NOTICE: **Do Not Cite or Quote.** Preliminary Comments submitted by individual members are for review and to assist meeting deliberations by the CAAC-ETBE/tBA committee. These comments do not reflect consensus advice or recommendations, and have not been reviewed or approved by the chartered SAB and do not represent EPA policy.

Dr. James Bruckner Preliminary Comments 8/9/17

Ethyl Tertiary Butyl Ether (ETBE) Charge Questions

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

The scientific literature search and screening strategy were clearly described on pp. xxvii – xxxiv of the IRIS document. This was a very thorough and effective approach to identify the most pertinent publications. Tables summarizing what appears to be the more important health effects information were constructed as recommended by the NRC (2011). This allows readers to survey and compare results/data available on particular health effects and species. I found the detailed evaluations of the 30 key study design and quality considerations useful.

3a. Noncancer Kidney Toxicity

The document's authors contend in lines 22-27 of p. 1-6 that individual components of chronic progressive nephropathy (CPN), a species-specific syndrome of aging rats, could occur upon ETBE exposure in humans. Furthermore, it is asserted that exacerbation of one or more of these manifestations of cell injury is relevant to the human kidney.

Response: This is speculation, as there are no human data for ETBE or TBA, nor a MOA for CPN in rats.

The document's authors conclude there was only evidence for "weak induction" of α2u by ETBE, as measurements of α2u in hyaline droplets were not made in enough dose groups for enough samples within groups.

Response: Medinsky et al. (1999) did not include data on individual kidney samples, but concluded: (1) there was an ETBE concentration-dependent increase in accumulation of protein droplets immunoreactive for α2u; (2) the α2u-containing droplet accumulation was directly related to the treatment-related increases in nephropathy and cell labeling index. Borghoff et al. (2001) also quantified α2u in each kidney sample from male and female rats that inhaled TBA for 10 days, and correlated α2u content with kidney cell proliferation.

The IRIS document's authors conclude that available data are insufficient to conclude that the α2u process is operative for ETBE

Response: I recommend the more comprehensive/extensive criteria of IARC (1999) be utilized for assessing the relevance of α2u as a MOA for ETBE's renal effects. I believe the ETBE data satisfy these criteria.

Table 1. Criteria for an agent causing kidney tumors through an α2u-globulin-associated response in male rats.

- Lack of genotoxic activity (agent and/or metabolite) based on an overall valuation of in vitro and in vivo data
- Male rat specificity for nephropathy and renal tumorigenicity
- Induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory
- Identification of the protein accumulating in tubule cells as α2u-globulin
- Reversible binding of the chemical or metabolite to a α2u-globulin
- Induction of sustained increased cell proliferation in the renal cortex
- Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets, α2u-globulin accumulation, cell proliferation)

Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis

Edited by C.C. Capen, E. Dybing, J.M. Rice & J.D. Wilbourn

IARC Scientific Publications No. 147

Lyon, France (1999)

3b. Noncancer Toxicity at Other Sites

Liver: It is pointed out that Kakehashi et al. (2013) presented findings indicating ETBE activates PPARα-, CAR- and PXR-mediated events in male F344 rat liver, and the chemical may induce liver tumors via such MOAs. The EPA document's authors ascertain that these data are inadequate to conclude that ETBE induces liver tumors via these MOAs.

Response: Alternatively, it might be stated that this is the first effort to address ETBE's MOA and that the findings, while advancing the state-of-knowledge, are inadequate at present to establish MOAs.

Increased weight, centrilobular hypertrophy and focal basophilic lesions have frequently been observed in the liver of rats in the subchronic and chronic ETBE studies. The document's authors opined that the liver weight increases were of uncertain toxicological significance. Kakehashi et al. (2013) measured increases in total hepatic P450, as well as elevations in mRNA expression of several CYPs in male F344 rats, but

these changes occurred only in response to a quite high oral dose of ETBE (1,000 mg/kg). Alternatively, the basophilic foci in liver could be of toxicologic consequence, particularly if they are progenitors of adenomas.

