxic MOA is highly speculative and has no quantitative relevance to acetaldehyde levels generated from realistic levels of human exposures to ETBE. Occupational exposures of 0.1 ppm ETBE have been reported in breathing zones of Japanese gasoline station workers and fuel tanker drivers, and these exposures are approximately equivalent to acetaldehyde systemic doses delivered from consumption of less than one drop of beer. This dose context comparison supports the conclusion that it is highly implausible that acetaldehyde from ETBE exposures contributes to human liver cancer risk.

- The External Review Draft assessment inappropriately considers the ETBE liver tumor promotion studies as primary evidence ETBE tumorigenicity. 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 (1000 mg/kg bw/day), a dose level that also exceeds metabolic saturation. This promotional activity has clear thresholds, with no evidence of promotion at a 300 mg/kg bw/day dose that is near or at an oral dose reflecting onset of nonlinear toxicokinetics. Thus, the available data supports the conclusion that the liver tumors observed following inhalation exposure of ETBE are most likely the result of the promotion of spontaneously initiated cells and as such have clear threshold dose-response relationships.

- Evidence of promotion of multi-mutagen-initiated tumorigenicity was observed in thyroid at oral gavage doses of 300 and 1000 mg/kg bw/day and in colon only at the high dose. No evidence of tumor promotion was observed in kidney, forestomach, urinary bladder or urethra. In a later single mutagen-initiated study, ETBE-induced liver promotion was restricted to the high dose of 1000 mg/kg bw/day, while the incidence of kidney adenomas was increased at 500 and 1000 mg/kg bw/day (however, combined adenoma/carcinoma incidence was not altered at any dose). It is important to note, however, that these studies do not provide meaningful evidence of ETBE carcinogenicity. Both assay designs were developed as screening assays of potential carcinogenicity hazard (but not risk), and were validated for target organ predictability against existing apical animal cancer bioassays. In the case of ETBE, however, which has two high quality apical rat carcinogenicity studies conducted by two routes of administration, the possibility of non-hepatic tumorigenicity (kidney, thyroid, colon) as suggested in the initiation-promotion assays was not confirmed in 2 apical animal bioassays. This combined evidence indicates that non-hepatic carcinogenicity identified in the 2-stage carcinogenicity assays is not relevant to ETBE carcinogen assessment, other than to provide possible supporting evidence that high-doses of ETBE exceeding metabolic saturation may have tumor-promoting activity. Such promotion responses are generally regarded as threshold-based MOA events.

- Derivation of RfC and RfD values based on urothelial hyperplasia in rats is inappropriate. Transitional cell hyperplasia observed with ETBE has been diagnosed as an exacerbation of chronic progressive nephropathy, a response that has no human clinical correlate. Kidney

toxicity in male rats is further confounded by an overlay of ETBE-mediated α-2u-globulin nephropathy, a MOA not relevant to humans. No other kidney lesions have been observed that are suggestive of functioning of other alternative MOAs.

- The External Review Draft assessment incorrectly concludes that there is inadequate information to draw conclusions regarding other health hazards that may be associated with ETBE exposure. There are reliable subchronic and chronic toxicity studies that evaluate many organ systems following oral and inhalation exposures to ETBE. In addition, specialized genotoxicity, neurotoxicity, functional immunotoxicity and mortality studies indicate that these endpoints are not hazards for repeated exposures to ETBE.

When the ETBE assessment considers these points, it will find:

1) ETBE is not a cancer concern for humans.
2) ETBE is a low concern to humans for chronic toxicity by inhalation and oral exposure.

Our conclusions are supported in our attached comments:

- LyondellBasell Comments on Charge Questions to the Science Advisory Board for the IRIS Toxicological Review of Ethyl Tertiary Butyl Ether. June 2017. Submitted July 31, 2017.

We urge the EPA and the augmented SAB Chemical Assessment Advisory Committee to review these and our detailed previous comments on the August 2016 External Peer Review Draft submitted to the EPA on October 31, 2016 and available in the EPA docket EPA-HQ-ORD-2009-0229-0044 (https://www.regulations.gov/document?D=EPA-HQ-ORD-2009-0229-0044).

LyondellBasell appreciates your consideration of our comments.

Regards,

LyondellBasell

Attachment

LyondellBasell Comments on Charge Questions to the Science Advisory Board for the IRIS Toxicological Review of Ethyl Tertiary Butyl Ether. June 2017

July 31, 2017

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods

Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

LyondellBasell Comment:

The External Review Draft has not noted the important publication of Eitaki et al. (J Occup Hlth. 53: 423-431, 2011) which provides a detailed analysis of ETBE exposures in gas station workers and gasoline tanker truck drivers. This paper offers important exposure data in a group of workers most likely to experience ETBE exposures, and confirms that ETBE exposures are very low – a geometric mean TWA exposure of 0.08 ppm in the breathing zone for gas station workers and 0.04 ppm for tanker drivers. Such high quality exposure is valuable as a context for framing decisions regarding use of animal toxicology datasets for derivation of ETBE health assessments.

As presented in earlier comments from LyondellBasell, the External Review Draft does not note that ETBE production in the United States is limited to a single, closed-system production site, and with essentially all production shipped to international markets. Importantly, There is NO *primary* evidence presented that ETBE was in fact detected in ground water or soil in the US in recent years. The "evidence" presented is the report titled "Summary Report on a Survey of State Experiences with MtBE and Other Oxygenate Contamination at LUST Sites," a Project of the New England Interstate Water Pollution Control Commission (NEIWPCC). EPA claims that based on this report, ETBE is "commonly detected" near LUST contamination areas. That is a *gross* exaggeration. The 2003 report makes unsubstantiated claims to have detected ETBE in a handful of states at the part per billion level in gasoline-contaminated groundwater and gives no references. There were very few cases where states reported that ETBE was detected in groundwater in 2002. Most were well below GC detection limit, in the 2-60 ppb range, the method used to supposedly detect it. According to this summary report, of the 36 states reporting oxygenates in groundwater in 2002, only 9 had hard data and 27 gave estimates. The 18% reported by EPA is the number of reports (3) of ETBE for gasoline releases only. In other words, only 3 reported cases (no evidence provided) of ETBE in groundwater at the part per billion level from hundreds of likely LUST cases in 2002. This is hardly a significant environmental concern. Furthermore, the EPA updated its UST standards in 2009 (requiring double hulled tanks with leak detection) and gave states until 2015 to comply. According to EPA, all states have now complied. So the likelihood of any soil or groundwater contamination from LUSTs by ETBE today is virtually non-existent.

It also is not clear why a repeat-dose neurotoxicity study was omitted simply because there were no consistent effects. Dorman et al. (1997) determined that there were no lasting neurobehavioral or neuropathology findings following inhalation exposure, although reversible neurobehavioral effects were noted at high dose levels. This suggests that the EPA may be placing more weight on positive findings than stronger studies that are negative.

2. Chemical Properties and Toxicokinetics

2a. Chemical properties. Is the information on chemical properties accurate?

LyondellBasell Comment:

The information on the chemical properties of ETBE appears to be accurate. However, the EPA should conduct a thorough quality control review of the tabular data and appropriate references for confirmation.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

LyondellBasell Comment:

The Supplemental Information, Section B.1.5 of Appendix B, includes reference to two ETBE-TBA PBPK models (Salazar et al., 2015 and Borghoff et al., 2016) and a review of these models by the EPA Pharmacokinetics Working Group (US EPA, 2017). Although noted that the Borghoff et al. 2016 model was used for the route-to-route extrapolation, it is not always clear that the modifications identified in the US EPA 2017 review were incorporated. As such when the question above is asked, is the use of the model appropriate and clearly described, including assumptions and uncertainties, the model version and parameter values that were used, and its limitations should be identified. Also, it is not clear in the US EPA 2017 assessment of these models that the modifications to the Borghoff et al. 2016 model demonstrate an overall better fit to the available ETBE and TBA tissue data following varying exposure scenarios and if model uncertainties might have influenced the route to route extrapolation used for estimating the oral slope factor.

As noted on page B-24 "For simulating exposure to drinking water, the water consumption was modeled as episodic, based on the pattern of drinking observed in rats (Spiteri, 1982). In particular, rats were assumed to ingest water in pulses or "bouts," which were treated as periods of continuous ingestion, interspersed with periods of no ingestion. During the active dark period (12 hours/day), it is assumed that 80% of total daily ingestion occurs in 45-minute bouts alternating with 45 minutes of other activity. During the relatively inactive light period (12 hours/day), it is assumed that the remaining 20% of daily ingestion occurs; the bouts are only assumed to last 30 minutes, with 2.5 hours between. This pattern is thought to be more realistic than assuming continuous 24 hours/day ingestion. The resulting ingestion rate for one exposure is shown in Figure B-2. "

It is assumed, but not clearly stated, that this description was incorporated into the Borghoff et al. 2016 model and used for the route-to-route extrapolation for calculating the oral slope factor for the cancer study. In the Borghoff et al. 2016 model, the drinking water exposure to ETBE was estimated as a daily dose (mg/kg bw/day) using the animal's body weights to calculate a constant infusion rate (mg/hour) into the gut lumen over a 12-hour simulation period. Although it is stated that the pulses or bouts over a 12-hour period is more realistic, there was no data to support that this was better at predicting ETBE and TBA tissue levels vs. the original description in the Borghoff et al. 2016 model. As such, any uncertainty with respect to the description of water consumption described in the model should be identified.

As stated previously, some of the features of the Borghoff et al. 2016 model were further clarified in the US EPA 2017 assessment with a list of suggested changes provided. However, it was never quite clear if these changes, when implemented in the Borghoff et al. 2016 model lead to improved predictions of the ETBE and TBA tissue data.

The final question "Are there additional peer-reviewed studies that should be considered for modeling?" It appears that all the peer-reviewed studies along with toxicokinetic study reports have been used in the development of the most updated model version (Borghoff et al., 2016) and considered in this assessment. However, now that the definitive ETBE and TBA elimination study is reported (Borghoff and Asgharian, 1996) it is not clear why the pilot studies by Sun and Beskitt (1995a,b) and Borghoff (1996) are still included. Based on data presented in the Supplementary – Appendix B, B.1 Toxicokinetics, EPA should reconsider the data that they discuss on Page B-5 and throughout the toxicokinetics section. Now that External Review Draft has included the definitive ETBE inhalation elimination study (Borghoff and Asgharian, 1996), it is not clear what the value of including these three pilot studies is since these data only represent an n=2, and in the case of the Sun and Beskitt studies, the n=2 is one male and one female rodent value.

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

<u>LyondellBasell Comment:</u>

Although the rate of ETBE metabolism could be justified as a choice for a dose metric, as commented in External Review Draft Section 3f.iii, the inhalation tumor data should not be used for derivation of an oral cancer slope factor. A high-quality rat drinking water conducted at the limit of water solubility (resulting in top doses of 542-560 mg/kg bw/day) did not result in any treatment-related tumors (Suzuki et al., 2012). Based on this, the PBPK model, using ETBE metabolism as the dose metric, would not be needed for route to route extrapolation. However, most critical to this evaluation is that the systemic dose of ETBE resulting from an inhalation exposure of 5000 ppm estimated as 842-860 mg/kg bw/day from the PBPK model. Thus, the inhalation dose is substantially greater than the daily dose resulting from drinking water exposure at the limit of water solubility, making use of such a dose irrelevant to the evaluation of oral drinking water risk estimates. The details of the toxicokinetic analyses are provided in Charge Question 4a.

Hazard Identification and Dose–Response Assessment

Comment on EPA's assessment of the toxicological studies and dose-response assessment, including whether there are additional peer-reviewed studies that should be considered.

3. Noncancer

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

LyondellBasell Comment:

Urothelial hyperplasia

The External Review Draft continues to inappropriately use urothelial hyperplasia in the kidney with a characterization of "high confidence" as the basis for derivation of the oral RfD and inhalation RfC. In its response to public comments addressing kidney effects (External Review Draft, Appendix D), the External Review Draft continues to suggest that urothelial hyperplasia is an ETBE toxicity response that is potentially independent of rat chronic progressive nephropathy (CPN) and thus could possibly occur in human kidney. The External Review Draft justifies its conclusion by incorrectly stating that because "…one of more those processes [within CPN] likely reflects some type of cell injury/cytotoxicity", it should be regarded as relevant to human kidney.

