Report of the Peer Consultation of the Potential Risk of Health Effects from Exposure to Tertiary-Butyl Acetate

Volume I

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Peer Consultation Organized by:
Toxicology Excellence for Risk Assessment
(http://www.tera.org/peer/)

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NOTE

This report was prepared by scientists of Toxicology Excellence for Risk Assessment (*TERA*) and reviewed by the panel members. The members of the panel served as individuals, representing their own personal scientific opinions. They did not represent their companies, agencies, funding organizations, or other entities with which they are associated. Their opinions should not be construed to represent the opinions of their employers or those with whom they are affiliated.

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Executive Summary

A panel of expert scientists met January 6-7, 2009 to conduct an independent peer consultation on a risk assessment text of tertiary-butyl acetate (TBAC). The assessment document was prepared by LyondellBasell Industries (the primary manufacturer of TBAC) under a voluntary agreement between Lyondell and the U.S. Environmental Protection Agency (EPA). Lyondell had petitioned EPA to exempt TBAC from regulation as a volatile organic compound and EPA requested that Lyondell conduct additional testing, assessment, and review of TBAC. The meeting was open to the public and was webcast on the Internet.

The peer consultation panel included a diverse group of scientists with training and experience in pathology, study design, neurotoxicity, genotoxicity, carcinogenicity, mechanisms of toxicity, metabolism and toxicokinetics, endocrine effects, reproductive and developmental toxicology, and risk assessment. The panel members reviewed the TBAC assessment document and the underlying toxicity data on TBAC to reach conclusions regarding hazard identification, dose-response assessment, risk characterization, and the need for further testing.

The authors of the risk assessment text presented information on the Lyondell-sponsored toxicity studies, noting that the mouse and rat subchronic study protocols were designed with input from the EPA and followed EPA study guidelines. In addition, a modified functional observation battery (FOB) thyroid hormone analyses and estrous cycle evaluation were conducted, and liver cell proliferation was assessed. The panel evaluated the study reports and additional tests and analyses and agreed that all of the Lyondell-sponsored appeared to be conducted according to good laboratory practice.

The panel then discussed noncancer endpoints and mode of action (MOA), as well as metabolic pathways and kinetics information for TBAC and its metabolite, tertiary-butyl alcohol (TBA).

The panel agreed that reproductive effects and developmental toxicity do not appear to be a concern for TBAC, but recommended further analyses of existing data to increase confidence in this conclusion, including use of historic or concurrent controls for the WIL (2008) study, a trend analysis of the Yang et al. (2007) study, and analysis of individual fetal body weights per litter from that study. In addition, the panel considered that a review and comparison of kinetics of TBA and TBAC might be helpful. The panel agreed that there was no indication of concern regarding immunotoxicity or need for further study of this endpoint for TBAC.

The panel discussed the choice of behavioral neurotoxicity as the critical effect, in particular the methods and statistical analyses for the mouse and rat FOB investigations. Panel members stated that the incidence measure of the neurotoxicity data did not reflect the percentage of animals affected; this resulted in a more-than-likely conservative benchmark dose (BMD) evaluation by the authors. The panel also questioned whether the hyperactivity endpoint in the mouse is an adverse effect, noting inherent problems regarding observation and measurement of hyperactivity in the mouse.

An assessment author presented information on the genotoxic and carcinogenic data for TBAC and TBA, noting that all the mutagenicity tests for TBAC are negative. No carcinogenicity studies have been conducted with TBAC. He noted the NTP (1995) carcinogenicity study on TBA found renal tubule cell adenomas in male rats. The renal tumors were suggested to have been caused by a mode of action involving alpha-2u-globulin, and therefore, are not relevant to humans. The panel discussed the genotoxicity data and the degree to which the collection of genotoxicity studies was sufficient to reach conclusions regarding the mode of action for the endpoint of interest – *in vivo* tumorigenicity.

All panel members felt that the overall weight of evidence indicates that TBAC is not likely to be genotoxic based on the available battery of studies typically required for hazard screening. Some panel members, however, felt that the data were too limited to conclude with certainty that genotoxicity would not play a role in the overall mode of action for potential tumors, because of the limitations in the ability of the current battery of *in vitro* and *in vivo* tests to fully assess the contribution of genotoxicity to *in vivo* tumor response. However, none of the panel members felt that additional standard genotoxicity assays were likely to contribute significantly to understanding the broader issue of tumor mode of action.

The panel discussed the renal tumors in male rats in the NTP (1995) TBA bioassay, along with the short-term renal effects data on TBAC, to determine whether TBAC is likely to act by the same mechanism as TBA, resulting in renal tumor formation in rats. The panel agreed that the TBAC data were sufficient to support the conclusion that alpha-2u-globulin accumulation occurs following TBAC exposure, and that this would be likely to be a driving MOA for renal tumors if a two-year bioassay in male rates were done with TBAC. The TBA data for renal findings consistent with alpha-2u-globlulin accumulation should be viewed as corroborative evidence for potential TBAC effects via this MOA. However, TBA alone may not explain fully the observed TBAC effects, based on evaluation of the dose-response behavior for renal effects of TBAC versus TBA. Panelists raised concerns that other active metabolites of TBAC might exist, or that TBAC itself might be an active moiety for renal effects. The panel also agreed that chronic progressive nephropathy (CPN) should at most be considered a contributing factor to the renal tumors for TBA (and thus potentially for TBAC if a chronic study were to be done), and not a separate MOA, since the body of literature for its relatedness to renal tumors is marginal. As to genotoxicity, the panel saw no evidence that genotoxicity is likely to be a rate limiting key event in contributing to observed tumors for TBA, nor would it be expected to be a key event for TBAC.

A dose-related increase in thyroid follicular cell adenomas was seen in female mice in the NTP (1995) drinking water study on TBA. The panel discussed the human relevance of these tumors and considered whether thyroid tumors would be expected in chronic studies with TBAC. An assessment author noted that no thyroid follicular cell tumors were seen in studies with methyl tertiary butyl ether (MTBE), which also metabolizes to TBA. The author did not think the response of TBA was based on a genotoxic MOA, since TBA is not genotoxic, and concluded that the evidence for TBA increasing thyroid follicular cell tumors in mice is weak and there is insufficient evidence to propose any MOA.

The panel discussed the evidence for a potential mode of action for thyroid tumor formation using the EPA (1998) guidelines for thyroid MOA, concluding that while not enough evidence fully supports this MOA, the response to TBA in mice appears to be that of a mild goitrogen operating by liver hypertrophy at high doses, resulting in a high liver clearance of the thyroid hormones. The panel suggested that whether or not the TBA thyroid tumors are considered to be spurious, the risk assessment authors need to present evidence for and against the hypothesis for the TBA tumors based on thyroid hyperplasia. The panel also noted that in general, thyroid tumors in rodents occur in response to increased hepatic hormone clearance to a much greater degree than in humans. Thus, a thyroid hyperplasia response in rodents may not be relevant for human carcinogenicity, particularly when the effects are observed only at high doses. The implications of high-dose effects need to be well understood in the context of other effects occurring.

The panel discussed whether the data are sufficient to indicate a common mode of action for TBA and TBAC. The panel concluded that liver effects appear to be similar between TBA and TBAC, but it is hard to do comparisons, due to differences in how some of the studies were performed. They noted that the liver effects seen are consistent with a MOA for thyroid tumors, but the authors need to analyze this in reference to EPA guidelines for thyroid tumor MOA.

The panel addressed the question of whether there are sufficient similarities between the toxicities of TBA and TBAC to allow the use of TBA chronic data to estimate TBAC chronic risks. They discussed the comparative data for several key endpoints and agreed that no further testing would be needed to address thyroid, reproductive, developmental or other endocrine endpoints, or non-cancer kidney endpoints.

The panel unanimously recommended that the authors organize the available data on TBA and TBAC to better evaluate the toxicological profile and understand kinetics differences, and to enable a more comprehensive look at all the available data. The panel recommended comparisons of TBAC to TBA kinetics, including differences in urinary metabolites; but did not think that full physiologically based pharmacokinetic (PBPK) models are needed. They recommended that the authors systematically compare the toxicities of TBA and TBAC side by side and consider including MTBE as well; and that a precursor-events-analysis may be sufficient. Arraying and analyzing the data will allow for better understanding of the differences in kinetics amongst the chemicals. The panel suggested that a side-by-side comparison of the appropriate critical effect(s) would also be very useful. Using the identified critical effect(s), the authors can then calculate benchmark doses and compare the results to determine dose concordance for TBA and TBAC.

In addition, the authors should evaluate the comparability between oral and inhalation routes of exposure for TBA and the high dose effects from the animal studies need to be put into context for low dose extrapolation for human exposures. With this information, the question of whether additional studies on TBAC are needed can be better answered. Possible additional work could be done to better elucidate kinetic information or perhaps conduct a shorter-term study with intermediate endpoints where kinetic information is also integrated. Either of these studies would better inform the dose-response assessment.

Several panelists stated that a two-year bioassay for TBAC would not necessarily be a useful additional study as it would only show the same tumors as seen with TBA and would not inform the dose-response assessment for TBAC. After some further discussion of this point, the panel unanimously agreed that a standard two-year bioassay (EPA or OECD protocol) for TBAC would not likely meaningfully inform the understanding of TBAC's toxicity for risk assessment purposes.

The assessment included derivation of acute and chronic reference concentrations (RfCs) for TBAC based on the 13-week mouse study (WIL, 2006a), with hyperactivity measured post exposure as the critical effect and benchmark dose (BMD) modeling used to calculate the point of departure. The panel thought that selection of the critical effect was premature at this time and recommended that the authors array all the endpoints and their effect levels to provide a complete picture and comparison of what is seen at the various concentrations and doses. They recommended that the authors then select appropriate endpoints for benchmark dose modeling before developing an RfC.

In conclusion, the panel did not think that a two-year rodent bioassay would contribute to further understanding of TBAC's toxicity for risk assessment. The panel suggested several ways that the authors could improve their analysis and presentation of the available data. The panel thought that that these improvements might identify key shorter-term or mechanistic studies that could be useful for assessing the risk of TBAC.

1. Participants

Sponsor

LyondellBasell Industries

Authors/Presenters

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Dr. Douglas McGregor, FRCPath

Peer Consultation Panel Members[1]

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[1] Affiliations listed for identification purposes only. Panel members served as individuals on this panel, representing their own personal scientific opinions. They did not represent their companies, agencies, funding organizations, or other entities with which they are associated. Their opinions should not be construed to represent the opinions of their employers or those with whom they are affiliated.

[2] Mr. Brown participated by teleconference.

***TERA* Staff**

Dr. Andrew Maier, Co-Chair
Ms. Ann Parker
Ms. Jacqueline Patterson

2. Background

This peer consultation meeting was organized by Toxicology Excellence for Risk Assessment (*TERA)*. *TERA* is an independent non-profit organization with a mission to protect public health through the best use of toxicity and exposure information in the development of human health risk assessments. The subject of this public peer consultation was a risk assessment document that evaluated the underlying toxicity data on tertiary-butyl acetate (TBAC) and reached conclusions regarding hazard and risk characterization. This risk assessment document was prepared by LyondellBasell Industries (the primary manufacturer of TBAC) under a voluntary agreement between Lyondell and the U.S. Environmental Protection Agency (EPA). Lyondell had petitioned EPA to exempt TBAC from regulation as a volatile organic compound (VOC) based on studies demonstrating its low photochemical reactivity. Given the potential for increased use of TBAC upon exemption from regulation as a VOC, EPA requested Lyondell conduct additional testing, assessment and review of TBAC. Lyondell has conducted additional toxicity tests and has prepared an assessment for chronic exposures, which was the subject of this peer consultation.[3] Lyondell and EPA are working cooperatively to assure that this assessment adequately addresses potential public health concerns. As part of their voluntary agreement with EPA, LyondellBassell selected and contracted with *TERA* to independently organize and conduct this peer consultation according to the guidance outlined in the voluntary agreement.