Developmental Toxicity: The document's authors conclude in lines 24 and 25 of page 1 – 100 that "available evidence is considered inadequate to draw conclusions regarding the developmental toxicity of ETBE".

Response: Does this statement means that available data are inconclusive, or that they are inadequate in scope to draw conclusions? Are additional studies/experiments of a particular type warranted to provide adequate data?

Male and Female Reproductive Toxicity: The document's authors conclude in lines 20-22 (page 1-76) and in lines 14-16 (page 1-88) that "available evidence is considered inadequate to draw conclusions regarding the male/female reproductive toxicity of ETBE, and male/female reproductive effects are not carried forward as a hazard".

Response: Again this conclusion leaves me wishing for a more definitive statement about ETBE's reproductive toxicity potential or lack thereof in laboratory animals and humans.

3d. Inhalation Reference Concentration for Noncancer Outcomes

Urothelial hyperplasia in male rats was selected as the basis for calculation of a RFC.

Response: This is a more appropriate index of nephrotoxicity than increased kidney weight, a non-specific index. The components of the composite UF of 30 are reasonable. Alternatively, exacerbation of CPN in female rats could be utilized as a toxic endpoint. As described previously, exacerbation of CPN, which is apparently augmented by α2u in male rat kidneys, is not relevant to humans. I have previously outlined my logic for support of α2u as a contributor to ETBE- and TBA-induced male rat nephropathies.

4b. Cancer Characterization

I. The authors of the ETBE IRIS document have done a reasonable job justifying their selection of the descriptor "Suggestive Evidence of Carcinogenic Potential". Saito et al. (2013) reported a statistically significant increase in adenomas (+1 carcinoma) in the liver of male F344 rats following 2 years of inhalation of 5,000 ppm ETBE. Chronic methyl tert-butyl ether (MTBE) exposure has been reported to cause liver tumors in male and female mice, but ETBE apparently has not been tested in mice.

Response: Although the document's authors assigned ETBE the designation "Suggestive Evidence of Carcinogenic Effects in Humans", alternatively the descriptor "Inadequate Information to Assess Carcinogenic Potential" could be considered. It should be

recognized that Saito et al. (2013) observed a significant increase in liver adenomas only at the highest (5,000 ppm) ETBE vapor level. The researchers estimated the rats' uptake during each daily 6-hour exposure to be 4,222 mg/kg, an extremely high dose. No hepatic effects were seen in oral bioassays at lower doses. Maltoni et al. (1999) saw no liver tumors in Sprague-Dawley (S-D) rats gavaged chronically with 250 or 1,000 mg/kg/day. Suzuki et al. (2012) observed no adverse liver effects in male or female F344 rats ingesting 28-560 mg/kg/day in their drinking water for 2 years.

II. The document's authors note that mode of action (MOA) information would be useful in judging the relevance of the animal data to human cancer hazard. Pertinent data are limited largely to a publication by Kakehashi et al. (2013), in which ETBE activates hepatic nuclear receptors linked to induction of certain cytochrome P450s (CYPs) involved in ETBE metabolism, as well as activation of PPARs. Oxidative stress, evidenced by increases in hydroxy radicals and 8-OHdG formation, is hypothesized as a MOA for liver tumorigenicity. Cell cycle arrest and apoptosis are also suggested as MOAs, although the study apparently did not last long enough for regenerative hepatocellular proliferation to be assessed. The IRIS document's authors concluded that this evidence is inadequate to establish a MOA for liver carcinogenesis. They therefore follow EPA default policy to conclude that the liver tumors induced by ETBE are "relevant to human hazard identification", and in the "absence of MOA information advocate linear extrapolation".