This External Review Draft conclusion is incorrect, however, in that a Pathology Working Group review of TBA-induced kidney lesions (Hard et al., 2011) and ETBE-induced kidney lesions (Saito et al., 2012, 2013) have unequivocally concluded that the urothelial hyperplasia response was integral to well-established pathological diagnostic criteria of CPN that is exacerbated by chemical treatment. Although the underlying MOA of CPN and its potential exacerbation by chemical treatment are not understood, the conclusive diagnosis of CPN is nonetheless regarded as not human relevant in that there is no known human kidney disease and/or injury correlate. Thus, because potential evidence of cell injury/cytotoxicity observed with TBA and/or ETBE only nested within a final CPN diagnoses, this response is not be regarded as human relevant.

The selection of urothelial hyperplasia as the key endpoint is *not valid* because the type of transitional cell hyperplasia observed in various chronic studies of ETBE is not a manifestation of chemically-induced renal toxicity, but a component of advanced CPN, a spontaneous disease process of rats. The transitional cell hyperplasia observed in rats in these cases is a morphologically distinct lesion affecting primarily the epithelial lining of the renal papilla. The initial change appears to be an edema-like outpouching of the papilla lining followed by collapse of the protruding, single layer of lining upon itself, sometimes conferring a multicellular appearance to the lesion. Despite the External Review Draft's contrary statement that CPN is unlikely to be associated with urothelial hyperplasia, this alteration is present in some degree ranging from trace to moderate in most rats with advanced CPN, and is particularly evident in

end-stage CPN, as an integral part of the CPN pathological spectrum. In fact, a Pathology Working Group review of the chronic kidney lesions observed in the chronic NTP drinking water bioassay of TBA (Hard et al., 2011), which were essentially equivalent to ETBE (Saito et al., 2012, 2013), unanimously concluded that:

> "…based on a characteristic morphology involving out-pouching of transitional cell epithelium from the papilla lining (Figs. 4 and 5), that the transitional cell hyperplasia in this 2-year study was a component of CPN and not a change directly induced by TBA. The urothelial lining of the kidney pelvis walls and ureteral mouth, when present in the kidney sections, showed no evidence of urothelial (transitional) cell hyperplasia" [note: the absence of urothelial hyperplasis in the kidney pelvis walls and ureteral mouth is supportive that non-CPN toxicity MOAs are not functioning.]

The association between this papillary lesion and advanced CPN has been reported by Hard and Khan (2004), Hard et al. (2009), and Frazier et al. (2012). This form of papilla lining hyperplasia has been described and illustrated (Figure 91, page 76S) in Frazier et al. (2012) under the term "chronic progressive nephropathy". The Frazier et al. (2012) publication represented a joint initiative sponsored by a number of international pathology bodies including the Society of Toxicologic Pathology (STP) from North America, the European Society of Toxicologic Pathology (ESTP), the British Society of Toxicologic Pathology (BSTP), and the Japanese Society of Toxicologic Pathology (JSTP). This publication had the purpose of harmonizing and standardizing the nomenclature and diagnostic criteria for proliferative and non-proliferative lesions of the rat and mouse kidney and lower urinary tract as part of the INHAND project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice). It should be noted that the year of publication for this document, 2012, post-dated the write-up of the studies on ETBE conducted by JPEC in 2008 to 2010. Consequently diagnostic guidance may not have been available at the time to enable the JPEC scientists to correctly refer to the papilla hyperplasia observed with ETBE as CPN-associated. The dose-related increase in severity of this lesion occurred because there was a relatively high severity of CPN across the groups of male rats, peaking at the high dose in the 2-year study (Suzuki et al., 2012, 2013). The occurrence of this lesion in male rats is explained by the difference in CPN severity between the two sexes, male rats having much higher grades of severity than the females, in which the severity grade did not reach the threshold for papillary hyperplasia development. The easier recognition of papillary lining hyperplasia compared with attempts to accurately and consistently distinguish between mild, moderate and marked grades of CPN severity is a likely explanation for the more impressive dose-related trend for "urothelial hyperplasia" than for CPN severity.

The morphological description of the urothelial hyperplasia associated with ETBE inhalation (Suzuki et al., 2012) and oral exposures (Saito et al., 2013) matches the hyperplasia of the papilla lining developing as a consequence of CPN of advancing severity. The description in these 2 publications is as follows: "low-grade multilayer thickening of the papillary epithelium, limited to small areas of the papilla, with protrusion into the pelvis lumen". In addition, Dr. Hard had the opportunity to view that hyperplasia as a member of the Pathology Working Group (PWG) that gave expert opinion on a selection of the slides from the JPEC studies of ETBE. There was

unanimous agreement by members of this PWG that the hyperplasia of the papilla lining in the ETBE studies was a typical outcome of advancing CPN (Cohen et al., 2011).

Papilla lining hyperplasia ("urothelial hyperplasia") usually develops in CPN of moderate (grade 3) to marked (grade 4) severity in the conventional 1-4 grading schema. Differences in the grades of CPN severity between males and females explains the observation of this lesion in male rats at the two highest doses in the chronic study of ETBE, but its absence in female rats at all doses (Suzuki et al., 2012, 2013). In the Suzuki et al. (2012) study, the mean severity grade of CPN in the high-dose males (10,000 ppm) was 2.4, implying that some rats had moderate or higher CPN. In contrast, the mean severity grade of CPN in the high-dose (10,000 ppm) female rats was only 1.5 (Suzuki et al., 2012), which is a lower severity grade than is associated with development of papilla lining hyperplasia. Expressed another way, Saito et al. (2013) recorded 38 male rats receiving 5000 ppm with either grade 3 or 4 CPN severity compared to only 10 female rats at that dose with those same grades of CPN severity.

Genuine urothelial hyperplasia does occur as a nephrotoxic response following exposure to some chemicals, but its morphology is quite different from the papillary hyperplasia associated with advancing CPN. Usually chemically-induced urothelial hyperplasia takes the form of focally thickened urothelium and/or solid nodules protruding from the pelvis lining, arising, not from the papilla lining which is a single cell layer, but from the multicellular urothelium opposite the papilla. For example, nodular urothelial hyperplasia can occur near the mouth of the ureter as a consequence of necrotic tip sloughing following severe papillary necrosis induced by ethoxyquin (Hard and Neal, 1992). ETBE exposure did *not* produce this morphological type of urothelial hyperplasia.

An attempt was made in the External Review Draft to provide evidence that the association between CPN and urothelial hyperplasia is weak (page 1-18, Tables 1-5, 1-6), by using contingency tables comparing occurrence of urothelial hyperplasia with CPN in individual rats, arranged by severity and analyzed by Spearman's rank correlation. The data on severity appeared to be derived from the NTP (1995) report. More meaningful strength of association would have been achieved by comparing presence or absence of urothelial hyperplasia with severity grade of CPN rather than comparing severity grades for both lesions. Experience with rat CPN indicates that urothelial hyperplasia is associated with increasingly severe CPN from moderate to marked severity grades, and not with the lower grades of CPN. The degree of severity of urothelial hyperplasia associated with advanced CPN on the other hand is invariably minimal to mild. These various aspects are quite evident in Tables 7 and 8 of the NTP (1995) report. Furthermore, it remains a fact that urothelial hyperplasia occurs as a component lesion of advanced stages of CPN. Chemically-associated hyperplasia of the renal pelvis lining manifests as foci of solid nodules of proliferating transitional epithelium (usually in the urothelial lining opposite the papilla) as illustrated in Figure 16 of the STP publication of Hard et al. (1995). In contrast, the "urothelial hyperplasia" occurring in advanced CPN and in the 2-year ETBE study (Cohen et al., 2011; JPEC, 2010) consists of edema-like outpouchings of the papilla lining (Hard et al., 2009), followed by collapse of the protruding, single layer of lining upon itself, sometimes conferring a multicellular appearance to the lesion. The lesion is illustrated in Figure 91 of Frazier et al.

(2012). Although generally referred to as hyperplasia of the papilla lining, this is not a proliferative lesion, and it should not be assessed individually, separate from CPN. Furthermore, no morphologically similar lesion has been found in human pathology atlases.

Acknowledging that the urothelial hyperplasia described by several authors in studies with ETBE is an outcome of advancing CPN is very important. This lesion was correctly diagnosed in an ETBE study by a team of expert pathologists with special expertise in toxicologic pathology of the kidney and urinary tract, and it is recognized as part of the spectrum of CPN pathology by a number of scholarly international toxicologic pathology bodies dedicated to setting accurate standards for diagnostic criteria of lesions observed in safety evaluation studies. Denying the accepted relationship between advanced CPN and papilla lining transitional cell hyperplasia, and attributing it instead to a nephrotoxic effect, would constitute a serious blunder in this IRIS assessment of ETBE.

The External Review Draft's response to public comments (Appendix D) also attempts to support its use of urothelial hyperplasia by citing FDA's use of CPN as a toxicity endpoint in the *draft* reviews of the human health risks of PDE's and MIBK. Importantly, the FDA reviews do not provide any detailed analysis of the lack of human relevance of CPN, and simply chose to rely on CPN as a conservative approach supporting a screening level risk assessment addressing health concerns associated with solvent contamination of pharmaceuticals.

Chronic Progressive Nephropathy

On **page 1-6, lines 21-23**, the authors of this External Review Draft now acknowledge correctly, that CPN has no renal counterpart in humans. But it is incorrect to state that CPN is *not* a specific diagnosis. Two publications of the Society of Toxicologic Pathology (and other scholarly bodies) in describing and setting diagnostic guidelines for non-proliferative lesions of the rat kidney (Hard et al, 1999; Frazier et al, 2012), have classified CPN as a special disease process, and a specific diagnosis. They have also advised that the component lesions of CPN should *not* be diagnosed separately but lumped together so that this important confounding entity can be properly identified and its influence on any chemical-related pathology be taken into account. On **page 1-35, lines 28-30,** the External Review Draft implies incorrectly that the aggregate term of CPN, which includes all the component lesions comprising CPN, is used for convenience and as a time-saver. It must be stressed again that the term CPN is a specific diagnosis for an extremely important confounding disease process that needs to be identified in the rat kidney as a single entity.

The External Review Draft now acknowledges that ETBE enhances CPN, but claims that exacerbation of any one of the component lesions of CPN, taken in isolation and out of context, might reflect a type of renal injury that could occur in humans. This is an inappropriate and invalid approach that is not applied to other complex toxicological entities such as α-2u-globulin nephropathy

BUN, Creatinine, cholesterolemia and kidney weights

In addition to urothelial hyperplasia being a human non-relevant endpoint, the Public Review Draft also suggests (p.1-78, l.27-33) that increased BUN, creatinine, cholesterol and kidney weights also are CPN-independent indicators or ETBE kidney toxicity. However, both BUN and creatinine are well known markers of advanced CPN, and cholesterolemia has also been reported to occur in advancing CPN (Berg, 1965; Coleman et al., 1977; Barthold, 1979). Increased kidney weights, either absolute or relative, are not specific for nephrotoxicity and therefore are also unsuitable for dose-response analysis and derivation of reference values.

The Public Review Draft noted that BUN, creatinine, and cholesterol concentrations in the blood of male rats were elevated in several studies, and of these, increased blood cholesterol was elevated in 3 of 4 studies (for example, Miyata et al., 2014). Because ETBE increased these serum measures at lower doses and in more studies than were associated with increased CPN, the Public Review Draft concludes that elevated serum cholesterol was an appropriate biomarker of a kidney effect induced by ETBE. It is well known that there is an increase in blood levels of cholesterol, creatinine, and BUN in CPN, and this is a reflection of the severity and progression of CPN, and is not necessarily dependent solely on chemical exacerbation of the disease process. In the chronic studies of CPN that recorded cholesterolemia, for example, CPN was of sufficient severity grade to affect cholesterol blood levels. These selected blood biomarkers are therefore, not specifically indicative of kidney alteration independent of CPN after exposure to ETBE.