The peer consultation panel was made up of scientists with expertise in the key disciplines necessary to evaluate the proposed approach. The panel members have training and experience in renal toxicity and tumors, pathology, study design, neurotoxicity, genotoxicity, carcinogenicity, mechanisms of toxicity, metabolism and toxicokinetics, inhalation toxicology, risk assessment, endocrine effects, reproductive and developmental toxicology, and mode of action. *TERA* was solely responsible for the selection of the panel members. Each panel member has disclosed information regarding potential conflicts of interest and biases related to the assessment and its sponsor. *TERA* carefully evaluated these disclosures when selecting panel members. Short biographical sketches and disclosure statements for panel members are provided in Appendix A.

The panel received the review package six weeks prior to the meeting to ensure adequate time to carefully review the documentation and prepare for the meeting discussions. The review package included the assessment document, an appendix of robust study summaries, and copies of references. *TERA* prepared a "charge" document derived from key questions that EPA and

[3] The exposure assessment section of the assessment will not be reviewed in detail by this panel.

Lyondell agreed should be asked of the panel. The following charge questions focused the panel's discussions:

1) Were the following toxicology tests for tertiary-butyl acetate (TBAC) performed in accordance with the agreed upon protocols and best laboratory practices?

- WIL Research Labs. (2006a). A 13-week subchronic inhalation toxicity study of tertiary-butyl acetate in CD-1 mice. Report WIL-14061. October 31, 2006.
- WIL Research Labs. (2006b). A combined 13-week subchronic inhalation toxicity study and reproductive toxicity screening test of tertiary-butyl acetate in rats. WIL-14060. October 31, 2006.
- WIL Research Labs. (2008). A Toxicity Study of Tert-Butyl Acetate (TBAC) in Mated Female Rats. WIL-14069. September 2, 2008.[4]
- WIL Research Labs (2007). A Two-week Inhalation Toxicity Range-finding Study of Tertiary-Butyl Acetate in CD-1 Mice. WIL-14053. March 6, 2007.
- ILS Inc. (2006) Quantitation of the Concentration of a2u-Globulin in Kidneys from Male Rats Exposed to Tertiary-Butyl Acetate (TBAC). ILS Study No.:C145-001. May 24, 2007.

2) Are there sufficient data to determine if tertiary-butyl alcohol (TBA) or TBAC are genotoxic or mutagenic? Does the weight of the evidence support the conclusion that either TBA or TBAC are genotoxic or mutagenic?

3) In previous chronic testing of TBA (NTP 1995), an increase in renal tumors in male rats was observed. Are the previous TBA data and the results of the 2006 toxicology tests for TBAC adequate to support the conclusion that a) the observed increase in renal tumors in male rats due to TBA exposure is related to the α2μ-globulin mode of action; b) that TBAC is likely to act by the same mechanism; and, c) that the renal tumors observed in male rats with TBA and, potentially, for TBAC are not relevant for human risk assessment? If these data are not adequate, what further tests are recommended to understand these effects?

4) In previous chronic testing of TBA (NTP 1995), a slight increase in mouse thyroid tumors was observed at the highest dose. Do the 2006 toxicology tests for TBAC provide any clues as to a potential mode of action for thyroid tumor formation or suggest that TBAC would also induce these tumors in mice? Are additional tests necessary to support or refute this conclusion? If so, which ones?

5) Do the 2006 toxicology tests and metabolic data for TBAC suggest that there are sufficient similarities between the toxicities of TBA and TBAC to allow the use of TBA chronic data to estimate TBAC chronic risks? Given the existing data for chronic exposure to TBA (NTP, 1995; 1997), the genotoxicity data for TBA and TBAC, and the metabolic data for TBAC, does the existing evidence support classifying either TBA or TBAC as human carcinogens? Is a 2-year bioassay for TBAC needed to make this determination, or is the existing evidence sufficient?

[4] This study was conducted independent of the voluntary agreement.

6) In the previous chronic testing of TBA and the 2006 toxicology tests for TBAC, effects on the liver were observed. Are the observed liver effects sufficiently similar to indicate a common mode of action (MOA) for TBA and TBAC? Given the metabolic and toxicokinetic profile of TBAC, do the data suggest that TBAC is acting through the TBA metabolite? If the data are not adequate to make this determination, what further tests could be conducted to reduce the uncertainty?

7) Do the data (including the 2006 toxicology tests for TBAC focused on the estrous cycle) support the conclusion that reproductive toxicity is not a key concern for TBAC? Do the data suggest that TBA or TBAC have an effect on reproductive or developmental function or induce hormonally-mediated reproductive effects? Are further tests required to reduce the uncertainty?

8) Do the data (including the 2006 immunotoxicology tests for TBAC and database for TBA immunotoxicity) suggest immunotoxicity concerns for TBAC? If so, are further tests required to understand these effects?

9) The assessment concludes that transient clinical signs of hyperactivity is the critical effect (i.e., lowest observed adverse effect) for calculation of toxicity reference values for TBAC. Is the selection of this as the critical effect for calculating reference values correct and supported? If not, what endpoint should be used?

10) In the Hazard Assessment for TBAC, were the acute and chronic toxicity reference values calculated in an appropriate manner?

11) In the assessment document, are the uncertainties fully described and appropriately characterized?

12) Are there additional issues or questions related to the toxicity or risk characterization of TBAC that should be discussed?

Members of the public were invited to observe the panel discussions by attending the peer consultation meeting in person or by viewing a live web cast. They were also given the opportunity to provide brief oral and written technical comments on the assessment document for the panel's consideration. No written public comments were received, however, one observer made technical comments during the meeting. A list of attendees is found in Appendix B.

TERA prepared this meeting report. The report summarizes the authors' presentations, the panel discussions, the authors' comments during the discussions, and comments from the public. The meeting report is a summary, not a transcript. Opinions and recommendations of the panel members are noted (although panelists are not identified by name). Panel members have reviewed the draft report, and their comments and corrections have been incorporated into this

final version. The authors also were given the opportunity to review the draft report to confirm the accuracy of their presentations and remarks. This report is available on the Internet at http://www.tera.org/Peer/TBAC/index.html.

3. Panel Introductions, Conflict of Interest, and Meeting Process

The meeting opened with a welcome by Ms. Jacqueline Patterson of *TERA*. She described the background and purpose of the peer consultation and the agenda for the meeting. Ms. Patterson noted that copies of panel members' biographical sketches and conflict of interest (COI) and bias disclosure statements were provided to all attendees (see Appendix B). The panel members then introduced themselves and noted whether they had additions or changes in their disclosure statements. None of the panel members had any substantive changes to their statements.

Dr. Dourson, the panel chair, then described how the meeting would be conducted. He explained that discussions would be organized around the charge questions and would follow the order in the agenda (see Appendix B). He noted that all panelists would have the opportunity to state their own positions on the charge items and panel members are encouraged to question one another to make sure that all the panel members and the authors understand the scientific basis for the panel's opinion. The panel will seek agreement, but if agreement is not reached, areas where panelists' disagree will be noted. Authors will make brief presentations and answer clarifying questions from the panel members. The authors will also be permitted to ask clarifying questions of the panelists so that they fully understand what is being suggested or said.

4. Introduction to Project and Studies Conducted

4.1 Author Presentations

Dr. Daniel Pourreau of LyondellBasell began the meeting with a brief explanation of the context for the peer consultation. He noted that the 2006 mouse and rat subchronic studies were conducted, and the risk assessment document was prepared, as part of a voluntary agreement between the EPA and Lyondell. Lyondell entered into the agreement in anticipation of greater production of TBAC as a result of its VOC exemption; its primary use is as a solvent in industrial processes and products. He thanked the panel for their efforts and indicated the authors and Lyondell will use the panel's comments and recommendations to determine if additional toxicity or exposure studies are necessary and to determine if TBAC poses a potential health risk.

Dr. Willem Faber, one of the assessment authors, then presented information on the Lyondell-sponsored studies. Slides of his presentation are found in Appendix C. He explained that the mouse and rat subchronic study protocols were designed by Dr. George Cruzan of ToxWorks and Dr. Douglas Wolf of the EPA. In addition, Drs. Abby Li and Susan Borghoff contributed to design of subsequent neurotoxicity and kidney evaluations, respectively. Dr. Faber noted that the rat subchronic inhalation toxicity study (WIL Research Labs, 2006b) followed OPPTS guidelines (OPPTS 870.3465, 870.3650, 870.6200, and 870.7800.), with the addition of evaluation of locomotor activity pre-exposure and on study week 12, as well as immune system

and kidney evaluations (including alpha-2u-globulin and tubular cell proliferation post necropsy). The mouse subchronic inhalation study (WIL Research Labs, 2006a) also followed OPPTS guidelines (OPPTS 870.3465). Clinical signs of hyperactivity were noted and a modified functional observation battery (FOB) was conducted on days 63-64 to better understand the clinical observations. Thyroid hormone analysis was done on study week 4; however, this could not be repeated at terminal sacrifice in the mouse study because not enough blood was left after histopathology. Estrous cycle evaluation was conducted and liver cell proliferation was also assessed. Dr. Faber explained how the clinical signs and functional observation battery were conducted, noting the difficulties of these evaluations in mouse inhalation studies, and the care taken to control exposures.

4.1.1 Clarifying Questions

Panelists asked several questions about the process for the FOB and clinical observations. Dr. Faber explained that the clinical observations were done three times a day and for the FOB, several technicians performed the observations at the same time. The technicians were blind to the treatment group for the FOB. Animals were removed from exposure chambers as soon as the blow down process (to reduce concentration to a safe level) was complete, approximately 30 minutes. All dose groups were evaluated at the same time post exposure and the animals were in their transfer cages for the testing.

4.2 Panel Discussion

4.2.1 Charge Question 1

The panel discussed charge question 1.

1) Were the following toxicology tests for tertiary-butyl acetate (TBAC) performed in accordance with the agreed upon protocols and best laboratory practices?

- **WIL Research Labs. (2006a). A 13-week subchronic inhalation toxicity study of tertiary-butyl acetate in CD-1 mice. Report WIL-14061. October 31, 2006.**
- **WIL Research Labs. (2006b). A combined 13-week subchronic inhalation toxicity study and reproductive toxicity screening test of tertiary-butyl acetate in rats. WIL-14060. October 31, 2006.**
- **WIL Research Labs. (2008). A Toxicity Study of Tert-Butyl Acetate (TBAC) in Mated Female Rats. WIL-14069. September 2, 2008. [5]**
- **WIL Research Labs (2007). A Two-week Inhalation Toxicity Range-finding Study of Tertiary-Butyl Acetate in CD-1 Mice. WIL-14053. March 6, 2007.**
- **ILS Inc. (2006) Quantitation of the Concentration of a2u-Globulin in Kidneys from Male Rats Exposed to Tertiary-Butyl Acetate (TBAC). ILS Study No.:C145-001. May 24, 2007.**

[5] [5] This study was conducted independent of the voluntary agreement.