Response: Several investigators have reported that ETBE induces certain hepatic CYPs in F344 and S-D rats. Both Turini et al. (1998) and Kakehashi et al. (2013) found: (a) induction of the CYPs responsible for ETBE metabolism; and (b) induction was limited to high ETBE doses. Thus, CYP induction and centrilobular hepatocellular hypertrophy are likely due to ETBE, but I agree that more experimental evidence is needed to support the contentions of Kakehashi et al. that oxidative stress, cell cycle arrest and apoptosis trigger regenerative cell proliferation. Nevertheless, I am troubled to be limited to EPA's default assumption policy, particularly for a very weak, non-genotoxic compound that has produced a benign tumor in one organ in one sex of one rodent species.

III. The IRIS document's authors state that the rodent bioassays of both MTBE and tert-butanol (TBA) could provide supplementary information on the carcinogenicity of ETBE.

Response: The results of the TBA bioassay should be carefully considered, since TBA is a major metabolite of ETBE in rats and humans. Both chemicals appear to have similar MOAs, in that high doses of each exacerbate chronic progressive nephropathy and cause accumulation renal protein droplets, α2u-globulin and mineralization that accompanied cell proliferation in male rats (Medinsky et al., 1999; Borghoff et al., 2001). Long-term

TBA exposure produced an increase in renal adenoma in male rats. This may also be possible for ETBE, judging from their similar MOAs.

Response: The utility of MTBE as a surrogate for ETBE carcinogenicty is quite limited. ETBE is dealkylated to acetaldehyde, which is quickly converted to acetate by aldehyde dehydrogenase. MTBE is dealkylated to formaldehyde. MTBE produces tumors in a variety of tissues in both sexes of rats and mice in contrast to ETBE. The IRIS document's authors point out in lines 14 & 15 of page 1-112 that the tumorigenesis bioassay results of ETBE, MTBE and TBA have little coherence.

IV. Although EPA's (2005) guidelines do not specify how to incorporate results from 2-stage initiation-promotion studies, it is pointed out that ETBE enhanced/promoted tumorigenesis in male rats pre-exposed to several initiators of carcinogenesis. Hagiwara et al. (2011) observed this phenomenon in male F344 rats pre-dosed with 4 potent genotoxicants. The EPA document's authors state that these findings suggest that persons exposed to mutagens and ETBE may be more susceptible to cancer than if they are exposed to ETBE alone.

Response: It should be recognized that the rats in the study of Hagiwara et al. (2011) received concurrent carcinogenic doses of 4 (or 5) genotoxicants prior to 300 or 1,000 mg ETBE/kg/day for 3 weeks. The higher ETBE dosage level was required to increase the incidence of most tumors. As mentioned previously, high doses of ETBE induce certain CYPs. The genotoxicants (diethylnitrosamine; N-butyl-N- (4-hydroxybutyl) nitrosamine; N-methyl-N-nitrosourea; and N-bis(2-hydroxypropyl) nitrosamine) administered by Hagiwara et al. (2011) all apparently require CYP-mediated metabolic activation. The enhanced tumorigenesis thus may have resulted from CYP induction and enhanced metabolic activation, although it is not clear whether ETBE induces the CYPs pertinent to these particular carcinogens.

I think it is reasonable to include the initiation-promotion data in the chemical cancer hazard characterization, in order to recognize the potential for such a chemical interactions exists. Nevertheless, this appears to be very unlikely under realistic exposure conditions, as the phenomenon is only manifest experimentally at extremely high exposure levels.

V. The IRIS document's authors conclude that "suggestive evidence of a carcinogenic potential for ETBE" is the most appropriate descriptor. This decision was based primarily on adenomas in the liver at one (the highest) dose in one sex of one species in one study. The authors also mention increases in focal pre-neoplastic liver lesions and the potential role of acetaldehyde as a causative moiety.