The Public Review Draft also has noted that numerous studies consistently reported an increase in absolute kidney weights after either oral or inhalation exposure to ETBE. Kidney weights, either absolute or relative, are non-specific indicators of kidney effects. The Public Review Draft prefers selection of increases in absolute kidney weights rather than increased relative kidney weights based on the rationale that absolute kidney weights are more reliable than relative kidney weights when accompanied by decreases in body weight gain. However, increased kidney weights, absolute or relative, are not a reliable indication that a chemically-induced adverse toxic event has occurred in the kidney. Increased kidney weights in the ETBE-exposed rats could be due to a variety of causes, such as non-adverse and compensatory renal processing and metabolism of the test article, urinary solute increase, and histopathological changes including hyaline droplet accumulation and linear papillary mineralization (LPM) in male rats, and CPN in both sexes.

If there is histopathological alteration in the kidney, an increase in kidney weights can be expected. The exposure of rats to ETBE exacerbated CPN in both sexes, or caused hyaline droplet accumulation associated with ETBE-induced α-2u-globulin nephropathy in male rats. These would be main factors resulting in increased kidney weights. In male rats exposed to ETBE, both of these factors would apply in subchronic studies, and CPN and papillary mineralization would affect kidney weights of the males in the chronic studies, including the 180-day study. In the female rats, CPN would also have an effect at these time-points. In the absence of histopathological change, as in some females in short-term studies of ETBE, increased kidney weights can be expected if the chemical is processed by the kidney. In our experience, when a xenobiotic is cleared by the kidney, there is an association with an increase in kidney weights. This is an adaptive response and may be due to metabolizing enzyme induction

in the renal tubules. Another adaptive response that could increase kidney weights is increased blood flow through the kidneys as a consequence of treatment. ETBE is metabolized to TBA and acetaldehyde, with TBA being the major systemically available metabolite of ETBE; TBA is processed through the rat kidney. Solvents can also increase solute and/or water retention in the kidney, resulting in an increase in kidney weights as another adaptive physiological response; TBA has solvent properties.

Because it cannot be assumed that an increase in kidney weights specifically reflects adverse renal toxicity, this non-specific endpoint is an inappropriate selection for deriving reference values for ETBE. This is particularly so when robust data indicate that an α-2u-globulin MOA and exacerbated CPN are functioning with ETBE exposures. Thus, altered kidney weights should not be a candidate for potential use in BMD modelling of non-cancer ETBE toxicity.

α-2u-globulin MOA

The External Review Draft MOA analysis has concluded that the data were insufficient to conclude that kidney effects in male rats were mediated by α-2u-globulin-associated nephropathy alone. This decision was based on an exaggerated interpretation of "data gaps" in the data, maintaining that most of the steps in the pathological sequence were not observed. This reflects a seriously flawed assessment of ETBE kidney effects, and suggests some obdurate prejudice against acknowledging the weight of evidence supporting this MOA in general.

α-2u-Globulin nephropathy is given much attention in the document, including a point by point assessment of the criteria, proposed by the EPA Risk Assessment Forum in 1991, needed to support acceptance of this process as a MOA in chemical carcinogenesis (EPA, 1991).
The External Review Draft essentially discounted this MOA as being irrelevant to ETBE (**page 1-35, lines 14-16**) because of the lack of fulfillment of criterion 3 in particular (**page 1-31**). This criterion requires demonstration in the male rat of several (but not necessarily all) additional steps in the α-2u-globulin histopathological sequence, induced by the test article in a manner consistent with an understanding of the pathogenesis of α-2u-g nephropathy. On **page 1-30, lines 36-37**, the document states that of the remaining **five** endpoints in the pathological sequence, only linear papillary mineralization and granular casts were observed. This is an exaggeration, and appears to reflect some underlying bias.

The three endpoints that were considered to be absent were necrosis, sustained cellular proliferation, and renal tubule hyperplasia. It is becoming more accepted that single cell necrosis as a recognizable morphological entity may not occur with weak inducers of α-2u-globulin nephropathy. Instead, cell loss appears to result from tubule cell exfoliation, a morphological event that is not always recognized by the study pathologist.

The absence of hyperplasia was considered in the External Review Draft to suggest that ETBE did not induce sustained cell proliferation. The intention of US EPA (2001) in listing hyperplasia as a lesion was to recognize what is now called atypical tubule hyperplasia (ATH), the preneoplastic precursor on the developmental pathway to renal tubule adenoma. As there were no renal tubule tumors induced by ETBE, foci of ATH would most likely be absent as well.

Their absence in the long term studies with ETBE is therefore in line with an absence of renal tumors and does not necessarily reflect an absence of sustained cell proliferation.

In fact, Medinsky et al. (1999) did provide data showing statistically increased, sustained tubule cell proliferation in male rats at 3 exposure levels, which the External Review Draft elected to discount because of a low, transient response in the female rats. At 1 week, 4 weeks, and 13 weeks, the doses of 5000, 1750, and 500 ppm showed statistical increases different from the control males at $p<0.001$, $p<.01$, $p<.01$, respectively. In contrast, the female rats showed a transient, lower statistical increase ($p<0.05$) at the highest dose at 1 and 4 weeks, which was not sustained for the 13 week time period. This unconvincing level of response in the female rats should not detract from the significance of the male response. It should be noted that Table 3 in the Medinsky et al. paper is incorrectly labelled. The subheadings for the male and female columns should be reversed. The correction was notified in Erratum published in *Toxicol Sci* in 2006 (see Medinsky et al., 1999 below).

Of the 5 endpoints that represent additional supporting data for an α-2u-globulin MOA, granular casts at the 13-week time-point, and linear papillary mineralization many months later in the 2-year study, following an initial phase of hyaline droplet accumulation in the cortex, are the most important observations for identifying an α-2u-globulin MOA. Granular cast formation in the straight proximal tubule where it narrows into the thin descending limb of Henle, and linear papillary mineralization in tubule lumens of the inner medulla, are both very distinctive lesions providing the most convincing histopathological evidence of α-2u-globulin nephropathy at subchronic and chronic stages. Granular casts are definitely indicative of cell loss from upstream proximal tubules, while linear papillary mineralization occurs because of accumulation of mineralized cell debris at the sharp U-turn in the thin descending limb of Henle. Both endpoints therefore signal the existence of cell loss, probably via exfoliation. While individually these specific lesions are not unique to α-2u-globulin nephropathy, their dual occurrence in the expected temporal sequence, along with hyaline droplet accumulation, is pathognomonic for α-2u-globulin nephropathy, and supports the weight of evidence favoring a role for this MOA in the male rat kidney response to ETBE.

In this version of the External Review Draft, the review of ETBE should have provided a more balanced assessment of the association of ETBE with α-2u-globulin nephropathy. The only pathological processes identifiable in the rat kidney following exposure to ETBE are α-2u-globulin nephropathy and exacerbation of CPN. There is absolutely no evidence among the published ETBE data to suggest that an unknown MOA is at work. Consequently, ETBE shows no potential to cause kidney effects relevant to humans. Therefore, the kidney is not a candidate for dose-response analysis, derivation of an RfD, or BMD modelling.

Specific comments on α-2u-globulin MOA addressed in earlier Lyondell Basell comments (2016) on the Public Review Draft that are still relevant to External Review Draft

Specific comments addressing the Public Review Draft analysis of the key events associated with the α2u-globulin MOA are as follows:

Public Review Draft p.1-29, l.20: "*The second criterion is to consider whether the protein in the hyaline droplets in male rats is α2u-globulin.* Line 227-28. *No statistical tests were performed on the results……did not test a sufficient number of samples within a dose group nor were enough dose groups tested…" The second criterion is to consider whether the protein in the hyaline droplets in male rats is α2u-globulin.* Line 227-28. *No statistical tests were performed on the results……did not test a sufficient number of samples within a dose group nor were enough dose groups tested…".* The purpose of immunohistochemical staining with antibody to α-2u-globulin is to confirm that the accumulated protein is α-2u-globulin. Just a few slides are sufficient for this purpose and to expect statistical analysis to be performed is unrealistic, demonstrating lack of experience with practical aspects of the α-2u-g syndrome. Immunohistochemical staining for α-2u-globulin can be a capricious technique and should not be used for grading a hyaline droplet response or for statistical analysis. Contrary to the EPA conclusion, the available data are quite adequate to fulfil the second criterion for identifying the accumulating protein as α-2u-globulin.

Public Review Draft p.1-29, l.33: "*The α-2u-g nephropathy third criterion is whether several steps in the histopathological sequence associated with α-2u-g nephropathy appear in male rats…. Of the remaining five endpoints….only linear papillary mineralization and granular casts were observed."* These two lesions are virtually pathognomonic for α-2u-globulin nephropathy, especially when occurring in tandem.

Public Review Draft p.1-30, l.7-9: *"However hyaline droplet deposition was observed in 80% of animals at all three inhalation exposure concentrations, compared with mineralization rates of 0, 2, and 12%..."*. This statement shows a lack of understanding of the grades of potency of α-2u-globulin nephropathy inducers. The review acknowledges that ETBE is a weak inducer of α-2u-globulin nephropathy and the degree of linear mineralization will be accordingly lower than with stronger inducers, but in contrast, hyaline droplet accumulation is invariably present after commencement of test article exposure. Hyaline droplets were also observed after 26 weeks of treatment (Miyata et al., 2014).

Detailed evaluation of the other available evidence supporting the third criterion α-2u-globulin.

(a) Public Review Draft p.1-30, l.12-13: *"Single cell death, exfoliation into the renal tubules, and necrosis were not observed in any study…"*. There is some emerging doubt concerning whether necrosis per se occurs in the initial stage of α-2u-globulin nephropathy, especially with chemicals of weaker potency. Cell loss appears to occur mainly through exfoliation of droplet-congested tubule cells. The term necrosis is not used in describing the α-2u-globulin nephropathy by the authors of the INHAND publication on diagnostic criteria for kidney lesions (Frazier et al., 2012). That the Japan Petroleum Energy Center (JPEC) study pathologists did not record tubule cell exfoliation with ETBE does not mean it was not present because it is an easily overlooked morphological alteration. However, the fact that granular casts were reported by Cohen et al. (2011) and Suzuki et al. (2012) means that tubule cell exfoliation did occur. Granular casts form at the junction of the outer and inner stripes of outer medulla where the pars recta narrows into the descending limb of Henle, due to the aggregation of droplet-congested cells that have exfoliated from the proximal

convoluted tubule higher up the nephron. In fact, Cohen et al. (2011) observed that the granular casts were "consistent with clusters of exfoliated proximal tubule cells that had been trapped at the OSOM/ISOM". Thus, it is clearly evident from these descriptions that cell loss through tubule exfoliation did occur in response to ETBE exposure.

(b) Public Review Draft p.1-30, l.20 and 22-24: *"Granular cast formation was observed in one study (Cohen et al, 2011)…Other studies did not report the presence of granular casts (JPEC 2008b, 2008c…"* (Repeated on page 1-31, lines 15-17*).* This is not correct because Suzuki et al. (2012) reported increased incidences of granular casts in males given 1600 ppm or higher levels of ETBE in the preliminary 13-week study, but not in females. These were presumably the same animals and lesions recognized by the Cohen et al. (2011) PWG. This evidence was reported in the Materials and Methods section of Suzuki et al. (2012) and shows that the PWG was not the only group to observe this pathognomic lesion.

(c) Public Review Draft p.1-30, l.33-36: *Observation of proliferation in both sexes suggests that this effect is not male-specific, and thus not α2u-g-specific. Furthermore, renal tubule hyperplasia was not observed in any 2-year study, suggesting that ETBE does not induce sustained proliferation.* Firstly, the Public Review Draft reviewers should be aware that in the Medinsky et al. (1999) paper regarding a 13-week toxicity study of ETBE, in Table 3 (Summary of labelling incidences in kidney), the columns for male and female rat response to cell proliferation are incorrectly labelled and the headings *males* and *females* should be reversed. In the female rats, increased cell proliferation was present only in one dose group (5000 ppm) and at only one time point (4 weeks). Significantly, there was no increase in female kidney at 13 weeks, in contrast to the males. Furthermore, the labelling index in the male rats was far more robust than that scored in the females and was evident at 1, 4, and 13 weeks as opposed to the absence of an increase in females at 1 and 13 weeks. The male response is consistent with hyaline droplet nephropathy, both increased cell proliferation and the hyaline droplet response being sustained over the 13-week period of the study. A modest increase in labeling index at just one dose and one time-point should not prejudice the other strong evidence underpinning the induction of α2u-globulin nephropathy by ETBE.