A panelist questioned what the charge question phrase "best laboratory practice" meant and the panel members all agreed that it would be better to reword the question to specify "good laboratory practice" instead, as this is well understood. One panel member closely reviewed the WIL study reports and found that these studies were conducted using good laboratory practice (GLP) and followed the protocols. Another panel member closely examined the ILS study report and was satisfied the methods were appropriate and done under GLP, but noted that technically these data should have been included into an amended report of the WIL (2006b) study report (as opposed to the stand alone report). An author said that they are working with the laboratories to have this done.

Another panel member questioned the statements regarding the certification for characterization and analysis of the test material and the meaning of the phrase "conducted according to unknown standards." An author explained that the test materials were provided by Lyondell and accompanied by a certificate of analysis. The certificate of analysis states that the analysis was done under good manufacturing practice (GMP). However, as the company laboratory is not within the WIL Research Laboratories, WIL could not make an evaluation of how the analysis was done beyond reading the providing lab's methods. Several panel members noted that it is not uncommon for test materials to be submitted under GMP and noted this way in the study report. One panel member believes that GMP is more rigorous than GLP. The panel agreed that the studies appear to be conducted according to GLP. The panel recommended an explanation be included regarding the chemical analysis statement (unknown standards) and including the ILS results to the original WIL study if possible.

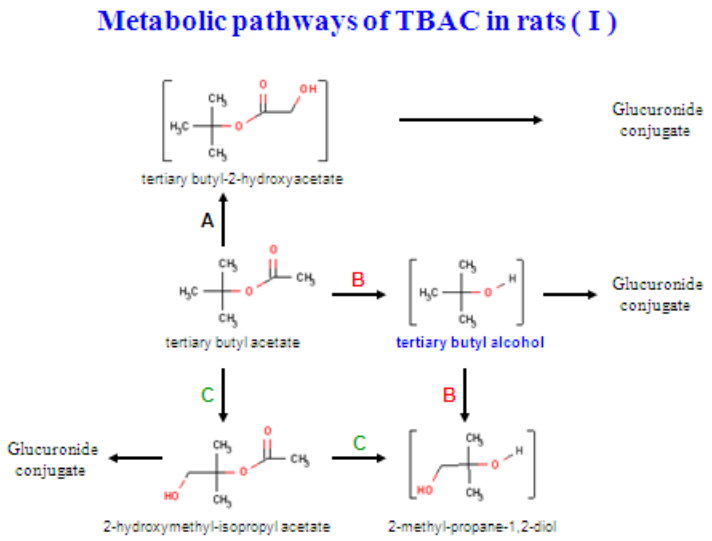
5. Metabolism/Kinetics and Noncancer Endpoints

5.1 Author Presentation

Dr. Willem Faber presented some key information related to noncancer endpoints and mode of action. For developmental toxicity he noted that a recent publication by Yang et al. (2007) identified developmental effects from TBAC, but the statistical analysis was unclear from the published report, particularly whether the analysis was based on the number of fetuses or litters. The Yang authors shared their individual animal data with Dr. Faber who identified some data entry errors. The Yang authors subsequently reissued corrected data tables, which changed the data interpretation slightly, but the Yang authors did not feel it necessary to issue a correction. These data were then re-analyzed by WIL Research Laboratories, which confirmed some of the findings in the original publication. The Yang study had minimal evaluation of the dams, leaving a question of whether the fetal effects were closely associated with maternal toxicity. To address this question, the sponsor contracted with WIL Research Laboratories, a laboratory with previous experience with TBAC, to examine maternal toxicity with the same dosing regimen as the Yang study; however, there was not enough time prior to completion of the risk assessment to also conduct fetal evaluations. The new study found that the number of pups per litter had an influence on the mean fetal body weight and that effects on fetuses were limited to the 1000 and 1600 mg/kg/day groups, with significant maternal toxicity at 800 mg/kg/day and above.

Dr. Faber also presented metabolic pathways and kinetics information. He briefly described three pathways (see figure below)– A) hydroxylation of the acetate group followed by

glucuronidation, a minor pathway; B) an esterase pathway that is major, particularly at higher concentrations; and C) a cytochrome P450 pathway that appears to dominate at lower concentrations. Pathways B and C are related, in that as C proceeds, any of the intermediates can be de-esterified to form the same metabolites as from pathway B (i.e. through tertiary-butyl alcohol). He noted that the de-esterification of TBAC differs from that of other simple aliphatic esters in that the nasal histopathology is not affected following the inhalation exposures, suggesting that TBAC is not a substrate for the esterases within the olfactory epithelium.



Dr. Faber discussed the figure from Groth and Freundt (1994) which displays concentrations of TBAC and TBA in the blood of rats exposed to TBAC in air (approximately 900 ppm). Blood levels of TBA and TBAC increased in tandem, with increasing duration of exposure. When exposure stopped at 255 minutes, the blood TBAC concentration immediately dropped, but the TBA blood concentrations leveled off before dropping more slowly than TBAC. These data show that after inhalation exposure to TBAC, the animals were still exposed to TBA for a significant period of time post-exposure. Forty-five minutes after the end of exposure, the blood levels of TBAC and TBA concentration were approximately 50% and 100% of their respective peak levels. These data suggest that during the clinical observations (conducted approximately 45 minutes post-exposure) in the 13-week study, the blood concentrations of TBA would be expected to be higher than the blood concentrations of TBAC. Clinical observations made during the exposure period would be expected to have blood concentrations of TBAC that are equal to or higher than those of TBA.

5.1.1 Clarifying Questions

Panel members asked a number of clarifying questions. One panelist noted that formaldehyde is a metabolite of methyl tertiary butyl ether (MTBE) and questioned if acetaldehyde is a metabolite of TBAC. An author responded that other aldehydes are hypothesized metabolites of TBAC, but acetaldehyde is not. A second author added that a meeting handout (see Appendix D) shows the sequence of steps involved in ester, e.g., TBAC, hydrolysis. In none of the steps is there a carbonyl group so the products of this reaction are not expected to have the reactivity

necessary to form products with nucleic acids and proteins, as would be the case with acetaldehyde. The panelist recommended that a statement to that effect be added to the document.

Another panelist noted that formaldehyde was formed by TBA *in vitro* in the Cederbaum and Cohen (1980) study and inquired if this was likely to occur *in vivo*. An author replied that based on the metabolic study they conducted, he would not expect that a hydroxyl radical attack on tert-butyl alcohol is likely *in vivo*. The panel member asked whether it is likely formaldehyde is formed under environmentally-relevant conditions. One of the authors noted that the Cederbaum and Cohen (1980) paper addresses the use of TBA in studies that are primarily on the effects of ethanol, in an attempt to distinguish actions attributable to (1) acetaldehyde or to (2) redox changes induced by the metabolism of ethanol. This usage is dependent on the assumption that TBA is not a substrate for alcohol dehydrogenase; therefore it cannot form an aldehyde or a ketone by dehydrogenation. The paper does not investigate production of acetone, and no data are reported on the formation of any metabolites of TBA other than formaldehyde. The paper reports formaldehyde produced *in vitro* at TBA concentrations around 35 mM, which is very high. The apparent Km is 30 mM, which is also very high and would seem to preclude this pathway from any functional relevance. Thus, while Cederbaum and Cohen demonstrated formation of formaldehyde, it was not under physiologically-relevant conditions.

Other clarifying questions included (1) whether a specific or general cytochrome P450 is involved with the metabolism and (2) if the ester hydrolysis of TBAC to TBA is hypothetical or proven experimentally. An author advised that cytochrome P450 in general is involved in metabolism and the ester hydrolysis is hypothesized from expected chemical reactions.

5.2 Panel Discussion

The panel discussed noncancer endpoints and charge questions 7, 8, and 9.

5.2.1 Charge Question 7

> **7) Do the data (including the 2006 toxicology tests for TBAC focused on the estrous cycle) support the conclusion that reproductive toxicity is not a key concern for TBAC? Do the data suggest that TBA or TBAC have an effect on reproductive or developmental function or induce hormonally-mediated reproductive effects? Are further tests required to reduce the uncertainty?**

A panelist led off by reporting that a careful review of the reproductive and developmental studies indicated that there were no effects on reproductive outcomes seen in any of the studies, despite reaching levels of maternal toxicity, and there was nothing in the studies to indicate that the estrous cycle was affected. There were no significant changes in any hormone level in the mouse or rat. Thyroxine levels in the male mouse high dose group at week 4 were the only hormone changes reported. This panelist noted that the assessment report text stated that the WIL 2008 study replicated the work by Yang et al., (2007); however, the former study did not fully replicate the latter study in that fetal parameters were limited to external examinations.

The panel discussed the merits of using historical controls compared to concurrent controls for the analysis of results of the WIL (2008) study. They discussed whether the use of historical controls was appropriate, whether resorptions occurred, and whether individual animal weights were taken into account (larger litter sizes usually result in lower individual newborn body weights). One panelist questioned the appropriateness of using the historical controls. Did the supplied historical control cover the timeframe during which the WIL 2008 study was conducted and did it contain only control rats that were dosed by gavage? The panelist advised that historical controls are typically not used in place of concurrent controls unless an obvious issue exists with the concurrent controls. Dr. Faber clarified that the historical controls were on a rolling 5-year period and included only oral gavage (with the same vehicle) studies. The first panelist suggested that the concurrent controls be statistically analyzed with the historic controls to determine if there is something unusual about the concurrent control group to support the decision not to use it. The panelist also inquired about the lack of trend analysis for the Yang et al. (2007) concurrent control and the historical control group. Other panelists questioned if the analysis would change the interpretation of the data. The first panelist said this type of analysis would not likely change interpretation of the study results, but would increase comfort with using the historical controls, and increase confidence in the selection of the fetal NOAEL.

A panelist recommended that *if* an additional developmental toxicity guideline study is desired, that a second species would not be necessary as there is nothing indicating TBAC is a selective developmental toxicant. The panelist thought that a prenatal oral gavage study (with doses less than 400 ppm) would help to establish a developmental NOAEL. Another panelist asked whether inhalation and oral kinetics information on TBAC is available and suggested that if not, the NSF International report on TBA (2003) might have information to further show how TBAC might act via the inhalation route. Dr. Faber noted that NTP reported that portal of entry effects are not expected for TBAC, thereby supporting use of oral study to evaluate the potential for developmental effects following inhalation.

The panel agreed that reproductive effects and developmental toxicity do not appear to be a concern for TBAC, but would like further analysis, as mentioned above, in order to be confident with this judgment. For example, the panel recommended that the use of historical controls for the WIL (2008) study be better justified with a comparison of historic and concurrent controls, as well as historic controls to the test groups. A trend analysis of the Yang data would be helpful and the LyondellBasell authors might seek to obtain the Yang study report so that individual fetal body weights per litter could be analyzed. In addition, a review and comparison of kinetics from TBA might be helpful.

5.2.2 Charge Question 8

8) Do the data (including the 2006 immunotoxicology tests for TBAC and database for TBA immunotoxicity) suggest immunotoxicity concerns for TBAC? If so, are further tests required to understand these effects?

One panel member reported that careful examination of the study report and data on organs related to immune function did not indicate any immunotoxicity effects. Another panelist thought the histological evaluation and separation by organ was very well done and exceeded

what is usually done for normal Tier 1 testing. The panel agreed that there was no indication of concern regarding immunotoxicity or need for further study of this endpoint for TBAC.

5.2.3 Charge Question 9

9) The assessment concludes that transient clinical signs of hyperactivity is the critical effect (i.e., lowest observed adverse effect) for calculation of toxicity reference values for TBAC. Is the selection of this as the critical effect for calculating reference values correct and supported? If not, what endpoint should be used?