Response: I believe "Inadequate Information to Assess Carcinogenic Potential" should also be given consideration as a descriptor. EPA (2005) guidelines state this descriptor is appropriate when: (a) available data are judged inadequate; and (2) there are differing results. Saito et al. (2013) saw an increased incidence of liver adenomas in male rats only at the highest (5,000 ppm) inhaled vapor level. Suzuki et al. (2012) found no increase in incidence of any tumors in rats ingesting high doses in their drinking water. Hagiwara et al. (2011) found no liver or kidney tumors in rats gavaged with 1,000 mg/kg/day for 23 weeks. Maltoni et al. (1999) saw no increase in liver tumors in S-D rats ingesting as much as 1,000 mg/kg/day.

The EPA document's authors mention the metabolism of ETBE to acetakdehyde as a potential MOA, though they determined that evidence is inadequate to conclude that ETBE induces liver tumors through a mutagenic MOA. It is noteworthy that enhance systemic genotoxicity was reported in the absence of aldehyde dehydrogenase-2 activity in transgenic mice (Weng et al., 2012). Nevertheless, it is important to recognize that the ETBE has not been found to be genotoxic in the preponderance of *in vitro* and *in vivo* test systems (McGregor, 2007; NTP 1995). ETBE's lack of genotoxicity is not even mentioned in Section 1.3. This is an important consideration in the selection of a descriptor and in the use of the default to utilize linear extrapolation in dose-response analyses.

It is appropriate to use the descriptor and dosage extrapolation for both oral and inhalation exposures. ETBE is well absorbed from the G.I. tract, although first-pass hepatic and pulmonary uptake may remove a substantial portion of low doses before they reach the arterial circulation. Systemic uptake of inhaled ETBE is more limited, but ETBE absorbed into the pulmonary circulation is rapidly distributed throughout the body.

4e. Inhalation Unit Risk for Cancer

The document's authors used a standard EPA approach: a multistage model to curve fit the data of Saito et al. (2013) to estimate a POD. Linear low-dose extrapolation was then utilized to estimate human cancer risk.

Response: The cancer risk assessment appears to have been conducted appropriately according to standard EPA policy. The result, a very low value for the inhalation unit risk for a lifetime of continuous ETBE inhalation, is what would be expected for any chemical for which there is not a firmly established MOA that supports a biologically-based dose-response model. It doesn't seem to matter that ETBE: (1) was not found to be carcinogenic in 2 of the 3 carcinogenicity studies; (2) caused a benign tumor in one organ of one strain of one sex of one species; (3) was tumorigenic at only an extremely high (5,000 ppm) exposure level; (4) was nephrotoxic and quite likely tumorigenic by male

rat-specific MOAs; (5) was not genotoxic in the preponderance of *in vitro* and *in vivo* test systems.

I am troubled that despite the overall weight of evidence, the decision was made by EPA staff that not enough is known about MOA, so linear low-dose extrapolation is evoked. I wish there could be an EPA cancer risk assessment process which would accommodate the ETBEs and TBAs of the world.

Tert-Butyl Alcohol (TBA) Charge Questions

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

The scientific literature search and screening strategy were clearly described on pp. xxvii-xxxiii of the document. This appears to be a very through and effective approach to identify pertinent publications. I was only able to locate one additional manuscript. Tables summarizing what appear to be the more important health effects information were constructed as recommended by NRC (2011). This allows the reader to survey and compare results/data available on particular effects and species. I found the detailed evaluations of the 12 key study design and quality considerations useful.

3b. Noncancer Toxicity at Other Sites

Developmental studies of TBA revealed decreases in body weight gain in offspring during postnatal days 2-10. A small increase in skull or sternebrae variation was observed in one study, but no skeletal malformations were reported. The modest effects appeared to be associated with altered maternal behavior, nutritional status or toxicity at high TBA doses, but the EPA authors concluded that it was not possible to discern fetal effects due to TBA from effects secondary to maternal dysfunction.

Studies of neurodevelopmental effects have been limited in number, have yielded inconsistent results, and have had significant limitations in study design and/or reporting. Therefore, it is clear, as stated, that information is inadequate to draw conclusions about neurodevelopmental toxicity.