Regarding hyperplasia, the Public Review Draft seem to be confusing *simple* with *atypical* tubule hyperplasia. The term "hyperplasia" covers both simple and atypical forms of tubule alteration. Simple tubule hyperplasia is usually a subchronic outcome of sustained tubule injury leading to sustained compensatory regeneration, but it is not a preneoplastic lesion. On the other hand, atypical tubule hyperplasia (ATH) is a chronically developing precursor proliferative focus on the pathway to developing into an adenoma, which in turn can progress into carcinoma. The fact that tubule hyperplasia was not observed in the ETBE studies can mean either that simple tubule hyperplasia did not develop in, for example, the 13-week studies of ETBE, or that no foci of ATH developed in the chronic (ie. the 2-year) study, consistent with the fact that no renal

tubule tumors were observed. This is certainly in keeping with ETBE being considered as a relatively weak inducer of this male-specific nephropathy, compared to a strong inducer like decalin, which induces in excess of a 20% incidence of renal tubule tumors.

(d) Public Review Draft p.1-31, l.29-32: "*Overall, the histopathological sequence has numerous data gaps such as the lack of necrosis, cytotoxicity, and tubule hyperplasia…..Therefore, the number of histopathological steps observed was insufficient to fulfil the third criterion*". This is a gross exaggeration and suggests a degree of prejudice. Multiple studies reported that ETBE induced hyaline droplets, and one group observed that those droplets had angular shapes characteristic of accumulating α-2u-globulin (Cohen et al., 2011). Granular casts were observed in a 13-week study by 2 independent groups (indicative of cell exfoliation), and linear papillary mineralization by several groups. Increased tubule cell proliferation was reported to be sustained over a period of 1 to 13 weeks. Development of renal tubule hyperplasia is *not* a necessary histopathological step for identifying α-2u-globulin nephropathy. When it does occur it is an outcome of that histopathological sequence. Thus, the absence of tubule hyperplasia (or renal tubule tumors) does not rule out an α-2u-globulin MOA – it just means that ETBE is in a lower range of potency than for example, decalin.

Public Review Draft p.1-32, l.4, EPA reliance on Doi et al. (2007) to support non- α-2u-globulin MOA: The Public Review Draft theme of mounting an argument against the α-2u-globulin MOA in general continues in this section by giving undue consideration to the limited paper by Doi et al. (2007). Using this paper, the Public Review Draft concludes that "*this analysis suggests that another MOA could be operative for inducing kidney tumors in male rats*." On little evidence, the Public Review Draft has given undue weight to a few unlikely hypotheses in the face of a wealth of data supporting α-2u-globulin as a valid MOA of no significance to human hazard assessment. There seems to be a bewildering reluctance for the Public Review Draft to acknowledge that inducers of α-2u-globulin can do so with a range of potencies. Weaker inducers (like ETBE) induce a relatively limited hyaline droplet response, a low incidence of mature granular casts (if any) because of insufficient cell loss through tubule cell exfoliation, with either no or minimal to mild linear papillary mineralization, and sometimes no statistically significant increase in renal tubule tumors or atypical tubule hyperplasia. Strong inducers of α-2u-globulin cause a strong hyaline droplet accumulation response, with frequent granular casts, moderate to severe linear papillary mineralization, and in excess of a 20% increase in renal tubule tumors. In between there is a range of variability from weak to strong.

In view of the way it has been used in the Public Review Draft argument, the Doi et al. (2007) paper warrants some comment. The results reported in a comparison of four inducers of hyaline droplet nephropathy by Doi et al. (2007) do not match the long-standing experience of the expert, Dr. Hard, and other pathologists knowledgeable in rodent kidney. Doi et al. (2007) conducted their study under the premise that the validity of the α-2u-globulin hypothesis was questionable. They compared the α-2u-globulin-related male rat kidney responses of two strong inducers that produced renal tubule tumors (d-limonene and decalin) against two weak inducers

that did not produce a statistically significant increase in renal tubule tumors (propylene glycol mono-t-butyl ether and Stoddard solvent IIC). There are at least 20 chemicals in the NTP TDMS database that were recorded as inducing hyaline droplet accumulation/nephropathy in the male rat kidney, representing a range of potencies. The Doi et al. (2007) study is limited as it lacks any examples of chemicals of intermediate potency in producing the response. This paper also treated the hypotheses of Melnick (Melnick ,1992; Melnick and Kohn, 1999) as possible alternatives, ignoring the facts that these hypotheses are lacking in any supporting data, and exemplary inducers of the α-2u-globulin nephropathy response *in vivo* do not injure renal tubule cells *in vitro* (Borghoff et al,, 1993). This is fully expected in that the *in vivo* kidney toxicity MOA for α-2u-globulin requires protein produced in liver.

Public Review Draft p.I-25-26, Table 1-6: Essentially this table sets out the reasons for discrediting ETBE induction of α-2u-globulin nephropathy by listing the steps that are interpreted as not being present in male rats exposed to ETBE.

(a) – *single-cell necrosis*. As mentioned previously, there is an emerging realization by some workers in this field (including some NTP pathologists) that necrosis per se does not necessarily occur in the early stage of α-2u-globulin nephropathy, especially with inducers of low potency.

(b) – *exfoliation of epithelial cells into the tubule lumen*. The fact that granular casts formed is an indication that exfoliation of tubule cells did occur in at least one subchronic study. The 13-week time point of Suzuki et al. (2012) needs to be added to the Cohen et al. line of this list representing the positive mark under the Results column.

(e) – *foci of epithelial hyperplasia*. It is not appropriate to include this lesion as a necessary step in the pathological sequence for α-2u-globulin nephropathy. Tubule hyperplasia, implying atypical tubule hyperplasia here, is the precursor of renal tubule adenoma, that is, it is a preneoplastic lesion that will only develop if the α-2u-globulin nephropathy response is sufficiently strong, directly reflecting the potency of agent.

Also, it is definitely not appropriate to include the cell proliferation investigated in the Medinsky et al. (1999) study box (e) under foci of hyperplasia. Hyperplasia of the type associated with moderate to strong inducers of α-2u-globulin nephropathy does not occur as early as in 13-week studies. However, unlike hyperplasia, cell proliferation does represent a required step in the α-2u-globulin nephropathy sequence and should be placed in a box dedicated to cell proliferation alone. This error again reflects inexperience with the details and practical aspects of the α-2u-globulin syndrome.

3b. Noncancer toxicity at other sites (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.

• **Liver effects: Suggestive evidence**

LyondellBasell Comment:

The cancer descriptor for ETBE of "suggestive" and should be changed to "not likely to be carcinogenic to humans", or possibly "inadequate evidence of carcinogenicity" because an increase in almost entirely benign liver tumors was observed only in male rats at a 5000 ppm inhalation exposure. Importantly, that exposure level exceeded the onset of nonlinear toxicokinetics at approximately 1800-2000 ppm ETBE (see Charge Question 4a). OECD and EPA cancer bioassay and risk assessment guidance emphasize that tumor responses limited to such metabolically-saturating dose conditions are not quantitatively relevant to human hazard or risk characterization when human exposures are well below the onset of metabolic saturation. This is certainly the case for ETBE, as the highest exposures to ETBE are generally associated with gasoline station workers and tank-truck drivers, and are well below 1 ppm. Thus, the External Review Draft inappropriately limits the discussion of the selection of potential cancer hazard descriptors only to the categories of "likely" and "suggestive".

As noted in the Preamble to IRIS Toxicological Reviews and in accordance with the EPA Cancer Risk Assessment Guidelines (2005), the category of "not likely to be carcinogenic in humans" should be considered when "…convincing evidence that effects are not likely by a particular exposure route or below a defined dose". ETBE is clearly not carcinogenic by the oral route in that a high quality drinking water bioassay showed no evidence of carcinogenicity up to the limit of solubility of ETBE in water. Thus, a classification of "not likely" by the oral route of administration is entirely justified. As noted above, ETBE also was carcinogenic by inhalation only at an exposure exceeding metabolic saturation, and the dose identified as the onset of nonlinear ETBE nonlinear toxicokinetics was substantially higher than real-world human exposures. This observation offers strong evidence that ETBE is not likely to be carcinogenic by the inhalation route "below a defined dose" (the dose at onset on nonlinear toxicokinetics).

- **Developmental toxicity: Inadequate evidence**
- **Male and female reproductive toxicity: Inadequate evidence**

LyondellBasell Comment (Reproductive and Developmental Toxicity)

Overview

The External Review Draft of ETBE reproductive and developmental toxicity has been updated considerably since the August 2016 draft document. Specifically, the section on reproductive toxicity in the 2016 document has been separated into separate sections on male and female reproductive effects in the 2017 External Review Draft document. Within the male reproductive toxicity section, data and statistical analysis from the Weng et al. (2014) publication has been included despite an inappropriate statistical analysis by the study authors. In the developmental toxicity section of the 2017 version, the table (Table 1-13) describing maternal toxicity in the 2016 version has been eliminated with no description of maternal toxicity in the developmental toxicity section whatsoever. Maternal toxicity information is only included within the female reproductive toxicity section, thus preventing the reader from forming any opinion on the

interrelatedness of maternal toxicity and developmental outcomes. This is a significant backwards step in removing pertinent maternal toxicity data from the developmental toxicity section of the External Review Draft document. In addition, the External Review Draft version assigns toxicological significance to the finding of increased skeletal variations (rudimentary lumbar ribs) in a single study (not replicated in other similar studies) that does not reflect current interpretation of these effects in the toxicology literature (e.g. Kimmel CA and Wilson JG (1973). Skeletal deviation in rats: malformation or variation? Teratology. 8(3):309–315).

OVERALL CONCLUSION FOR REPRODUCTIVE TOXICITY

In conclusion, the availability of a one-generation reproductive toxicity study, a two-generation reproductive toxicity study, specialized studies investigating ovum fertilization and sub-acute hormone responses and histopathological evidence for reproductive organs from numerous repeated-exposure studies (both with oral and inhalation routes of exposure) allows toxicologists to reach a conclusion regarding the ability of ETBE to cause reproductive toxicity. The only evidence of an adverse effect is from one study (Weng et al., 2014) with multiple flaws. *The External Review Draft should acknowledge the depth and rigor of the available studies and state that ETBE, based on the available evidence, does not cause reproductive toxicity.*

OVERALL CONCLUSION FOR DEVELOPMENTAL TOXICITY

The External Review Draft conclusion regarding ETBE developmental toxicity hazard as "inadequate to draw conclusions" is not supported by a review of the available studies for this endpoint. Currently, there are two prenatal developmental toxicity studies in rats and one in rabbits, all testing ETBE up to 1000 mg/kg bw/day (the current limit dose level for developmental toxicity according to the EPA Test Guideline OPPTS 870.3700). In addition, there is one one-generation reproductive toxicity study and one two-generation reproductive toxicity study that can be used to assess developmental toxicity during the postnatal period in growing animals. *The amount of information from these five studies allows for a robust assessment of developmental toxicity hazards associated with ETBE exposure.*

In addition, the removal of the pertinent maternal toxicity data that should accompany the developmental outcome data from this section into the section for female reproductive toxicity is a tremendous step backward in allowing the reader to understand any possible relationship between the degree of maternal toxicity observed and the developmental outcomes observed. If the data had already been collated within the female reproductive toxicity section it would have been very simple to also present it in this section, allowing an analysis of maternal toxicity and developmental outcome to be a straightforward exercise. Removal of the maternal toxicity data from this section suggests that 1) maternal toxicity has no bearing on developmental outcome (known not to be true) and 2) the reader should ignore the maternal toxicity data while trying to understand the developmental toxicity data.