Discussions of this area began by panelists asking for clarification from the authors regarding the protocols and methods for the mouse and rat functional operation battery (FOB) investigations, including handling and transfer of animals, technician responsibilities and daily procedures, and ramp up and blow down of inhalation chambers. An author described how the animals were housed and transferred, and also the roles and responsibilities of the lead technician and technicians. The author explained that mice and rats behaved very differently when exposed to TBAC, which has a strong odor, and when they are put in inhalation chambers the rats were observed to curl up, put their noses in their armpits and go to sleep (exposure took place during day time hours when rats normally sleep). Mice, on the other hand, ran around and explored during exposure. The technicians were aware of the animals' treatment groups during their exposure and post exposure observations for clinical signs. As most of these studies are done with rats, the rat protocol included rat locomotor activity at week 12 (typical plastic box cage with beam breaks counted) and FOB conducted pre-exposure and at weeks 3 and 12. The original mouse protocol did not include locomotor activity or FOB assessments; however, when the technicians noted clinical signs of hyperactivity in mice during and post exposure, a blinded FOB was added for the mouse at study day 62-63 to corroborate the clinical observations. Because the mouse study did not original call for the modified FOB, only post-exposure results are available. An additional difference between species was that the modified FOB for the mouse tested the animals after exposure terminated for the day, while for the rat the testing was done prior to the day's exposure. A panel member provided the table below as a summary of tests conducted in these studies.

Species	**Tests Prior to 1st Exposure**	**Tests 0-2.5 hours post exposure**	**Tests approx. 18 hours post exposure (tested immediately prior to exposure)**
Rats	clinical exam FOB locomotor activity	clinical exam FOB	clinical exam locomotor activity
Mice	clinical exam	clinical exam FOB	clinical exam

The author noted that significant findings were limited to an increase in motor activity counts in the last 15 minutes period in male rats exposed to 1600 ppm and clinical signs of hyperactivity and excessive grooming in mice immediately after exposure. The majority of the mouse effects

were seen at 1600 ppm, with a number of animals showing these effects at 400 ppm, and an occasional observation at 100 ppm. A panel member noted that the 400 ppm dose level in both mouse and rat is important and that greater understanding of the 100 ppm results is desirable. Observations of hyperactivity at the 100 ppm dose were not statistically significant but there was some indication of activity.

Panel members asked a number of additional clarifying questions. A panel member pointed out that there may be an issue regarding timing of FOB testing (15 minutes to 2 ½ hour range) that may impact results between exposure groups of mice. An author indicated that the animals were removed at the end of the exposure period but prior to the blown-down period in an attempt to collect FOB data from mice with blood concentrations similar to during exposures. Another panelist asked what positive control was used and an author indicated that amphetamine is the usual positive control and that the laboratory was very well qualified for this type of study. In response to another panelist's question about dose selection, the authors explained that the dose groups were selected after examination of results of the two-week study, which tested acutely lethal doses of 2500 ppm and below. No differences in sensitivity between the mouse and rat were seen in the two-week study. A panel member asked about the purity of the test material. An author noted that TBAC is 99.7% pure; with small amounts of diisobutylene, TBA, water, and isobutylene. Trace metals are below detection limits (<1 ppm). [Note: post meeting, Dr. Pourreau of LyondellBasell clarified that they have run tests for total non-volatile residue (NVR) and results show that total NVR (including metals) are below 0.01 ppm.]

The panel sought clarification and discussed a number of issues related to the locomotor activity procedures, statistical analysis, and results. The authors called upon Dr. Abby Li of Exponent to help them in answering the panel's questions. Dr. Li had assisted the authors in the evaluation of the neurotoxicity results and preparation of the assessment. Dr. Li clarified that the study authors conduct repeated measures of analysis of variance (ANOVA), looking for interactions between dose levels and whether the dose by time interaction is significant. A linear trend analysis was done, and if found significant, the highest dose level was removed and the analysis repeated with lower dose levels until there was no significance. No Dunnett's test appears to have been done. A panel member pointed out that this analysis makes the assumption that dose response would be a straight line, but perhaps it really is a nonlinear effect whereby low doses of a central nervous system depressant produce increased motor activity via disinhibition, and the higher doses produce decreased motor activity. Looking at the data for various endpoints in the study may reveal some patterns that are other than linear. Another panel member suggested taking a step back and noted that the measures are very variable and with no statistical significance and few animals in the later weeks (~10). The panelist asked whether exploring this line of reasoning further was of value. Others recognized the large variability, but did not want to dismiss the results outright due to lack of statistical significance, when the group size may be the problem. Additionally, the lack of statistical significance for the analysis conducted does not address the concern that the analysis itself was predicated on assumptions of a linear dose response.

The panel examined the data tables on incidence of hyperactivity in mice that were used for the derivation of the RfC closely, and one questioned calling hyperactivity at 1600 ppm an adverse effect, when the modified FOB results were negative. Another panelist noted that hyperactivity

in the mouse is not a common critical effect, noting that EPA's IRIS database does not have even one chemical for which this is the critical effect. A panelist noted that when looking at neurotoxicity effects, one should look at patterns and whether they make sense, and evaluate if there is consistency. In this case, there is no pattern and so one needs to question whether this is a meaningful effect. While the assessment authors cannot be faulted as they chose an effect with observations at a lower concentration than other endpoints, the uncertainty in this data set is high, and the panelist questioned whether selecting this endpoint is making best use of the data available. Others agreed that the mouse data are not convincing and suggested looking at the rat study. Additional panelists agreed, noting that the increase in motor activity counts in the last 15 minutes period in male rats exposed to 1600 ppm is a LOAEL.

The panel also discussed increased adrenal gland weights (relative to body weight) in female rats at 400 and 1600 ppm in the WIL (2006b) study, as well as at 1600 ppm in the Yang et al. (2007) study. One panel member suggested looking more closely at the adrenal gland; that perhaps some subtle changes in histology of the adrenal cortex is involved and suggested examining which subregion of the cortex was hypertrophied. The panel member suggested this alteration may indicate alteration in the production of mineralocorticoids, which are important for blood pressure and kidney function. As this is occurring in the female, it is not a secondary effect related to kidney damage (which was not seen in females). Another panel member did not think the adrenal effects were important; however, noting that nothing was seen in the clinical pathology, sodium and potassium levels were normal, as was urine specific gravity. This panel member thought the effects seen are a common stress response with high dose chemicals.

Another panel member noted that something appears to be happening between 400 and 1600 ppm in these laboratory animals, and if one only looks for a single critical effect one may miss the full picture. Some of the effects discussed are interrelated and an examination of all effects from a broader perspective might be helpful. The panelist suggested that the authors describe the dose-response relationship and what is happening in different systems (i.e., adrenal, liver, kidney, thyroid, and CNS) at each dose level. The panel agreed that given the uncertainty with the mouse results and hyperactivity endpoint, that a broader look at what effects are seen at increasing dose would be helpful for determination of the point of departure and derivation of the RfC.

6. Genotoxicity, Carcinogenicity, and Mode of Action

6.1 Author Presentation

Dr. McGregor presented information on issues related to genotoxicity and carcinogenicity. He noted that other compounds that metabolize to TBA, such as MTBE, were also evaluated for their relevance to TBAC's genetic toxicity and carcinogenicity. He noted that MTBE and other compounds that are ethers of TBA are relevant to TBAC because these compounds are oxidized by cytochrome P450 to produce TBA. Further metabolism of TBA includes direct glucuronidation or oxidation, first to 2-methyl-1,2-propanediol, which can then be further oxidized to 2-hydroxyisobutyrate, most probably via 2-hyroxy-2-methyl-proprionaldehyde.

Dr. McGregor noted that results of all mutagenicity tests on TBAC were negative. Genetic toxicity and mutagenicity studies on the TBAC metabolite, TBA, also were negative, except for a study by Williams-Hill et al. (1999), which indicated a significant response in *Salmonella typhimurium* TA102 with a maximum response at about 2000 ug/plate. Two other GLP-compliant studies in different laboratories, using doses ranging from 15 to 5000 ug/plate and including the strain *S. typhimurium* TA102 amongst others (McGregor et al., 2005) could not replicate the Williams-Hill observations. A third study that also extended the dose range to 5000 ug/plate with *S. typhimurium* strains (but not TA102), Zeiger et al. (1987), also did not report a significant effect. He also noted that the results of the Comet assay (Tang et al., 1997) should be disregarded as it was a single experiment and has not been confirmed or repeated in any other study.

Dr. McGregor presented information from the NTP (1995) carcinogenicity study on TBA, noting that the renal tubule cell adenomas and carcinomas were limited to male rats; they did not occur in female rats or in male or female mice. He explained that the pattern of exposure of renal tubule cell adenomas was suggestive of a mode of action involving alpha-2u-globulin. This protein is synthesized in male rat livers, and then secreted into the blood, where it can bind with certain exogenous chemicals. This altered protein is excreted in the kidney, but a large proportion is re-absorbed in the proximal tubule, where it tends to accumulate in lysosomes and forms microscopically recognizable hyaline droplets that eventually lead to death of renal tubule cells. He listed the key events as described in the assessment document. He noted that increased proportion of renal cortex tubule cells with hyaline droplet accumulation was also seen with TBAC exposed male rats, but not female rats or male or female mice (Huntingdon Life Sciences, 2000a; WIL Research Labs, 2006b). Dr. McGregor also discussed chronic progressive nephropathy (CPN) from the NTP study on TBA, noting that adenomas are associated with end-stage CPN and the effect of chemical exposure is to aggravate the disease process, resulting in earlier damage to the kidney tissue and animals dying from kidney failure.

Dr. McGregor brought two errors in the document to the attention of the panel. The paragraph on the top of page 52 discussing reevaluation of renal tumors should be moved to the bottom of page 41. On page 48, reference to P1 in the first paragraph should read P2.

6.1.1 Clarifying Questions

None.

6.2 Panel Discussion

The panel discussed the charge questions related to genotoxicity, carcinogenicity, metabolism, and mode of action in the following order 2, 3, 4, 6, and 5.

6.2.1 Charge Question 2

2) Are there sufficient data to determine if tertiary-butyl alcohol (TBA) or TBAC are genotoxic or mutagenic? Does the weight of the evidence support the conclusion that either TBA or TBAC are genotoxic or mutagenic?

A panel member opened the discussion with an overall summary of the data as presented in the sponsor's document. This panelist felt the document presentation was reasonably comprehensive. The reviewer indicated agreement with the assessment's conclusion that TBAC was negative for genotoxicity in the studies examined and if there were mutagenic metabolites formed from TBAC, the collection of current studies did not identify this as a concern. In summarizing the data for TBA, the panelist noted several shortcomings related to the single positive Ames assay (Williams-Hill, 1999), including inadequate documentation of the specific Ames protocol used and inadequate presentation of the actual data. Moreover, the cytotoxic potency indicated by William-Hill and colleagues was not consistent with other bacterial mutagenicity studies (Zeiger et al., 1987; McGregor et al., 2005), which showed no cytotoxicity at even higher concentrations. [Note the panelist was an author of one of these studies]. This suggested to the panelist that there were differences in test material used among the studies. Another reviewer added that the Williams-Hill et al. (1999) study did not report information on test material purity, which adds to this uncertainty.

A panelist asked other panel members to comment on whether the rationale provided in the document was appropriate for decreasing the emphasis on the TBA Comet assay (Tang et al., 1997). A panel member responded that this was difficult to determine based on the presentation of the data in the Tang publication, but that the assay is more accurately considered a measure of alkali-labile sites (which can arise from many sources in addition to direct DNA reactivity), and thus agreed with the assessment authors that its impact for assessing risk is unclear.