Reproductive studies of TBA have yielded little of concern. A slight decrease in sperm motility in one study was not seen in other assessments. No adverse effects were observed in 1- or 2-generation studies of ETBE, although no 2- generation reproductive studies of TBA have been reported. There appears to be little cause for concern.

2. Inhalation Reference Concentration for Noncancer Outcomes

I concur that the NTP (1995) 2-year drinking water bioassay of TBA was the most appropriate study from which to select an adverse effect on which to base the inhalation RfC. Despite the vapor level-related increase in severity of chronic nephropathy in male rats, the findings cannot

be used due to the male rat specificity of these effects. Thus, it is reasonable to use a PBPK model to estimate the inhalation exposure that would produce the same internal dose of TBA in the rat as that associated with chronic nephropathy in female rats in the aforementioned NTP (1995) bioassay of TBA. TBA must be assumed to be the bioactive moiety, due to the absence of information on the nephrotoxic potential of its metabolites. The composite uncertainty factor of 100 is reasonable.

4b. Cancer Characterization

The document's authors considered two cancer descriptors: "Likely to be Carcinogenic to Humans" and "Suggestive Evidence for Carcinogenic Potential." They concluded the latter designation was appropriate, although chronic TBA exposure resulted in increased incidence of tumors in more than one species, sex and organ.

Response: I agree with selection of "Suggestive Evidence for Carcinogenic Potential for most of the reasons given by the authors. A third descriptor "Inadequate Information to Assess Carcinogenic Potential" should also be considered. The document's authors concede in their Summary of Evidence of Carcinogenicity (Section I.3.2) that TBA-induced renal tumors may be associated with α2μ-Globulin (α2μ-) nephropathey and with chronic progressive nephropathy (CPN), MOAs specific to the male rat. The authors also concede a well-recognized point by Hard *et al.* (2009) that α2μ and TBA-exacerbated CPN can work in concert to enhance cell proliferation and ensuing formation of renal tumors in male rats.

In their MOA Analysis-Kidney Effects Section (p.121-139) the IRIS document authors conclude it is difficult to disentangle other potential mechanisms from α2μ and CPN processes, and that renal tumors may be attributable to other , yet unspecified process. The authors then adapt the default that "renal tumors induced by TBA are relevant for human hazard identification."

Response: Defaulting in this situation, in my mind, runs counter to making a decision based on the "weight of scientific evidence." There is a reasonable amount of pertinent data, the balance supporting α2μ and CPN as co-MOAs specific to male rat renal tumors. There may <u>always</u> be other <u>processes</u> involved in the sequence events that culminate in carcinogensis. No amount of research will ever provide enough evidence to include or <u>exclude all possible MOAs</u> and <u>avoid the default</u> assumptions of potential human relevance and low dose linear extrapolation. TBA's low potency, male rat-specific MOAs, and almost virtual lack of genotoxicity *in vitro* and *in vivo* are important considerations in this deliberaton.

If renal adenomas in male rats drop out of consideration, the thyroid follicular adenoma in female mice is the only tumor to consider. There is some evidence of thyroid-pituitary disruption. Wu and Farrelly, *Amer. J. Therap*. <u>13</u>:141-144 (2006) point out the species differences and limitations of using rats and other rodents as animal models for predicting human risks of thyroid cancers. I am not an expert in this field and defer to others on the committee.

McGregor (2007) emphasizes that the finding of TBA-induced thyroid follicular adenoma (NTP, 1995) lacks independent confirmation.

4e. Inhalation Unit Risk for Cancer.

The oral data on thyroid follicular adenomas in mice cannot be extrapolated to inhalation exposure of humans due to lack of a PBPK model for TBA or ETBE for mice. I also agree with the decision not to extrapolate from oral data on kidney ademomas in male rats to predict tumor risks in humans. This is an interesting and unexpected decision, when the document's authors previously conclude in the MOA analysis that "renal tumors induced by TBA are relevant for human hazard evaluation", since renal tumorigenesis may be attrituable to yet unspecified processes, in addition to α2μ and CPN.