As per the US EPA developmental toxicity test guidelines (Section (2) Evaluation of study results, Subsection (iv) states "In the evaluation of study data, the litter should be considered the basic unit of analysis". In several places within Table 1-16 of the 2017 IRIS assessment for ETBE, this fundamental rule is ignored and data is presented on a per fetus basis. For example,

within the section on "Prenatal Survival" of Table 1-16, the entry for Aso et al., (2014) is preimplantation loss data is presented and analyzed on the total number of preimplantation losses without any correction for the differing number of litters available for analysis (range of 20 to 22 litters/group). While the "% preimplantation loss" value is divided by the total number of corpora lutea, the analysis is not conducted on a per litter basis as per the EPA Test Guidelines.

The concern for preimplantation loss within the developmental toxicity studies should be tempered by the knowledge that the dams were not treated during the majority of the preimplantation period. Implantation occurs in the Sprague Dawley rat between gestation days (GD) 5 and 6 and typically these studies wait to start treatment until GD 6 to insure no interference with implantation. In these studies, treatment started on GD 5, allowing for the slight possibility for interference with implantation. The lack of consistency for a possible effect on implantation suggests that the preimplantation data from Aso et al. (2104) is not of concern.

It is truly disheartening to observe the External Review Draft retreating on their previous assessment in the 2016 draft document on the interpretation of the increase in rudimentary lumbar ribs found in the Aso et al. (2014) publication. Examination of the data and discussion within publications by Chernoff et al. (1991) and Kimmel and Wilson (1973) reinforces the previous opinion described within the 2016 draft assessment. Chernoff et al. (1991) viewed these rudimentary lumbar ribs as transient and Kimmel and Wilson (1973) reported no correlation with the presence of rudimentary lumbar ribs with teratogenic potential unless the rib was much longer than usual. Consideration of data for risk assessment purposes includes replication of effects between studies of similar design (the effect was not noted in the other rat developmental toxicity study (Gaoua, 2004) and comparison of the effect to data contained within historical control (HC) databases (study incidence was well within the HC range). Considering this increase in rudimentary lumbar ribs as 1) treatment related and 2) useful for hazard identification purposes does not contribute to the overall more complete analysis and interpretation of the developmental toxicity database for ETBE in the External Review Draft.

Specific Comment: Male Reproductive Toxicity

The authors of the External Review Draft of ETBE male reproductive toxicity have introduced the testicular pathology reported in Medinsky et al. (1999) without a responsible critique of the study methodology, including the use of an incorrect fixative (10% neutral buffered formalin) for fixing the testes (Bouin's or Davidson's fixative should have been used as per the EPA Test Guidelines OPPTS 870.3650, 870.3550, 870.3800 and 890.1500).

In 1996, Bond et al., reported the results of a 90-day vapor inhalation study conducted with ETBE. An increased incidence of degeneration of the seminiferous epithelium was reported following exposure to either 1750 or 5000 ppm of ETBE (6 hrs/day, 5 days/week). All of the male rats in each group (11/group) were described as having degeneration of the testes with the number with "sloughed epithelium" being 7, 3, 3, and 7 for the 0, 500, 1750 and 5000 ppm groups, respectively (Table 25 of the report). In Table 26 of the report, the percent of seminiferous tubules with spermatocyte degeneration was 2, 2, 8, and 13% and lumenal debris was 2, 1, 3, and 1% for the 0, 500, 1750 and 5000 ppm groups, respectively. There were no

differences between the exposed groups and the control group in terms of percent of seminiferous tubules in specified developmental stages (Table 27). The standard deviation and ranges for the spermatocyte degeneration for the 0, 500, 1750 and 5000 ppm groups were 1, 2, 4, and 11 (standard deviations) and 1-4, 1-7, 3-16, and 0-39% (ranges of percent animals affected).

Testicular histopathology results are highly dependent on the methods used to obtain them and therefore whenever results such as these are reported, it is critical to examine the methods used. The subchronic study was designed to investigate the effects of repeated exposures of ETBE with emphasis on effects in the liver, kidney and nervous system. Consequently, all of the tissues collected for histopathological examination were preserved in 10% neutral buffered formalin (NBF) including the testes. The rat testes are covered by the tunica albuginea, a tough, fibrous capsule. The current EPA and OECD Test Guidelines for repeated-exposure and reproductive toxicity testing require the testes to be preserved in stronger fixatives such as Bouin's or Davidson's solutions since fixation in 10% neutral buffered formalin (NBF) results in fixation artifacts. The proposed reasons for this are that the NBF is not strong enough to penetrate the tough tunica albuginea while the stronger fixatives such as Bouin's easily penetrate this fibrous capsule. The fixation artifacts that are reported to occur with testes fixed with NBF are sloughing of the seminiferous epithelium and cells within the lumen (luminal debris), the exact findings reported in the ETBE study.

The ETBE study also reported a staging exercise for the testes of the male animals. Unfortunately, the study design used the testes from the male rats from the repeated exposure portion of the studies where the testes were fixed in NBF rather than the testes from the neurotoxicity subgroup where the testes were fixed with glutaraldehyde using the whole-body perfusion technique. The authors state that they performed testicular staging according to Hess (1990). However, Hess (1990) specifically refers to staging rat testes using perfusion-fixation (rather than immersion fixation) and embedding the testes in plastic (rather than paraffin as was done in the ETBE inhalation study). The authors did not find any effects on stages within the testes but used the incidence data gathered as part of the staging exercise for "percent of the seminiferous epithelium with spermatocyte degeneration" to describe an adverse effect on the testes.

It is important to recognize that the design of the 90-day repeated exposure study was to describe adverse effects in organs following repeated exposures. It was not designed to measure testicular function. Measures of the testicular function are often more sensitive measures of adverse effects. For example, animals with an increased incidence of "percent of the seminiferous epithelium with spermatocyte degeneration" would be expected to have decreased sperm counts in both the testes and the epididymis.

Given the unfortunate use of NBF as the fixative for the testes, the limited group size and lack of measures of testes function suggests that the Bond study should at best, be considered a screen for possible affects in the testes. It is important to note that there were no other indications of testicular effects (i.e. no effects on testes weight, no effects on any accessory sex organs and no gross findings). The lack of effect on the accessory sex organs suggests that the endocrine function of the testes was normal. The authors of the report appear to understand and appreciate

the limitations of this study design for describing effects in the testes with the following statement appearing in the Discussion Section of the report: "The impact of the response of the testes to ETBE would best be measured by sperm count, motility assessment and breeding studies" (Page 78 of the report).

It is not possible from the Bond study report to ascertain if the testicular staging exercise was added after the in-life portion of the study was completed or exactly how the "percent of the seminiferous epithelium with spermatocyte degeneration" data was collected and analyzed statistically. The wide range of values suggests that these effects may have been limited to a few animals in the mid- and high exposure groups.

The External Review Draft should realize that the testes pathology data from Medinsky et al. (1999) are suspect and use the data from the oral one and two-generation reproductive toxicity studies (Fujii, et al., 2010 and Gaoua, 2004) as the definitive evidence of possible ETBE effects on the testes, as both the Fujii and Gaoua studies exposed the male rats longer to ETBE and used the correct fixative for fixation of the testes.

The publication of Weng et al. (2014) is titled "Assessment of the reproductive toxicity of inhalation exposure to ethyl tertiary butyl ether in male mice with normal, low active and inactive ALDH2". This publication reports a series of experiments that utilized aldehyde dehydrogenase 2 gene (*Aldh2*) knockout mice (KO), *Aldh2* wild type mice (WT) and *Aldh2* heterogeneous mice (HT) with (theoretically) no, normal or low *Aldh2* gene (product) activity, respectively. These different *Aldh2* genotypes were exposed to ethyl tertiary butyl ether (ETBE) by inhalation (two experiments) in order to examine whether *Aldh2* genotype status affected the toxicity of ETBE in these mice.

There are two major problems with the Weng et al. (2014) publication. The first is the inappropriate use of a basic statistical analysis. Within the Methods Section of the paper it states:

"Multiple comparisons between the control and exposure groups were performed using one-way analysis of variance (ANOVA), following by Dunnett's post hoc test. Student's t test was applied for comparisons between averages of two samples. The minimal of level of significance chosen was $p < 0.05$."

However, Dunnett's test is performed by computing a Student's t-test for each treatment group where the t-test compares the treatment group to a single control group. Since each comparison has the same control in common, the procedure incorporates the dependencies between these comparisons. In particular, the t-statistics are all derived from the same estimate of the error variance which is obtained by pooling the sums of squares for error across all (treatment and control) groups. Dunnett's test addresses the important probability of Type I error issues found with multiple-comparison procedures.

The major flaw in the Weng et al. (2014) paper is that they (correctly) conduct a Student t-test procedure within the Dunnett's post-hoc test that incorporates the dependencies between these comparisons. However, this statistical test does not provide them with statistically significant differences (e.g. Table 1, Figures 2, 3, 4 and 5 within Weng et al., 2014) so they resort to a

second Student t-test procedure "*for comparisons between averages of two samples*" that does not correct for the dependencies between these comparisons nor does it use the correct error variance term for multiple comparisons. This violates a basic rule of statistical analysis when several treatment groups are being compared to a single control group.

Furthermore, close inspection of the relative organ weight values revealed minimal to no change between the treated and control group values. For example, the KO Control group (13-week study) relative epididymal weights were 0.26 ±0.01 (SD) versus 0.24 ±0.02 for the ETBE 1,750 ppm group yet this difference is stated to be statistically significant at $p \leq 0.05$ (Table 1) using a pair-wise Student's T-test. A general rule of thumb is that for group means to be different, they should be greater than 2 SD (standard deviations) apart. It is obvious from inspection of the data in Table 1, there is no difference between the respective control groups and their appropriate exposure groups, when the appropriate statistics are used.

The second major problem with the Weng et al. (2014) publication is that the data does not support the theoretical basis for the observed effects. The theoretical basis behind this experiment is that during the process of ETBE metabolism, acetaldehyde is formed within the cells of the animal. Normally, the acetaldehyde that is formed within the body (from any source) is rapidly further metabolized by enzymes (including *Aldh2*) to form acetic acid (acetate ion) which is then utilized as a carbon source within endogenous metabolic pathways. The authors of the Weng et al. (2014) paper postulated that the mice with the KO or HT genotype would exhibit exacerbated toxicity from ETBE due to the reduced clearance and metabolism of acetaldehyde within those mice.

The 9-week experimental results are interesting in that it appears for the sperm number measurements, the HT mice in the ETBE 500 ppm exposure group are more severely affected (over 50% reduction compared to the respective HT control group) when compared to the KO ETBE 500 ppm exposure group (only a 23% reduction compared to the KO control group). If the basic premise of the investigation is correct, the KO mice should be experiencing higher internal exposure to acetaldehyde (presumably the proximate testicular toxicant and genotoxicant) and therefore the KO group should experience more dramatic effects than the HT group. This finding is replicated when the ETBE 200 ppm groups are compared between the HT and KO groups as well.

The lack of a dose-response relationship for sperm numbers within the KO mice in the 13-week study and the increased sensitivity of the HT mice when compared to the KO mice in the 9-week study strongly suggest that the basic hypothesis of the authors is incorrect. The motile sperm analysis also reveals similar problems, starting with a remarkable lack of variance for this endpoint, although in this instance the authors switch from using a standard deviation measure to a standard error measurement (this allows the error bars to appear smaller on the bar graphs). Again, there is a lack of a dose-response relationship in the KO mice in the 13-week study and again, the HT mice appear to be more severely affected than the KO mice in the 9-week study; results that do not fit with the underlying hypothesis of the study authors.

The histopathological data presented in Figure 1 and Table 2 is suspect as well. Focal atrophy of the seminiferous tubules is a common lesion in laboratory rodents. It is imperative that a rigorous protocol be followed, including a predetermined number of fields within each slide, blinding of the pathologist to the treatment status and compilation of several independent pathologists prior to arriving at any conclusions regarding an increased incidence of seminiferous tubule atrophy. Since this lesion is typically widespread throughout the testes (when related to treatment), a much better measure or indicator of its presence is a change in testes weight rather than focal areas observed under a microscope. The author's seem to question their own methods and results describing the findings as "borderline" and "slight" in the comparison that was conducted. Finally, they only used a very small sample size for this part of the experiment and the results are highly questionable. Even the photomicrograph presented shows perfectly normal seminiferous tubules immediately adjacent to the affected tubules, something one would not expect with a treatment-related lesion. The lack of dose-response relationship within the KO treated groups suggests that there is no relationship between the external exposure metric (varying between 500 and 5000 ppm; 10-fold) and the incidence of the very slight to slight seminiferous tubule atrophy observed histologically.