Another panelist agreed with the first reviewer's conclusion that TBAC is likely negative for genotoxicity and mutagenicity hazard screening purposes. This reviewer however, commented on several points of disagreement with the first panel member on the existing battery of genotoxicity studies, providing additional considerations for several studies. This panelist concluded from the data set as a whole that both TBAC and TBA are probably not genotoxic *in vitro* from a hazard screening perspective. Whether they are also non-genotoxic *in vivo* cannot be determined from the available data. The second panelist first provided comments on the TBAC studies. The panelist noted that the bacterial mutagenicity study (Huntingdon Life Science, 2000b) for TBAC was negative. The mouse lymphoma study (Huntingdon Life Science, 2000c) was also negative, but the study has some uncertainties because the variation of the testing protocol used is the least likely to yield a positive result. In the bone marrow micronucleus test (Huntingdon Life Science, 2000c) it was a little unclear whether adequate doses were given to induce bone marrow toxicity. As a result, this reviewer felt this study might be limited for hazard screening purposes, and also pointed out that an effect in the bone marrow would not necessarily translate to other target organs *in vivo*.

The panelist also commented on uncertainties affecting the interpretation of several of the assays for TBA. This panelist felt that the mutation assay in mouse lymphoma cells (McGregor et al., 1988) was not completed in the optimum way limiting conclusions that could be drawn, but thought that redoing the assay could yield a negative result as well. The sister chromatid exchange assay did not provide a measure of cytotoxicity in the summary report available (NTP, 1995). This panelist agreed with some of the comments from another panelist regarding poor documentation in the Williams-Hill et al. (1999) bacterial mutagenicity study. The panelist did

note that because of the dose spacing involved it was not conclusive that the Williams-Hill et al. (1999) study showed discordant cytotoxicity compared to the other mutagenicity studies. Regarding the Comet assay, the panelist agreed that the assay type is limited, but it cannot be discarded solely on the basis that the finding was not repeated.

The first panelist also provided comments following review of the recent DNA adduct study by Yuan et al. (2007) that reported the formation of DNA adducts in the lung, liver, and kidneys following TBA dosing, as measured by a very sensitive technique (accelerated mass spectrometry). Adduct levels were not higher in the kidneys than other organs, and were not measured in the thyroid, suggesting lack of concordance of DNA adducts with tumor targets. However, these measured data in the kidney were for mice and not rats (which is the species for which kidney tumors were reported). This panelist recommended that the Yuan study be added to the assessment document. The panelist concluded that for TBA, the data were less conclusive than TBAC regarding negative genotoxicity, even so, some ability to cause DNA damage (e.g., adducts) would not necessarily translate to mutagenic events. An assessment author noted that there are additional uncertainties related to the interpretation of the Yuan study. The analytical technique used involved the reduction of samples of DNA to carbon before measurement of radioactivity. Hence, there is no way of identifying where any adduct may be situated. The only indication of DNA purity was the A_{260nm}/A_{280nm} absorption ratio, which was 1.80. Under current practices, this would not be considered sufficient for the demonstration of adducts. Nucleotide or base separation and identification of the adducted residue would be required. The author added that the level of adducts were very similar in mouse kidney, lung and liver and that no tumors have been reported in any of these organs in chronic mouse studies with TBA. Consequently, the author indicated that the utility of the findings was not evident. A panelist agreed that these additional points support discounting the usefulness of this study for risk assessment.

Panel members agreed that TBAC is not genotoxic in the tests that are typically required for hazard screening and more genotoxicity testing is not needed. Overall, the panel agreed on the interpretation of most of the genotoxicity studies from the context of a hazard screening standpoint. In a few cases panel members had differences in opinions regarding the robustness of individual studies for evaluating the potential genotoxicity endpoints they were designed to evaluate. In particular, there were differences in confidence among panel members in the sensitivity of several of the TBA studies. For the micronucleus study (NTP 1995) panel members differed in their confidence that a sufficiently high dose was used and the degree to which the negative results would be useful to make conclusions for the *in vivo* tumor targets. A panelist noted that although the mutation study in mouse lymphoma cells (McGregor et al., 1988) was conducted according to accepted protocols, it was noted that more sensitive protocols or higher test concentrations could have been used. But this panelist and another noted that even if further testing was done using more sensitive protocols, such testing would likely continue to yield a negative result. Panel members also agreed on significant limitations in the bacterial mutagenicity study by Williams-Hill (1999), but disagreed on the degree to which these limitations would be sufficient to entirely discount the positive finding reported in this study.

The panel also discussed the broader issue of the degree to which the collection of genotoxicity studies was sufficient to reach conclusions regarding the mode of action for the endpoint of

interest – *in vivo* tumorigenicity. Some panel members felt that that the overall weight of evidence clearly indicates that TBAC is not genotoxic and that since the typical battery of studies had been done; the data are adequate to conclude that a genotoxic mode of action is not a significant contributor to the potential tumor responses. Other panel members, reflected on the limitations in the ability of the current battery of *in vitro* and *in vivo* tests to assess the contribution of genotoxicity to the tumor response, and felt that the data were too limited to conclude with certainty that genotoxicity does not play a role in the overall mode of action for tumors. However, none of the panel members felt that additional standard genotoxicity assays were likely to contribute significantly to understanding the broader issue of tumor mode of action.

6.2.2 Charge Question 3

> **3) In previous chronic testing of TBA (NTP 1995), an increase in renal tumors in male rats was observed. Are the previous TBA data and the results of the 2006 toxicology tests for TBAC adequate to support the conclusion that a) the observed increase in renal tumors in male rats due to TBA exposure is related to the α2μ-globulin mode of action; b) that TBAC is likely to act by the same mechanism; and, c) that the renal tumors observed in male rats with TBA and, potentially, for TBAC are not relevant for human risk assessment? If these data are not adequate, what further tests are recommended to understand these effects?**

The panel discussed the renal tumors in male rats in the NTP (1995) TBA bioassay, along with the short-term renal effects data on TBAC, to determine whether TBAC is likely to act by the same mechanism as TBA, resulting in renal tumor formation in rats. A panelist noted that for TBAC itself, three or four of the seven criteria for demonstrating a mode of action related to alpha-2u-globulin accumulation (established by IARC [1999] and Hard et al. [1993]) were met. The panelist added several points that support this MOA hypothesis: (1) TBAC is not genotoxic in the available hazard screening battery, (2) the observed nephropathy and renal tumorigenicity for TBA were specific to male rats, (3) hyaline droplet accumulation was observed with TBAC and this precedes tumorigenesis, and (4) the droplets from the TBAC study contain alpha-2u-globulin as measured in histochemistry and an ELISA test. Chemical binding of TBAC to alpha-2u-globulin has not been measured, however, nor has induction of sustained increased cell proliferation in the renal cortex. In addition, similarities in dose-response of tumors and histopathological endpoints cannot be judged, absent a tumor study with TBAC. Based on the data and analysis, this panelist felt that there is sufficient evidence that the alpha-2u-globulin MOA applies to TBAC. A second panelist voiced agreement with the analysis presented by this reviewer, and several panelists suggested that the assessment explicitly list the IARC criteria and present the data relative to the individual criteria in a systematic fashion.

A panelist indicated that comparison of the dose-response information for hyaline droplet formation appeared to suggest that TBAC induced alpha-2u-globulin accumulation at a lower dose than TBA. Although uncertainties in this conclusion could in part reflect difference in kinetics across dose routes among the available studies, the differences in absorption for TBAC versus TBA would not likely account for the full difference. Another panelist added that additional quantitative information on TBAC kinetics would be helpful. Based on the points

raised by the first panelist, the second panelist indicated that the risk assessment would not be able to rely on data for TBA as the key basis for an argument for the renal tumor MOA, since there might be other active metabolites or TBAC itself might be an active moiety for renal effects. The second panel member recommended, in the context of this endpoint as well as other effects, to line up the array of effects data for both TBAC and TBA to allow for easier evaluation of arguments regarding the toxic moiety. The authors agreed that the kinetic data are limited and cautioned panelists from drawing too many conclusions about the predominance of various metabolic pathways from the urinary metabolite data shown in Table 14 in the document.

A panelist also questioned the contribution of the chronic progressive nephropathy (CPN) to the observed tumor response with TBA. This panelist noted that the literature is not convincing for a relationship between CPN and TBA renal tumor formation and does not agree with the conclusion that CPN is a viable MOA hypothesis for TBA. A second panel member agreed, and suggested that at most CPN might be considered a contributing factor, rather than a separate MOA. This reviewer felt that CPN would be a non-specific response to many nephrotoxicants. Moreover, the relationship between CPN and renal tumors is unclear.

After some additional discussion, the panel agreed that the TBAC data were sufficient to support the conclusion that alpha-2u-globulin accumulation is the driving MOA for the observed male renal tumors with TBA. The TBA data for renal findings consistent with alpha-2u-globulin accumulation should be viewed as corroborative evidence for potential TBAC effects via this MOA. However, TBA itself may not explain fully the renal tumor effects, based on evaluation of the dose-response behavior for renal effects of TBAC versus TBA. The panel also agreed that CPN should at most be considered a contributing factor to the renal tumors from TBA (and thus, potentially for TBAC, if a chronic study were to be done), and not a separate MOA, since the body of literature for its relatedness to renal tumors is marginal. As to genotoxicity, the panel saw no evidence that genotoxicity is likely to be a rate limiting key event in contributing to observed tumors. Based on these conclusions, a panelist asked what additional information would be needed to eliminate genotoxicity as potentially contributing to observed tumors. Another panel member indicated that target-organ specific *in vivo* genotoxicity data would be an important addition to the data set.

7. Thyroid and Liver Effects, Metabolism, Use of TBA Data for TBAC

7.1 Author Presentation

Dr. Douglas McGregor presented information related to the thyroid follicular cell tumors in the TBA NTP study. He explained that adenomas (but not carcinomas) were seen in mice, but not in rats, from the NTP drinking water bioassay for TBA, with a dose related increase for females, but not males. No thyroid follicular cell adenomas were seen in mice exposed to MTBE (which also metabolizes to TBA) by inhalation. The MTBE inhalation dose was calculated to be about 1.5 times greater in the inhalation study than the TBA dose in the drinking water study (based on a default respiratory volume of 1.8 L/hr for mice [Gold and Zeiger, 1997]). Therefore, the evidence for the increased incidence of thyroid follicular cell tumors in female mice of the NTP drinking water study being caused by TBA is weak and inconsistent. Dr. McGregor did not

think that this could be a genotoxic response since TBA is not a genotoxin, although a mode of action involving indirect interference with thyroid hormone production might be considered. The LyondellBasell's assessment document reviews the few data available to try to propose a mode of action for the thyroid tumors, but he concluded that the evidence for TBA increasing thyroid follicular cell tumors in mice is weak and there is insufficient evidence to propose any MOA.

7.1.1 Clarifying Questions

A panelist asked whether the difference in findings between TBA and MTBE might reflect a strain difference. The author indicated that the strains of mice were different and while the dose routes also were different, the dose rates were likely to be very similar and some similarities in the carcinogenic outcome could be reasonably expected. In response to another panel question, the author noted that the mouse TBA incidence at the highest dose is outside the range of the NTP historical controls. Other panelists questioned how far outside the historic range the highest dose is, and consulted Table C4B of the NTP Blue Book [NTP, 1995]), which indicated that the historic controls for female mouse are 0-5% (+/- 4.4%). Therefore, the female mouse results are outside the historic range. A sponsor noted that NTP does not have historic control data for noncancer endpoints, but they could go back and do that evaluation.

7.2 Panel Discussion

The panel discussed charge questions related to the thyroid and liver effects, as well as metabolism and use of TBA data for TBAC and whether a two-year bioassay is needed.

7.2.1 Charge Question 4

> **4) In previous chronic testing of TBA (NTP 1995), a slight increase in mouse thyroid tumors was observed at the highest dose. Do the 2006 toxicology tests for TBAC provide any clues as to a potential mode of action for thyroid tumor formation or suggest that TBAC would also induce these tumors in mice? Are additional tests necessary to support or refute this conclusion? If so, which ones?**

The panel discussed the mouse thyroid tumors from the TBA NTP study and the thyroid effects data on TBAC. A panelist began the discussion by stating that a slight change in circulating T4 levels was seen in the mouse 13-week study with TBAC, but with no changes in thyroid morphology to indicate that the thyroid gland was over stimulated. Although the T4 levels may suggest something going on, such levels are known to be variable. This panelist agreed with the assessment authors that the TBA thyroid tumors appear to be a spurious finding. Another panelist countered that the rates of nonneoplastic lesions in TBA-treated female mice were 33% for controls and 80% for the high dose (see page 249 of NTP report); a less dramatic increase was also seen in males. Thus, some evidence exists for histopathology of the thyroid. The EPA (1998) guidelines for understanding thyroid MOA were suggested by a third panelist as a way of analyzing the thyroid effects. The guidelines list eight criteria, but focus on five of these as being the most important considerations. The panelist noted that some evidence for a thyroid

growth-stimulation MOA for TBA, albeit not very strong, exists for four of the five important areas:

- Hormone changes: one statistically significant decrease in T4, other suggestions of hormones changing, but these are not statistically significant.
- Cell growth: focal growth is evident, but this is not generalized hyperplasia as expected with thyroid stimulation.
- Site of action: some evidence exists that tumors may occur via increased thyroid hormone catabolism due to hyperplasia of the liver.
- Dose concordance: the doses that evoke tumors also cause these minimal hormone changes, cell growth, and liver hyperplasia.
- Reversibility: little evidence exists for this criterion.

This panelist suggested that the authors step through these EPA criteria and the resulting conclusion might be that not enough evidence supports this MOA for TBA. This panelist thought, rather, that the TBA response in mice was that of a mild goitrogen operating by liver hypertrophy at high dose, but that the evidence for this is not definitive. The first panelist noted however that male rats tend to be more responsive to goitrogens and generating tumors, but in this case, female mice exposed to TBA appeared to be more responsive. Also, there was no evidence of changes in TSH in the TBA studies. This panelist suggested that this may simply be a liver clearance effect, a possibility for a small organic molecule. The third panelist noted that if an MOA based on liver hypertrophy is considered, the authors would need to further review hypertrophic measures such as liver size and weight. Another panelist stated that first one has to establish if the effect is real. If a pattern exists, it is at very high doses. In the TBAC studies, liver effects were seen in the high dose female rats and in mice at the 600 ppm dose, and also early in the two-week studies. While some data contribute to the weight of evidence for potential modes of action, one should consider this information systematically in the context of its relevance to risk assessment. A pattern at high doses may not matter for risk assessment. Several panelists suggested showing the sequence of changes in liver for TBA and TBAC from short-term to long-term studies and that the authors identify the main metabolic pathway for low and high exposures. One of these panelists also suggested that looking across species is important, and suggested presenting assimilated data across species, sexes, and length of observations for TBA, TBAC, and MTBE. This panelist did not disagree with the conclusion that the thyroid effects might be spurious, as there is some evidence for thyroid effects, but the picture is not clear and if anything is happening, it is at high doses which may not be relevant to human exposures. Another panelist agreed.

The panel suggested that whether or not the TBA thyroid tumors are considered to be spurious, the risk assessment authors needs to lay out evidence for and against the hypothesis for tumors based on thyroid hyperplasia. The implications of high dose effects need to be well understood in the context of other effects occurring. Overall, TBA is perhaps a weak goitrogen. The panel members noted that thyroid tumors in rodents occur in response to increased hepatic hormone clearance to a much greater degree than in humans. Thus, a response in rodents may not be relevant for human carcinogenicity, particularly when the effects are observed only at high doses.

7.2.2 Charge Question 6

6) In the previous chronic testing of TBA and the 2006 toxicology tests for TBAC, effects on the liver were observed. Are the observed liver effects sufficiently similar to indicate a common mode of action (MOA) for TBA and TBAC? Given the metabolic and toxicokinetic profile of TBAC, do the data suggest that TBAC is acting through the TBA metabolite? If the data are not adequate to make this determination, what further tests could be conducted to reduce the uncertainty?

One panelist suggested that few additional studies are needed. The panelist noted that the increased liver weight was seen at the highest dose in the subchronic inhalation study and also at the 750 ppm concentration in the two-week study in mice. The results are not surprising, and these effects look similar to those seen for TBA, but it is difficult to compare the two chemicals' studies. The panelist continued that the authors should be careful about interpreting the data from just a hazard perspective and should revise the text to present a mode of action context, particularly taking into account the relevance of high dose effects to low dose extrapolation.

This panelist continued that if the authors are going to rely on data for cancer from a metabolite, then a quantitative kinetic comparison of data between parent and metabolite should be considered, including potency of the metabolite in relation to parent compound. Very short term investigations measuring an early key event for the metabolite directly and for the various parent compounds could be conducted to gain some idea of the extent of metabolism (quantitatively) to the putatively toxic metabolite (TBA) for the different compounds in comparison with their effects. The authors could also compare benchmark doses for subchronic neurotoxicity endpoints for TBA and TBAC to provide some indication of relative potency for that endpoint. Such an analysis would likely vary by endpoint. Another panelist asked if this analysis would include histological evaluation, and pointed out that there may be differences in preparation of slides, pathologists, and judgments of severity. The first panelist acknowledged the difficulty in comparisons, but noted that one makes as much out of the available data as one can. Thus, one might conduct a short-term investigation to get preliminary information, or perhaps very early effects to compare TBA and TBAC kinetics.

The panel discussed metabolism of TBAC (see figure in Section 5.1). One panel member indicated that the proposed metabolic pathways make sense, but one needs to quantify how much TBAC is metabolized to TBA. If it is a 1:1 relationship, and toxic effects are comparable, then the authors' contention that the toxicity of TBA can be used for TBAC is more reasonable. If the kinetic comparison is not 1:1 and effects are still comparable, what would that mean? Another panelist responded that different metabolites might be causing different effects. The data presented are not convincing that TBA is causing all the observed effects (as discussed above in the context of the renal effects). If the esterase pathway (B pathway) dominates, then this panelist would be more convinced. A third panelist added that in order to use TBA results as a surrogate for TBAC toxicity, one would have to demonstrate that an adequate quantity of TBA is generated *in vivo* from TBAC. Looking at the pathways, one would have to see that TBAC metabolizes to 2-hydroxyisobutyric acid (HiBA), predominantly through TBA. The panelist asked what percentage of the total is represented by each pathway, noting that some of the information in the text on alpha-2u-globulin suggests that the TBA metabolite of TBAC may not

account for all effects. As noted during the discussion of renal effects, the assessment author had indicated that the availability of robust data for quantification of the metabolic pathways is too limited to provide such precise estimates. Another panelist pointed out that TBAC can metabolize to HiBA without ever going through the TBA pathway.

A panel member suggested that one could design studies to measure an early event in some tissue for both TBA and TBAC. Approximately equal results would provide some confidence that TBA is the active moiety. However, if the effect is quantitatively less for TBA, then TBAC or some metabolite for TBAC, other than TBA or its downstream metabolites, is likely to be causing effects. Another panelist noted that on page 12 of the assessment document, a 75 minute lag time is evidence suggesting that the conversions of TBAC to TBA was not immediate. One of the authors responded that the TBAC conversion to TBA is different than with other acetate esters; TBAC conversion appears to be much slower and elimination of the resulting alcohol is also much slower. The author thinks that the toxicity will be unequivocally linked to TBA, however, since the available number of metabolizing enzymes that yield TBA is very large. Moreover, the effects seen – alpha-2u-globulin and the thyroid---are effects that reflect cumulative damage. Referring to the work of Groth and Freundt (1994), which compares TBA and TBAC in blood, the author noted that since elimination curves are so long, the lag time does not make a difference. This author continued that a PBPK model in rats, humans and mice for both TBA and TBAC would be ideal, but questioned what endpoints investigated would contribute significantly to a dose response assessment for human health. The alpha-2u-globulin is not relevant to humans and rodents are more sensitive than humans for thyroid effects. In the end, other endpoints are more sensitive and ultimately would be used for the dose response assessment. The author noted that even if one developed PBPK models for TBA and TBAC, they would not resolve whether any of these effects were due to TBAC or TBA. The TBAC PBPK model would have a large portion devoted to TBA production and elimination. The CNS effects observed with both compounds have a large amount of variability. Therefore, it would not ever be possible to "subtract" or remove the TBA component from the TBAC component with any degree of certainty. Nor is it possible to inhibit the metabolism of TBAC to TBA (and corresponding metabolites) since the enzymatic systems responsible are too numerous and widespread within the organism. Inhibition of all of these enzymatic systems would kill the test subject outright, regardless of TBAC or TBA exposure.

One panelist agreed that TBA is an inseparable part of TBAC, but again questioned whether TBAC might cause toxicity that TBA does not. Another panelist added that other effects such as hyperplasia and various nephropathies were seen in the kidney besides those associated with alpha-2u-globulin. These toxicities may be due to another mechanism not seen with TBA. The authors agreed that other effects were seen in the kidney, but that in developing their report they decided to use the TBA data for just the genotoxicity and carcinogenicity endpoints, for which TBAC data were lacking. For other endpoints, the authors relied directly on the TBAC data.

One panelist suggested the development of a comparison of the toxicology profiles of TBA and TBAC to provide some confidence that the toxicology profiles are similar. From there, one can to some extent do a rudimentary dose/response comparison.

The chair summarized the panel's recommendations:

- There appear to be similar liver effects between TBA and TBAC, but it is hard to do side by side comparisons, due to differences in how some of the studies were performed.
- The liver effects seen are consistent with a MOA for thyroid tumors, but the authors need to analyze this in reference to EPA guidelines.
- Comparisons of TBAC to TBA kinetics are needed including differences in urinary metabolites; but this comparison does not need to be to the extent of a full PBPK model.
- Compare the toxicities of TBA and TBAC side by side; precursor events analysis may be sufficient.
- High dose effects from the animal studies need to be put into context for low dose extrapolation for human exposures.

Furthermore, the panel did not think that further testing was needed at this time. Rather it recommended that the authors first start with a general profile toxicology comparison of TBA and TBAC. Then they should compare the available kinetic data on TBA and TBAC, and compare inhalation to oral routes to provide insight into differences among routes. With this information, they could then compare potency between TBA and TBAC for selected endpoints, or, at the least, for neurotoxicity.