The usefulness of measuring sperm parameters (sperm head count and sperm motility) with small sample sizes (n=5 mice/group in the thirteen week exposure and 5-9 mice/group in the nine-week exposure) is questionable. Typically, group sizes of twenty animals per group are required to have any reasonable statistical power in these types of studies. In addition, the 10-fold increase in external exposure level (from 500 ppm to 5000 ppm ETBE) did not result in differences in sperm head numbers in the KO mice in the 13-week study. The sperm head values are typically reported as total values and as values per gram of tissue. In this instance, the authors only provide the values per gram of tissue.

The method used to measure sperm motility utilized a machine that can be quite complicated to those not familiar with its use. Since the machine can also be used for clinical purposes, it has many settings that are typically not used in laboratory settings. Typically, laboratory animal labs that measure sperm motility use settings that determine motile and immotile sperm. In this instance, the laboratory further divided the motile sperm population into rapid, medium and slow motile sperm. This is an unreliable measure since individual motile sperm can speed up and slow down during the analysis and it is unclear how the machine decides to count those instances (it all depends on how you set the "windows" or parameters of the machine (not described within the paper). The numbers were then again analyzed statistically. When these types of analyses are conducted, it often appears that the investigators are dividing the data into "subsets" to reach statistically significant conclusions when the aggregate data do not support those findings.

Figures 2 and 3 report the same data since one is the inverse of the other (either a sperm is motile or not). Figure 4 reports the "rapid" sperm and the pattern described above (failure of a dose-response relationship in the KO mice exposed for 13 weeks and HT mice being more severely affected than the KO mice in the 9-week exposure) is replicated in all three of these graphs.

The collection of sperm data in mice is somewhat unusual as rats are typically used for studies that collect male reproductive endpoints. However, there are a few studies available that provide

a useful historical control database for mice in general and specifically for C57BL/6 WT mice. In a series of seven studies collected at WIL Research Labs (Now Charles River)(E. Sloter, personal communication) using CD-1 mice, the sperm concentration within the left testis and epididymis were 128.9 ±16.92 and 534.5 ±130.36, respectively (mean ±SD). The range for each of these measures was 118.1-153.7 and 285.9-645.4, respectively. While this strain is different from that used in the publication, it is important to note the sperm head numbers (124-127) within the ETBE KO treated groups (that are suggested to represent an effect) is almost exactly the mean value reported by WIL Research Labs for the control CD-1 mice. Either there is a very large strain difference between CD-1 mice and C57BL/6 mice or there is a difference between the laboratories in their ability to correctly measure this endpoint. It should be noted that WIL Research Labs has conducted literally thousands of studies using this machine to measure sperm counts over many years and with several different species.

A strain difference between the CD-1 mice and C57BL/6 mice is not unexpected as CD-1 mouse strain is a maximally outbred strain and therefore has greater reproductive success and higher fertility than an inbred strain. In comparison, C57BL/6 mouse strain is a maximally inbred strain and therefore is used for testing by NTP and also when a knockout mouse strain is desired. Maximally inbred mice strains have lower reproductive success and reduced fertility as a consequence of their inbred status. In this instance, the Weng et al. (2014) paper is reporting higher values of sperm counts in the C57BL/6 maximally inbred strain than is being reported by others in the maximally outbred CD-1 mouse strain (WIL Research Labs). This is the exact opposite of what would be expected given what is known about these two mice strains and their fertility.

In terms of sperm motility, the HC database for CD-1 mice from WIL Research Labs reports an 85.5% (SD ±3.86) from seven studies (as would be expected from a maximally outbred mouse strain). In two studies from Charles River Lab (CRL; J. Charlap, personal communication) using C57BL/6 WT mice, the two mean sperm motility values reported were 57.5% (±14.8) and 56.9% (±17.2), suggesting a lower fertility measurement in the maximally inbred strain. Taking these values at face value, this data suggests a confirmation of what is known regarding the fertility of these two mice strains based upon their breeding programs and genetic makeup. Both of these laboratories have extensive experience conducting these types of measurements.

The sperm motility values for the WT control group in the Weng publication reports higher sperm motility values for the C57BL/6 mice (approximately 70% for the 13-week study and 85% for the 9-week study). These are very high values for this maximally inbred strain of mouse. Furthermore, the ETBE exposure groups (WT, HT and KO mice) that are reported to be "affected" by ETBE exposure, have sperm motility values that are very high compared to the C57BL/6 control groups reported from CRL. This comparison with mice data from these other laboratories suggests that the sperm motility measurements from the Weng publication are suspect and should be considered unreliable.

In conclusion, the effects reported within the Weng et al. (2014) paper should be viewed with a high degree of skepticism as they do not support the primary premise put forth by the authors. The lack of a dose-response relationship in the KO mice in the 13-week study and the increased

sensitivity of the HT mice when compared to the KO mice in the 9-week study simply do not support the basic premise of the experiment. Without further investigation of the functional effects of removing the *Aldh2* gene on the physiology and metabolic capacity of the mice, it is impossible to understand what is occurring following the ETBE exposure. The effects described are very slight and are not what would be expected if internal levels of acetaldehyde were appreciable in these animals. Comparison of the sperm values reported by the authors with those from more established investigators suggest that the data reported for these endpoints is suspect and does not correlate with what is known about this strain of mouse. It is suggested that the data from the Weng et al. (2014) paper be removed from the draft assessment until the issues noted above can be resolved.

The increased estradiol findings from de Peyster et al. (2009) deserve discussion. These increases in the 1,200 and 1,800 mg/kg bw/day groups occurred at dose levels causing significant toxicity, including death. It is reasonable to assume that the "normal" physiology and metabolism of these animals would be markedly perturbed with significant hepatic and renal effects expected based upon findings from other studies. Since estradiol is normally produced in male rats of this age, ETBE-induced toxicity to the liver and kidney may reduce the "normal" metabolism, conjugation and clearance of this endogenous hormone within these animals. Hormone measurements noted at dose levels producing lethality and other signs of toxicity are rarely relevant to effects noted at much lower exposure concentrations.

Specific Comment: Female Reproductive Effects

The External Review Draft for ETBE female reproductive toxicity section contains information from the developmental toxicity studies as well as the reproductive toxicity studies. The assessment questions the strength of the data for maternal toxicity in these studies based upon 1) statements regarding rabbit body weights measures within the EPA developmental toxicity guidelines and 2) "inconsistent" results in maternal body weight changes during gestational exposures. Closer inspection of the data does not support this interpretation. While the weights of sexually mature rabbits plateau prior to use in these studies, reductions in maternal body weight gain (BWG) or weight loss are still indications of maternal toxicity in this species. This is difficult if not impossible to tell from the study data as it is currently presented within the draft assessment as body weight and body weight changes values are not presented alongside feed consumption values. Examination of the BWG data from the rabbit study exposure period demonstrates that the dams within the 1000 mg/kg bw/day ETBE group had a 54% decrease in BWG (0.12 kg) when compared to the BWG for the control group (0.26 kg), a clear indication of adverse effects within the dam. In addition, decreased feed consumption was noted in the dams from the 1,000 mg/kg bw/day group during the period of reduced BWG corroborating the adversity of the event (data not included within Table 1-15).

Regarding the differing effects on maternal BWG within the rat studies with gestational exposure, the External Review Draft contains a critical error. The External Review Draft fails to separate and recognize the difference between ETBE exposures that start on GD 5 versus those that start 10 weeks prior to mating and throughout mating and gestation. Looking at the two prenatal developmental toxicity studies in rats and considering only the BWG data during the

exposure period, there are consistent effects on maternal BWG at 1000 mg/kg bw/day of ETBE during the period of exposure (GD 5 through 19). In the case of the data from Aso, et al. (2014) publication (JPEC, 2008), there was an 8% decrement in maternal BWG during GD 5 – 19 when compared to the control value. In the other rat developmental toxicity study (Gaoua, 2004a), exposure to the same dose level during the same period of gestation caused a 17% decrement in maternal BWG, a statistically significant change when compared to the control value. While these two studies differ in magnitude of effect, this difference is not unusual for two studies conducted in different laboratories on different continents. The pattern of effect is however, the same.

The External Review Draft compared these maternal BWG data from these two prenatal developmental toxicity studies with the P0 dams from the one generation reproductive toxicity study published by Fujii et al. (2010) (JPEC 2008) and the P0 and F1 dams from the two generation reproductive toxicity study by Gaoua (2004b). The External Review Draft highlights variability and differences in maternal BWG responses in those two studies to the two prenatal developmental toxicity studies. However, the draft assessment failed to consider the differences in the onset of the dosing regimen between these differing types of studies. The dams in the reproductive toxicity studies had the advantage of treatment for ten weeks prior to mating, allowing for these animals to induce metabolic and elimination pathways to clear the chemical, well before becoming pregnant. The dams within the developmental toxicity studies received the ETBE exposure for the first time on GD 5 with no induction or acclimation period. This is an important point as developmental toxicity can occur following a single exposure during a sensitive period of gestation/development and the prior induction of metabolic elimination pathways within the dam could be important to both the maternal and conceptus response.

The External Review Draft is remiss in not trying to corroborate the studies that did report decrements in BWG during gestation with decreases in feed consumption during those periods. Since BWG and feed consumption are directly related, examination of the feed consumption data would allow for a better understanding of the cause-and-effect for the decreases in BWG.

Finally, the External Review Draft includes a lot of information on ovarian weights without any discussion on normal factors that can significantly affect the weight of the organ. In order to correctly understand ovarian weights and potential differences between groups, it is critical to understand what stage of the estrus cycle the female rat is in at the time of necropsy. Indeed, this is a requirement contained within the US EPA test Guidelines OPPTS 870.3800 and 890.1450. Without the information regarding estrus cycle stage, the tables describing ovarian weights are impossible to interpret.

3c. Oral reference dose for noncancer outcomes. Section 2.1 presents an oral reference dose of 5x10–1 mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

<u>LyondellBasell Comment:</u>

See detailed comments to Charge Question 3a. Selection of urothelial hyperplasia in male rats is not appropriate in that this lesion is an integral component of CPN, and an endpoint not regarded as clinically relevant to human.

In addition, a high-quality chronic drinking water bioassay conducted at the limit of water solubility of ETBE, and the data from this study should thus be preferred for selection of endpoint(s) for non-cancer outcomes. Use of the oral bioassay results is entirely consistent with recommendations in the EPA Cancer Risk Assessment Guidelines, which emphasize that studies can be conducted to "…clarify and describe the variability to be expected because of differences in ….route of exposure." Since high quality chronic studies are available in rats for both oral and inhalation routes, the potential for such inter-route variability on study outcomes is fully characterized by the apical bioassays by both routes of administration. Importantly, PBPK modeling of the comparative oral versus inhalation internal doses indicates that inhalation exposures results in higher internal doses than can be obtained in drinking water exposures conducted at the limit solubility. Thus, it is not appropriate to use PBPK modeling to extrapolate oral toxicity from an inhalation study when a high-quality oral study is available.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 9 x 100 mg/m3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

LyondellBasell Comment:

See detailed comments to Charge Question 3a. Selection of urothelial hyperplasia in male rats is not appropriate in that this lesion is an integral component of CPN, and endpoint not regarded as clinically relevant to human.