7.2.3 Charge Question 5

> **5) Do the 2006 toxicology tests and metabolic data for TBAC suggest that there are sufficient similarities between the toxicities of TBA and TBAC to allow the use of TBA chronic data to estimate TBAC chronic risks? Given the existing data for chronic exposure to TBA (NTP, 1995; 1997), the genotoxicity data for TBA and TBAC, and the metabolic data for TBAC, does the existing evidence support classifying either TBA or TBAC as human carcinogens? Is a 2-year bioassay for TBAC needed to make this determination, or is the existing evidence sufficient?**

One of the observers at the meeting, Dr. Douglas Wolf of EPA, explained that EPA's cancer guidelines make no presumption for any particular mode of action for a tumor, only that tumors arising in rodents are relevant to humans, unless some contrary data are available. The guidelines recommend that all relevant data should be reviewed, an MOA should be hypothesized, and an understanding of key events should be made in order to evaluate relevance to humans. A linear or nonlinear dose response assessment is then conducted, based on understanding of this biology. Panelists asked whether the intent of the voluntary agreement effort is to evaluate shorter-term studies and build a collective understanding of other compounds and principle metabolites, in order to determine whether chronic exposure studies for TBAC were needed. The observer explained that the goal of the TBAC effort was to use a tiered testing approach and to evaluate results from the subchronic studies and compare the relative spectrum of effects between TBA and TBAC to judge if there is sufficient information to make an assessment, or whether additional work is needed. A panelist recommended that instead of focusing exclusively on hazard testing, one should consider the nature of testing that is required based on the pattern of effects and weight of evidence for hypothesized modes of action.

The panel then discussed whether a two-year cancer bioassay for TBAC was needed. One panelist thought there may be sufficient justification not to do a cancer bioassay, but information

has not been presented in the assessment document in a format to fully support this conclusion. Another panelist suggested further discussion of whether the information from the two 13-week studies could be used to determine if any effects of TBAC, if not already seen with TBA, are a concern for human health. What might the known long-term toxicity of TBA not predict for the unknown long-term toxicity of TBAC? After some additional discussion the chair indicated that not all panel members think the information is organized in the text in order to answer this question overall and suggested the panel first address the question by examining the key toxicity endpoints that had been previously discussed.

One panelist stated that for endocrine toxicity and reproductive and developmental effects, the conduct of a two-year bioassay for TBAC is irrelevant. This panelist saw no value to do a two-year bioassay to further clarify the thyroid effects, including tumors, or other endocrine responses. Another panelist agreed that a two-year bioassay for TBAC may not be needed, but this did not address the kinetic comparisons, which are important in any subsequent dose-response assessment. After a brief further discussion, the chair noted unanimous consensus on the lack of need for a two-year bioassay to address thyroid, reproductive, developmental, or other endocrine endpoints.

On the kidney non-cancer endpoints, one panelist stated that a two-year bioassay was not needed to clarify the outstanding questions, but that a comparison of doses between TBA and TBAC should be included as part of a larger picture approach, as previously suggested. This comparison could be done with existing studies and the integration of available data. Another panelist agreed, but suggested looking at differences in dose and route. For example, some differences in toxicity between TBA and TBAC in the 13-week studies might be due to different dosing regimens. If so, the uncertainties due to these differences might be mollified, such that a two-year study for TBAC is not needed. After a brief further discussion, the chair noted unanimous consensus on the lack of need for a two-year bioassay to address non-cancer kidney endpoints.

On the kidney cancer endpoints, one panelist stated that the apparent increase in potency of TBAC relative to TBA (for alpha-2u-globulin) is fairly strong evidence that a two-year bioassay would only serve to confirm that the MOA is the same and, therefore, no benefit would be gained from an additional study. Another panelist thought, however, that a comparison between relative potency of TBA and TBAC should be performed; this panelist was not convinced that TBA explains all of TBAC toxicity. Studies short of a two-year bioassay might show some effects beyond kidney tumors.

Another panelist recommended looking at the whole toxicity picture rather than by endpoint. This panelist thought that the rationale for using TBA as a surrogate for TBAC may exist, but the data have not been presented in an informed and tiered fashion. This panelist suggested that the authors first step through hazard identification of the critical endpoint(s) and then evaluate the MOA(s). The available kinetic data should be fit in as needed to identify where gaps exist. Then if necessary, a shorter-term study on the endpoint of interest for TBAC could be conducted. A second panelist agreed, but suggested to first develop a side-by-side profile as suggested earlier, then identify the critical effect(s), comparing these effects quantitatively with benchmark doses (BMDs); and then look at kinetic information to explain differences, if any. This information

should allow one to answer whether a two-year bioassay or some shorter study is needed. The first panelist agreed and noted that if profiles between TBAC and TBA are quite different after the shorter-term studies, then one would have to look at what other toxicity TBAC might pose.

An EPA observer noted that the panel concluded that genotoxicity is essentially negative for TBAC, and given that, asked whether the panel was aware of any compounds with a 90-day study where a two-year chronic study picks up different effects at lower concentrations. A panelist responded that at the NTP, generally if an agent was negative in genotoxicity bioassays and essentially little toxicity was seen in the 90-day study, the chemical was not tested in the two-year bioassay, unless there was some major structural alert or the chemical was produced in very large volumes and there was a regulatory imperative. The panelist asked if positive findings in additional *in vivo* genotoxicity testing were found, would this diminish the panel's conclusion regarding rat kidney or thyroid tumors. This panelist went on to state the fact that a potentially positive genotoxicity study does not mean the tumors are from a genotoxic MOA. The panelist explained the distinction between mutagenicity and genotoxicity, noting that genotoxicity reflects anything that touches DNA. However, not all that touches DNA leads to a resulting mutation and it is rare to have a chemical that does not show some DNA damage response. Generally, if one finds adducts in one tissue, one will find them in others, unless the material is highly reactive or metabolized and does not move to other tissues. Seeing adducts does not indicate a mutagenic mode of action. In particular, this reviewer does not think that the Comet assay is a good predictor of mutagenicity, although others do.

A second panelist added that if you found mutations in a tissue, then a temporal and dose concordance analysis is needed. Whether or not genotoxicity is the operating MOA is not simple box checking yes or no. Another panelist noted that the discussion was confusing hazard characterization and dose response. Issues in target tissue relate more to dose response assessment. If the panel recommends a long-term bioassay, then it needs to be designed to provide information relevant to dose-response assessment.

Several panelists thought that a cancer bioassay would not necessarily be a useful additional study as it would only show the same tumors as seen with TBA and would not inform the dose response assessment for TBAC. One of these reviewers suggested considering additional work to tease out kinetic information or perhaps conduct a shorter-term study with intermediate endpoints where kinetic information is also integrated. Either of these studies would better inform the dose-response assessment.

To answer the charge question, the panel unanimously agreed that the authors should organize the available data on TBA and TBAC to better evaluate the toxicological profile and understand kinetics differences. Such effort might lead to additional study recommendations. Specifically, the panel suggested:

- The authors compare the toxicological profiles of TBAC and TBA side-by-side, and consider including MTBE as well. From this comparison, the appropriate critical effect(s) may be identified.
- Array and analyze the data to understand differences in kinetics.

- Based on this critical effect(s), the authors can then calculate benchmark doses and compare the results to determine dose concordance for TBA and TBAC.
- Evaluate the comparability between oral and inhalation routes of exposure for TBA.

With the results of the above work, the panel then suggested that the authors consider what additional work, if any, would be recommended to inform the quantitative risk assessment.

The chair then asked the panel specifically:

> "Is a standard 2-year bioassay (EPA or OECD protocol) for TBAC likely to meaningfully inform our understanding for risk assessment?"

The panel unanimously agreed that it would not.

8. Derivation of Reference Values

8.1 Author Presentation

Dr. Will Faber briefly presented information regarding the derivation of the reference values, and in particular the benchmark dose calculations (see slides in Appendix C). Dr. Faber (with the help of Dr. Abby Li of Exponent) compared the critical effects in the available studies and they determined that several endpoints were not relevant to human health (e.g., renal tubule cell adenomas and the thyroid follicular cell adenomas). They determined that acute neurotoxicity endpoints that were collected from the mouse 13-week study (WIL, 2006a), and in particular the hyperactivity measured post-exposure, were the most appropriate endpoints to model for the acute and chronic reference values. They identified the 400 ppm concentration as the LOAEL and 100 ppm as the NOAEL. Dr. Michael Gargas and Mr. Chris Kirman of The Sapphire Group assisted the authors with benchmark dose modeling of this data set. Incidences of hyperactivity in mice from post-exposure observations from the first three weeks of the study were used to derive a BMD. An Effect Concentration (EC) producing a 10% increase in extra risk of 237 ppm was calculated, with 150 ppm as the LEC10 (the 95% lower confidence limit) for an acute RfC. Results from the remaining weeks (4-12) were used for the chronic RfC (46 ppm EC10 and 31 ppm LEC10). For the acute BMD, the gamma model was determined to have the best fit, while for the chronic BMD, the quantal linear model fit best.

8.1.1 Clarifying Questions

A panelist asked for an explanation of the basis for selection of the models, noting that the quantal and multistage results looked similar. Dr. Faber indicated he believed The Sapphire Group considered the AIC, p-value, and the EC10/LEC10. Another panelist indicated that the visual fit and residuals analysis are usually also evaluated. The panel recommended that the authors better explain the justification for model selection in the text.

Another panelist asked whether neurotoxicity data for TBA were reviewed and considered for the TBAC assessment. Dr. Faber confirmed that the assessment did not present nor consider

TBA data on neurotoxicity. TBA data were considered in relation to genotoxicity and carcinogenicity only.

8.2 Panel Discussion

Following this presentation, the panel discussed derivation of the reference concentration and charge questions 10, 11 and 12.

8.2.1 Charge Question 10

10) In the Hazard Assessment for TBAC, were the acute and chronic toxicity reference values calculated in an appropriate manner?

Panelists asked for further clarification of tables in the assessment document that summarized the incidence of hyperactivity in mice from the WIL (2006a) study, and how they were used to create the dose incidence figure in the presentation (Slide 3, see Appendix C). The author confirmed that in Table 27 of the assessment, the incidence reflects the number of times any animal was observed exhibiting hyperactivity within each 7-day period (week). Incidence is *not* necessarily the number of animals showing the effect. The "n" reflects the number of animals on test that week. A single animal may account for multiple observations of hyperactivity within a given day or week, and therefore the incidence reported could be greater than the number of animals on test. In Table 28, the "n" is the average number of animals on test for that time period. For example, during weeks 1-3, the average number of animals at the 1600 ppm dose was 59, reflecting that one animal had died. After 5 weeks, a portion of the animals were sacrificed for blood analysis, which resulted in reduced numbers for weeks 4-9 (18-19 average animals per dose group). In Table 28 the column "average % with hyperactivity" is the number of times hyperactivity was observed, divided by the average number of animals on test for that time period. It is **not** the average percentage of animals exhibiting hyperactivity.

Panel members discussed that using the neurotoxicity data by itself is problematic, due to the incidence measure not reflecting the percentage of animals affected, questions whether the hyperactivity endpoint in the mouse is an adverse effect, and inherent problems regarding observation and measurement of hyperactivity in the mouse. A panelist observed that the 1600 ppm dose level seems to consistently produce hyperactivity effects in both rats and mice, but thought how the authors used the data was very conservative. Others agreed, and expressed concern and discomfort with use of the mouse hyperactivity results alone being used for the critical effect and derivation of an RfC.

The panel discussed other endpoints to determine if a different critical effect would be more appropriate. A panelist noted that increased liver weights and adrenal effects were seen at 1600 ppm and perhaps 1600 ppm would be an effect level, with 400 ppm protecting for all effects seen, including developmental and reproductive toxicity endpoints. Looking at the Yang et al. (2007) study, the 800 mg/kg/day LOEL, when converted to an inhalation concentration (assuming 100% bioavailability) results in an equivalent concentration of 400 ppm. An assessment author pointed out that the Yang et al. (2007) study was oral gavage and therefore

peak concentration and area under the curve need to be considered in extrapolating across routes of exposure. A panelist indicated that this issue reinforces the need for more toxicokinetic data.