4. Cancer

4a. Cancer modes-of-action in the liver. **As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR*α*, PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.**

LyondellBasell Comment

This charge question is too limited for addressing whether consideration of only the stated receptor-mediated pathways are adequate for assessing if the ETBE-induced liver tumors in male rats are relevant to human hazard identification. Several comprehensive reviews have emphasized that rodent carcinogenicity responses restricted to high test doses may have questionable human health hazard and risk relevance (Foran et al., 1997; Slikker et al., 2004a,b; Barton et al., 2006; Carmichael et al., 2006; Doe et al., 2006). High-dose specific saturation of

metabolic processes, including toxicokinetics, may result in transition to modes of action unique to those high dose levels and/or represent non-dose proportional augmentation of modes of action operating at lower animal doses. If the onset on nonlinear toxicokinetics is well separated from human exposures, effects noted above the onset nonlinear toxicokinetics are not regarded as quantitatively relevant to humans (Saghir et al. 2012; Bus, 2017; OECD, 2012). As noted in comment on Charge Question 1, worst-case occupational breathing zone ETBE exposures have been determined for gas station workers (pumping gas) and gas tank truck drivers, and those exposures (geometric mean exposures of 0.08 and 0.04 ppm, respectively) are substantially lower than the inhalation exposure estimated for onset of nonlinear toxicokinetics in rats. Also as noted in comment to Charge Question 1, the likelihood of ETBE exposure via drinking water contamination from LUST is very low both in possible numbers of affected sites as well as reported ETBE concentrations (in the low ppb range).

Consistent with the expert reviews noted above, the EPA Cancer Risk Assessment Guidelines (2005) have emphasized that changes in toxicokinetics with increasing dose may result "…*in important differences between high and low dose levels in disposition of the agent or generation of its active forms. These studies play an important role in providing a rationale for dose selection in carcinogenicity studies.*" Similarly OECD (2012) Guidance on Conduct and Design of Carcinogenicity Assays emphasizes that *"Available toxicokinetic data (ADME) should always be taken into account when selecting dose levels for a chronic toxicity or carcinogenicity study. . . Many toxicokinetic processes influencing absorption, distribution, elimination and metabolic activation or detoxication may become saturated at higher doses, resulting in systemic exposures to parent compound or metabolites that would not be expected in the real life human exposures for which risk assessments are needed. . . There is broad acceptance that the top dose should ideally provide some signs of toxicity such as slight depression of body weight gain (not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours. Excessive toxicity at the top dose level (or any other dose level) may compromise the usefulness of the study and/or quality of data generated. Criteria that have evolved for the selection of an adequate top dose level include:* ***in particular*** [emphasis added] *toxicokinetics; saturation of absorption; results of previous repeated dose toxicity studies.*

OECD guidance (2012) for dose selection for chronic studies explicitly expands on the conclusion that toxicity limited to doses exceeding metabolic saturation is not quantitatively relevant to human hazard or risk by stating:

> "Although top dose selection based on identification of inflection points in toxicokinetic nonlinearity may result in study designs *that fail to identify traditional target organ or body weight effects* [emphasis added], it must be appreciated that metabolic saturation in fact represents an equivalent indicator of biological stress [to the MTD]. In this case, the stress is evidenced by appearance of non-linear toxicokinetics rather than appearance of histological damage, adverse changes in clinical chemistry, haematology parameters or decrease in body weight gain."

Bus (2017) has recently commented on the pragmatic inferences associated with implications of this guidance as:

> "It can be argued that if *post-hoc* ADME experimentation shows the hazard response of concern to occur only at doses exceeding a KMD [the Kinetically-Derived Maximum Dose, defined as the dose reflecting onset of nonlinear toxicokinetics], these responses should then logically be regarded as quantitatively non-relevant to human risk on KMD considerations alone. Put another way, if the bioassay had been originally implemented with current guidance recommending *a priori* KMD considerations, it would have been completely acceptable to have limited the top dose to a KMD with the result that the MTD-only identified hazard(s) would not have been found."

Thus, when non-dose-proportionate toxicokinetics are considered as a key event of the overall MOA of a chemical response, the high-dose specific liver tumorigenicity of ETBE can be concluded as non-quantitatively relevant to humans.

As noted in earlier LyondellBasell comments (2016) on the Public Review draft, ETBE metabolism in rats is saturated at inhalation exposures ≥ 1750 ppm. ETBE saturated metabolism and ensuing nonlinear toxicokinetics are readily evidenced by an increase in the percentage of exhaled volatile organics at 1750 and 5000 ppm exposures that was associated with a concurrent decrease in the percent of radioactivity excreted into urine at these same exposures. Borghoff and Asgharian (1996) reported that the percentages of the absorbed dose eliminated as exhaled volatiles recovered in exhaled air or as radioactivity recovered in urine of male and female F344 rats up to 48 hr following single nose-only exposures to ^{14}C-ETBE was as follows:

	Mean Percent Eliminated (4 rats/group)		
Elimination Pathway	500 ppm	1750 ppm	5000 ppm
Male Exhaled Volatiles	28	41	51
Male Urine	59	53	45
Female Exhaled Volatiles	32	52	64
Female Urine	59	41	30

Using ETBE PBPK model (Borghoff et al., 2016), ETBE metabolic saturation was estimated to occur between 1800 to 2000 ppm, in excellent agreement with the experimental data of Borghoff and Asgharian estimating saturation at ≥ 1750 ppm (1996). Importantly, the PBPK model also estimated a total ETBE absorbed dose of 842-860 mg/kg/day at the 5000 ppm 6 hr/day inhalation exposure. The approximate 850 mg/kg bw/day inhalation dose, the only exposure resulting in liver tumors, is substantially higher than the top daily doses of 542 (males) and 560 (females) mg/kg/day in drinking water containing ETBE at the limit of water solubility. These results indicate that the inhalation tumorigenic dose cannot be plausibly attained in drinking water exposures, strongly supporting the conclusion that it is not toxicologically justified to estimate potential oral cancer risks from model extrapolation of the higher-dose inhalation tumor response.

In response to public comment (External Review Draft, Appendix D), EPA observes that "Metabolic saturation would only lead to a disproportionate increase in toxicity if it is the parent chemical, ETBE in this case, that is the proximate agent." This conclusion is incorrect. Evidence of toxicokinetic saturation based only on plasma parent compound analyses nonetheless indicates that the overall toxicokinetic profile has been profoundly perturbed, setting into motion altered tissue distribution and kinetics of both parent compound and metabolites. Such alterations of both parent and metabolite tissue dosimetry can initiate non-identified and novel high-dose specific MOAs, and/or dose-disproportionately amplify subthreshold MOAs functioning at lower doses causing only high-dose toxicity expression. For example, given the structure of ETBE, it is not implausible that direct binding of ETBE to α-2u-globulin in part accounts for the kidney toxicity of ETBE. Thus, high-dose specific elevation of plasma ETBE could non-dose disproportionately elevate the male rat kidney toxicity response.

The limitation of the hepatic tumor response to the toxicokinetically-saturated and high-exposure only dose indicates that the liver tumor response is not quantitatively relevant to humans based on the observation that real-world human exposures to ETBE are substantially less than the likely onset of the inflection point of nonlinear toxicokinetic behavior at approximately 1750-2000 ppm ETBE. Humans are not exposed to these extremely high concentrations of ETBE at which metabolism is saturated. The ACGIH has set an occupational Threshold Limit Value (TLV®) of 25 ppm ETBE (ACGIH, 2015), however, actual breathing zone exposures of Japanese gasoline station workers and fuel tank drivers have been reported as less than 0.1 ppm (Eitaki et al., 2011). In addition, exposures to ETBE via drinking water are likely to be very low and below those associated with occupational scenarios (see Charge Question 1).

<u>Consideration of Alternative MOA: ETBE is not Genotoxic</u>

The conclusion that ETBE tumors are not quantitatively relevant to humans is also consistent with the evidence that ETBE is not genotoxic. The External Review Draft has inappropriately concluded that overall evidence for ETBE genotoxicity is "inconclusive" (Table 1-13). In earlier LyondellBasell comment (2016) on the Public Draft version of the ETBE assessment, the following detailed comment was offered regarding the negative genotoxicity of ETBE:

> "The genotoxicity of ETBE has been extensively investigated across a variety of test systems. This includes the standard battery of tests used in regulatory genetic toxicology such as the bacterial gene mutation test (Ames test), an *in vitro* test for cytogenetic damage in mammalian cell cultures, a forward gene mutation test in Chinese hamster ovary cells and multiple tests for cytogenetic damage in mammalian bone marrow cells. The above studies were conducted with applicable testing standards/guidelines available at the time. The results from these studies were uniformly negative. The above battery of tests is considered adequate for the global registration of a pesticide or a human pharmaceutical, but yet, it was surprising that the agency's draft assessment concluded that only "[l]imited studies have been conducted to understand the genotoxic potential of ETBE" (page B-65, line2). In particular the draft assessment states that "[k]ey studies in terms of chromosomal aberrations, DNA adducts etc. are missing" (page B-65, lines 11-12). It is not entirely clear as to why the draft assessment did not consider the available

cytogenetic tests (one *in vitro* and several *in vivo* bone marrow micronucleus tests) as constituting tests for chromosomal damage. Furthermore, it is not a common practice in the field to consider DNA adducts determination as a "key" investigation when all apical genotoxicity endpoint are negative.

Additional evidence supporting the lack of genotoxicity for ETBE comes from the studies conducted on tertiary-butyl alcohol (TBA), an *in vivo* metabolite of ETBE. TBA has been extensively investigated for genotoxicity (see 2016 EPA IRIS Draft Assessment on TBA) and the weight of evidence suggests that TBA is devoid of genotoxic potential (McGregor et al., 2010).

The genetic toxicology database on ETBE is summarized in the following table:

Summary of Genetic Toxicology Studies

No.	Study Type	Endpoint Investigated	Result
1	Bacterial Reverse Mutation Assay	Gene Mutation	Non-genotoxic
2	*In vitro* CHO/HPRT assay	Gene Mutation	Non-genotoxic
3	*In vitro* chromosomal aberration assay in CHO cells	Clastogenicity	Non-genotoxic
4	*In vivo* assays for cytogenetic damage (Total of 7 assays)	Clastogenicity and aneugenicity	Non-genotoxic

It is interesting to note that the External Review Draft states that the genotoxicity database on ETBE is inadequate for " (a) the coverage of the studies across the genotoxicity tests needed for proper interpretation of the weight of evidence of the data; (b) the quality of the available data" (page B-65, Section B.2.2.4). However, as presented above, ETBE was evaluated for all apical endpoints of regulatory interest (i.e., gene mutations, clastogenicity, and aneugenicity) and using quality standards such as protocols compliant with guidelines existing at the time and following GLP standards. Thus, neither the coverage of the endpoints nor the quality of the data should be of concern. The External Review Draft also states that key studies in terms of chromosomal aberrations are missing. This statement does not accurately reflect the wealth of information available from multiple in vivo micronucleus tests, which are essentially considered by the genetic toxicology community as being equivalent to tests for chromosomal aberrations.

Thus, the available genotoxicity database on ETBE is robust and sufficient to draw a firm conclusion that ETBE is not likely to be an *in vivo* genotoxicant and as such the liver tumors observed in the inhalation studies are not likely mediated by a mutagenic MOA."

4b. Cancer characterization. **As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes**

that there is *suggestive evidence of carcinogenic potential* for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Does the classification give appropriate weight to the results from initiation–promotion studies? Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.

LyondellBasell Comment:

Consideration of "promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure" is not appropriate for characterizing the cancer hazard or risk of ETBE. Conduct of initiation-promotion studies are stated by the EPA Cancer Risk Assessment Guidelines (2005) as intermediate-term dosing studies that "*screen for carcinogenic and preneoplastic effects* [emphasis added]." Thus, when high-quality apical bioassays of carcinogenic potential are available, as is the case for ETBE by both oral and inhalation routes in rats, the value of such screening studies is largely restricted to informing the MOA and are not appropriate for inferring health hazards or risks in the presence of chemical-specific apical cancer bioassays.

The External Review Draft response to public comment (Appendix D) inappropriately and simplistically states that because tumors and preneoplastic were observed at gavage doses (likely resulting in differential toxicokinetics relative to drinking water) less than 1000 mg/kg/day in the initiation-promotion assay, ETBE has the potential to induce tumorigenic responses below 1000 mg/kg/day by MOAs that might be operative at lower oral doses. However, these assays were conducted with co-treatment with potent genotoxic initiating agents, and thus the resulting tumorigenic outcomes do not inform ETBE hazard or risk characterization. This is particularly so since apical oral and inhalation bioassays of ETBE identified only liver tumors at a metabolically-saturating inhalation exposure dose, and none after drinking water exposures conducted up to the limit of water solubility. These apical assays clearly serve as the primary evidence of ETBE carcinogenicity potential.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states:

"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for quantitative analysis, and whether the Saito et al. (2013) study is suitable for this purpose.

LyondellBasell Comment:

The above quote from the EPA Cancer Risk Assessment Guidelines (2005) clearly indicates that when a determination of "suggestive" evidence has been made, derivation of quantitative tumor analyses as reflected by calculation of cancer slope factors "may only be useful for some purposes" such as "providing a sense of magnitude and uncertainty of potential risks, or setting research priorities." In contrast to this guidance, the External Review Draft simply states that the inhalation liver tumor data "were suitable for quantitative analyses." This re-phrasing of the guidance recommendation leaves risk assessors with the inference that the derived slope factors are indeed acceptable for actual use as quantitative estimates of human cancer risks. Leaving such an inference is clearly misleading given the clearly stated EPA Cancer Risk Assessment Guidance.

The recent report of Bogen and Heilman (2015) affirms the comparative-only value of the oral ETBE oral slope factor. Thus the oral slope factor of 0.001 per mg/kg-day ranks in the lower 10% of slope factor potencies of 81 oral slope factor values listed by EPA (2013) IRIS. Importantly, however, as noted in comments to Charge Question 3b (liver), the External Review Draft should have considered a cancer classification of "not likely to be a human carcinogen" on grounds of the negative drinking water study and a tumor response from inhalation exposures that was restricted to doses above metabolic saturation.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10–3 per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

<u>LyondellBasell Comment:</u>

As noted in comment on Charge Question 3a (liver), ETBE was not carcinogenic in a drinking water study up to the limit of water solubility, and thus ETBE cannot reasonably be regarded as a carcinogen by the oral route and thus these negative data are not appropriate for derivation of an oral cancer slope factor. Extrapolation to oral liver cancer from inhalation-only liver tumors seen only at the 5000 ppm inhalation exposure is clearly not justified in that the estimated systemic dose of ETBE at this exposure level (approximately 850 mg/kg bw/day) exceeded those attainable at the maximum drinking water dose (542-560 mg/kg bw/day), and occurred only at a metabolically-saturating dose.

The External Review Draft has selected the rate of metabolism as the appropriate dose metric for estimating the ETBE liver neoplastic response. However, the External Review Draft uses the inhalation exposure concentration (mg/m^3) from the 6 hr inhalation exposure and adjusts the dose for a 24 hr/7 days a week to make the dose equivalent for the internal BMC and BMCL. The adjustment to the $BMDL_{10\%}$ results in a lower rate of metabolism correlated with the 10% BMR for neoplastic response, and therefore incorrectly lowers the resulting estimate of the POD and $BMDL_{HED}$ for drinking water. It would be more appropriate to adjust based on the rate of dose (mg/kg/h) than the total dose for the 24 hr versus 6 hr inhalation exposure in order to keep the rate of metabolism associated with a given response the same among exposure scenarios.

We agree with the External Review Draft's conclusion that an adjustment factor for the inhalation and oral unit risks should not be applied based on concern that individuals deficient in ALDH2 might be potentially more sensitive. The available information on acetaldehyde's potential role in ETBE-induced liver tumors is inadequate for risk assessment decisions. In addition, occupational exposures to ETBE in gasoline station workers and fuel tanker drivers are generally less than 0.1 ppm, and equate to acetaldehyde generation approximately equivalent to consumption of less than one drop of beer (see Charge Question 5 comment). This dose context to commonly consumed alcoholic beverage suggests it is highly implausible that ETBE exposures would result in adverse outcomes even in ALDH2 deficient susceptible subpopulations.

4e. Inhalation unit risk for cancer. Section 2.4 presents an inhalation unit risk of 8 x 10–5 per mg/m3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

LyondellBasell Comment:

Derivation of an inhalation unit risk is highly questionable given that liver tumors were restricted only to male rats, consisted almost entirely of benign tumors (9/10 total tumors observed), and were entirely restricted to the top inhalation 20,900 mg/m3 exposure that was distinctly the above the onset of ETBE nonlinear toxicokinetics. All three of the observations combine to support the conclusion that ETBE-induced rat liver tumors represented less than "suggestive" evidence of animal carcinogenicity and more consistent with a classification of "not likely" to be a human carcinogen below an unrealistically high human dose.

5. Question on Susceptible Populations and Lifestages

Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

LyondellBasell Comment:

The hypothesis that individuals with diminished ALDH2 activity represent a reasonable potential susceptible population is not biologically plausible based on that observation that the total dose of acetaldehyde resulting from metabolism of reasonable human ETBE exposures is minuscule. Assuming 100% conversion of absorbed ETBE to acetaldehyde, the acetaldehyde metabolically delivered from 8 hr exposures to 0.1 ppm ETBE observed in gas station workers or gas tank truck drivers is equivalent to acetaldehyde formed from consumption of 0.003 ml of beer (or 16% of one drop of beer assuming 50 µl/drop. (LyondellBasell, 2016). If such extremely low ethanol exposures presented a true risk of liver cancer, even in ALDH2 susceptible populations, signals of ethanol-induced liver cancer would be readily detectable in the human population.

This substantive dosimetry limitation to the plausibility of possible susceptibility of ALDH2 deficient individuals was identified in earlier LyondellBasell comments (2016) on the Public Review draft of the ETBE assessment, but is not addressed in the current External Review Draft (including response to public comments in Appendix D).

Detailed analysis of the ALDH2-deficient hypothesis form LyondellBasell (2015)

The discussion of toxicokinetics on p.1-49 also states that the ETBE metabolite, acetaldehyde, "…has been suggested to be a contributor to ethanol-related liver toxicity and cancer", citing Setshedi et al., 2010). However, this statement does not acknowledge the important dose-context caveat offered for ethanol liver cancer by Setshedi et al. (2010): "The data strongly support the concept that *chronic and excessive alcohol consumption* [emphasis added] contributes to and probably promotes progressive liver disease and HCC." Thus, in order to better understand the potential for acetaldehyde produced from ETBE metabolism to be a plausible biological mechanism of ETBE-induced liver cancer, it is constructive, if not necessary, to examine the context of acetaldehyde production resulting from ETBE exposures relative to acetaldehyde formation resulting from consumption of beverage alcohol. *Such a contextual dose comparison reveals that it is highly implausible that acetaldehyde derived from ETBE exposures is a realistic contributor to liver cancer in humans.*

Nihlen et al. (1998) have reported a high quality inhalation toxicokinetic study of ETBE in 8 male volunteers exposed for 2 hr to 0, 5, 25 and 50 ppm ETBE under conditions of light exercise. Under these exposure conditions, the overall respiratory uptake of inhaled ETBE was 32-34%, and 45-50% of the total absorbed dose was exhaled as ETBE. The Nihlen et al. (1998) dataset provides the basis to estimate potential acetaldehyde formation from a relatively high 2 hr 50 ppm ETBE exposure (e.g., the 8 hr Threshold Limit Value (TLV) for occupational exposure to ETBE = 25 ppm) relative to that from consumption of alcoholic beverages. Based on the total inhaled ETBE and associated respiratory uptake data reported in Nihlen et al. (1998), McGregor (2007) calculated a total ETBE systemic absorbed dose of 1.8 mg/kg bw for a 2 hr 50 ppm ETBE exposure, which translates to a total dose of 126 mg ETBE (1.23 mmoles) for a 70 kg volunteer. A 1.23 mmole ETBE dose would generate the same mmole dose of acetaldehyde assuming 100% metabolism of ETBE. Also assuming that consumed ethanol is 100% metabolized to acetaldehyde, 1.23 mmoles of total consumed ethanol equals 56.8 mg ethanol. One can of beer (12 oz, 355 ml) containing 5% alcohol delivers a total ethanol dose of approximately 14 g or 14,000 mg ethanol (CDC, 2016). Thus, consumption of 1 can of beer delivers an acetaldehyde dose approximately equivalent to that resulting from a 2 hr exposure to 12,300 ppm ETBE. More reasonably, consumption of 1 can of beer is equivalent to a 2 hr 24,600 ppm ETBE exposure in that Nihlen et al. (1998) determined that 45-50% of the absorbed ETBE dose is exhaled as parent compound, thus allowing only 50% of absorbed dose available for metabolism to acetaldehyde. However, because such high ETBE exposure equivalents are also likely to exhibit metabolic saturation in humans, ETBE inhalation exposures might never be able to deliver acetaldehyde systemic doses equivalent to consumption of a single alcoholic beverage.

Conversely, a 2 hr 50 ppm ETBE exposure is equivalent to consumption of approximately 0.004 of a can of beer (1.4 ml). It is important to note, however, that actual breathing zone exposures in

Japanese gasoline station pump workers and fuel tanker drivers have been shown as generally less than 0.1 ppm ETBE (Eitaki et al., 2011). Assuming 100% conversion of absorbed ETBE to acetaldehyde, the acetaldehyde metabolically delivered from 0.1 ppm ETBE exposure is equivalent to acetaldehyde formed from consumption of 0.003 ml of beer (or 16% of one drop of beer assuming 50 µl/drop).

These dose comparisons indicate it is highly implausible that systemic acetaldehyde doses resulting from ETBE occupational and general population exposures contribute as a mode of action to increased human liver cancer risk, and particularly when alcohol-induced liver cancer risk has been associated only with chronic abuse-level alcohol consumption. If such ETBE exposures were a true contributor to liver cancer risk, the very much larger systemic dose of acetaldehyde delivered from even extremely modest consumption of alcoholic beverages (e.g., volumes substantially less than even a single beer) should have readily detectable liver cancer signals in the general population. As stated in Setshedi et al. (2010), high volume alcohol abuse is required to detect an increase in hepatocellular carcinomas.

6. Question on the Executive Summary

The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

<u>LyondellBasell Comment:</u>

As noted in comments across all the Charge Questions, selection of kidney toxicity for derivation of the oral and inhalation RfD and RfC, and use of male rat liver tumors for derivation of an inhalation cancer slope factor, is highly uncertain and do not approach a high level of confidence. Likewise, the highly speculative conclusion that acetaldehyde metabolized from ETBE presents a potential risk to susceptible humans with ALDH2 deficiency is unwarranted when considered in context to general population ethanol consumption.

<u>Additional Miscellaneous Comments Addressing EPA Responses to Public Comment (Appendix D)</u>

Comment [LyondellBasell]: Numerous questions and concerns related to specific aspects of PBPK modeling described by Salazar et al. (2015) and as implemented in public comment draft were received.

EPA Response: EPA has adopted the newly available Borghoff et al. 2016 model, as summarized in Appendix B.1.5−B.1.6 and U.S. EPA (2017).

LyondellBasell Response (July 2017): EPA should clarify throughout the document the final model version and parameter values used, especially for route to route extrapolation purposes for calculating an oral slope factor for the cancer study.

LyondellBasell Response (July 2017):

Supplementary – Appendix B, B.1 Toxicokinetics

General comments concerning the data sets used for PBPK modeling

- Page B-4, lines 23-28. It is stated that "Urine samples were also analyzed for *tert*-butanol metabolites HBA and MPD, and $^{14}CO_2$ was measured in exhaled air." It should be clarified that ^{14}C-ETBE-derived radioactivity was exhaled as volatile organics, in which ETBE and TBA was quantitated, and exhaled as $^{14}CO_2$ measured. The $^{14}CO_2$ exhaled was < 3% of the total absorbed dose.

- Pages B-5 and throughout the toxicokinetics section the studies cited by Sun and Beskitt (1995a,b) and Borghoff (1996) are discussed. Now that EPA has included the definitive ETBE inhalation elimination study (Borghoff and Asgharian, 1996), it is not clear what the value of including these three pilot studies where the data only represents an n=2 and in the case of the Sun and Beskitt studies, the n=2 is one male and one female rodent value.

- Throughout this section, the inhalation exposure concentrations were converted from ppm to mg/m3 units. This conversion is acceptable but should be referenced in a footnote. On page B-4, the exposure concentrations for the Borghoff and Asgharian (1996) ETBE study are expressed as ppm. It is important to the reader that there is consistency with how these exposure concentrations are expressed

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