A panel member suggested that the authors' case would be strengthened by display of the data for the array of endpoints and comparison of effects at equivalent concentrations. Rather than present a single critical effect, the panel recommended that the authors should consider the entire database and present NOAELs and LOAELs on all the endpoints (e.g., liver, kidney, thyroid, adrenal, developmental, neurotoxicity) in a table so that the reader can see the array of effects. In this way, a fuller understanding of toxicity in the animal studies may emerge. Another panelist suggested that the authors could then select appropriate additional endpoints to calculate BMDs, and provide support that the selection of the neurotoxicity endpoint is a very conservative choice that will encompass any other possible toxicity.

The panel agreed that the use of the neurotoxicity hyperactivity endpoint by itself was not appropriate and recommended the authors array all the data to provide a more complete picture of toxicity of TBAC in the animal studies. They also reiterated their earlier recommendation that toxicokinetic studies be analyzed and/or conducted to show how the formation of TBA from the various pathways of exposure can be integrated into the understanding of the critical effect.

The panel discussed uncertainty factors, recognizing the difficulty and limitations of choosing appropriate factors without clearly identifying the critical effect and dose level. However, panel members discussed the authors' selection of uncertainty factors for use with the neurotoxicity endpoint. The authors used a total uncertainty factor of 100. They selected a factor of 3 for extrapolation from animals to humans (UF_A). This is the default value recommended by EPA (1994) as the RGDR (Regional Gas Deposition Rate) methods were used to account for toxicokinetic differences between species. The default value of 10 was used for intra-human variability (UF_H), and a value of 1 for the subchronic-to-chronic extrapolation (UF_S). Since benchmark dose modeling was used and the effect was mild, no adjustment was made for use of a LOAEL (UF_L). Finally, a factor of 3 was selected for data base uncertainties (UF_D).

Two panel members clarified for others that the RGDR is a dosimetric adjustment used by EPA, several U.S. states and consulting firms for a variety of sponsors to adjust the laboratory animal concentration to an equivalent human concentration and replaces the kinetic half of this uncertainty factor. The RGDR is 1, which leaves a 3-fold factor (1/2 log 10) for uncertainties in toxicodynamics. Another panelist noted that the factor of 3 for kinetic variability might be appropriate and to remove it from the full factor of 10, one might need to know what metabolite is causing the adverse response.

A panel member asked whether the subchronic uncertainty factor is needed for derivation of the acute RfC. Other panelists agreed it is not, but noted that the authors need to make the case that the level chosen for the acute RfC will protect for longer-term effects, that is, that one would not expect to see more toxicity in longer term studies at lower doses.

A panel member also questioned the selection of one for UF_S for the chronic RfC, as the principal study was of subchronic duration. An author noted that there was no consistent trend with time between weeks 4 and 13, indicating that longer durations would not result in effects at

lower doses. Another panel member agreed with the authors' selection of 1, because the array of data indicates that one would not expect to pick up a more sensitive endpoint in a longer-term study. A second panel member favored a UF_S of 3, however, because one cannot be sure that the trend of the critical effect would continue the same out to a chronic duration. However, this same panelist thought that database uncertainty factor should be 1, instead of 3, thereby resulting in a total UF of 100, the same as the authors'.

Another panelist questioned using only a factor of 3 for database uncertainty, given the uncertainties in the overall data base for TBAC, as have been discussed during the meeting and specifically with relation with TBA. Panel members explained the common practice for the database UF with one mentioning that the TBA issues would not generally be addressed by this factor, and another noting that what is usually considered is the availability of five types of studies (subchronic in two different species; developmental toxicity in two species; and a reproductive/two-generation study). Another panelist noted that while the two-generation study is missing, it is not a concern because there was nothing in the one-generation study that indicated a two-generation study was needed. For developmental toxicity, there were no anomalies and no reason to think that TBAC would not produce maternal toxicity in another species or that one would see malformed fetuses. It was suggested that this reasoning be explicitly spelled out in the report. Several panelists were of the opinion that all these considerations of the array of data should be considered together in determining the database uncertainty factor and suggested that looking at the whole picture, an uncertainty factor of 1 would be the most appropriate factor for database.

In conclusion, the panel thought that selection of the critical effect was premature and recommended that the authors array all the endpoints and their effect levels to provide a complete picture and comparison of what is seen at the various concentrations and doses. They recommended that the authors then select appropriate endpoints for benchmark dose modeling and if the hyperactivity is modeled again, it should be modeled as a continuous data set. With regard to the uncertainty factors used by the authors with the hyperactivity endpoint, the panel reached unanimous agreement as follows:

> UF_A – factor of 3 is appropriate, but the authors need to better explain the RGDR and summarize any insights from the toxicokinetic data
> UF_S – factor of 1 could be appropriate but needs further justification in the text; otherwise 3.
> UF_H – default factor of 10 is appropriate.
> UF_L – factor of 1 is appropriate with use of BMDL
> UF_D – factor of 1 is appropriate, but the data need to be arrayed and considered in total, to present a more complete picture to justify moving from the default of 10.

Thus, the overall factor would be 30 or 100, depending on UF_S selection (see discussion above).

A panelist asked about the RfC calculated from human eye irritation data that was also presented in the assessment document. An author noted that they presented this information to demonstrate that the RfC based on hyperactivity in mice would be protective for other endpoints,

including the human eye irritation endpoint. The panelist suggested this needs to be explained as does the basis for use of an uncertainty factor of 2 in that RfC derivation.

8.2.2 Charge Question 11

11) In the assessment document, are the uncertainties fully described and appropriately characterized?

The panel discussed uncertainties and made suggestions for further describing them throughout the meeting and these are covered under the individual questions above.

8.2.3 Charge Question 12

12. Are there additional issues or questions related to the toxicity or risk characterization of TBAC that should be discussed?

The panel members did not have any additional questions or issues. They did provide a few additional suggestions:

- Tables should be fully described and should be able to stand on their own so that the reader need not refer back to the text.
- Within the exposure section, which the panel did not review, there were tables missing and some were mis-numbered. These should be fixed and the authors might also explain why they used California models.
- Providing a diagram of structures of TBAC and related chemicals would be helpful.

In conclusion, the panel did not think that a two-year rodent bioassay would contribute to further understanding of TBAC's toxicity for risk assessment. The panel suggested several ways that the authors could improve their analysis and presentation of the available data. The panel thought that that these improvements might identify key shorter-term or mechanistic studies that could be useful for assessing the risk of TBAC.

9. References

Cederbaum, AL; Cohen, G. (1980). Oxidative Demethylation of T-Butyl Alcohol by Rat Liver Microsomes. Biochemical and Biophysical Research Communications 97(2):730-736.

EPA (Environmental Protection Agency). (1994). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. US EPA, Office of Health and Environmental Assessment, EPA/600/8-90/066F.

EPA (Environmental Protection Agency). (1998). Health Effects Test Guidelines, OPPTS 870.7800, Immunotoxicity. US EPA, Prevention, Pesticides, and Toxic Substances. EPA 712-C-98-351.

Gold, L.S., Zeiger, E. (1997). Handbook of Carcinogenic Potency and Genotoxicity Databases, CRC Press, Boca Raton, 754 pages.

Groth, G; Freundt, KJ. (1994). Inhaled tert-Butyl Acetate and its Metabolite tert-Butyl Alcohol Accumulate in the Blood During Exposure. Humans and Experimental Toxicology 13:478-480.

Hard, GC; Rodgers, IS; Baetcke, KP; Richards, WL; McGaughy, RE; Valcovic, LR. (1993). Hazard Evaluation of Chemicals that Cause Accuymulation of (alpha-2u)-Globulin, Hyaline Droplet Nephropathy, and Tubule Neoplasia in the Kidneys of Male Rats. Environmental Health Perspectives 99:313-349.

HLS (Huntingdon Life Sciences). (2000a). Tertiary-Butyl Acetate. 14 Day Repeat Dose Snout Only Inhalation Toxicity Range Finding Study in Rats. Lyondell Chemical Worlwide, ACR 036/003332.

HLS (Huntingdon Life Sciences). (2000b). Tertiary Butyl Acetate Bacterial Mutation Assay. Lyondell Chemicals Worldwide, ACR 039/002499.

HLS (Huntingdon Life Science). (2000c). TBAC In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes. Lyondell Chemical Company, ACR040/002265.

IARC (International Agency for Research on Cancer). (1999). Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. Eds: Capen, CC; Dybing, E; Rice, JM; Wilbourn, JD. IARC Scientific Publications No. 147. Lyon, France. Available on-line at: http://monographs.iarc.fr/ENG/Publications/pub147/IARCpub147.pdf

ILS Inc. (2006). Quantitation of the Concentration of a2u-Globulin in Kidneys from Male Rats Exposed to Tertiary-Butyl Acetate (TBAC). ILS Study No.:C145-001. May 24, 2007.

McGregor, DB; Brown, A; Cattanach, P; Edwards, I; McBride, D; Caspary, WJ. (1988). Responses of the L5178Y tk+/tk- Mouse Lymphoma Cell Forward Mutation Assay II: 18 Coded Chemicals. Environmental and Molecular Mutagenesis 11:91-118.

McGregor, DB; Cruzan, G; Callander, RD; May, K; Banton, M. (2005). The Mutagenicity Testing of Tertiary-Butyl Alcohol, Tertiary-Butyl Acetate, and Methyl Tertiary-Butyl Ether in *Salmonella typhimurium*. Mutation Research 565:181-189.

NSF International. (2003). t-Butanol. CAS # 75-65-0. Oral Risk Assessment Document. Available for a fee at http://www.techstreet.com/cgi-bin/detail?product_id=1094024.

NTP (National Toxicology Program). (1995). *Toxicology and Carcinogenesis Studies of t-Butyl Alcohol in F344/N Rats and B6C3F1 Mice* (Tech. Rep. Ser. No. 436; NIH Publ. No. 95-3167). Research Triangle Park, NC: NTP.

NTP (National Toxicology Program). (1997). Toxicity Studies of t-Butyl Alcohol (Tech. Rep. Ser. No. 53; NIH Publ. No. 97-3942). Research Triangle Park, NC:NTP.

Tang, G; Wang, J; Zhuang, Z. (1997). Cytotoxicity and Genotoxicity of Methyl Tert-Butyl Ether and Its Metabolite to Human Leukemia Cells. Chin. J. Prev. Med. 31(6).

Williams-Hill, D; Spears, CP; Prakash, S; Olah, GA; Shamma, T; Moin, T; Kim, LY; Hill, CK. (1999). Mutagenicity Studies of Methyl-tert-butylether using the Ames Tester Strain TA102. Mutation Research 446:15-21.

WIL Research Labs. (2006a). A 13-week subchronic inhalation toxicity study of tertiary-butyl acetate in CD-1 mice. Report WIL-14061. October 31, 2006.

WIL Research Labs. (2006b). A combined 13-week subchronic inhalation toxicity study and reproductive toxicity screening test of tertiary-butyl acetate in rats. WIL-14060. October 31, 2006.

WIL Research Labs. (2007). A Two-week Inhalation Toxicity Range-finding Study of Tertiary-Butyl Acetate in CD-1 Mice. WIL-14053. March 6, 2007.

WIL Research Labs. (2008). A Toxicity Study of Tert-Butyl Acetate (TBAC) in Mated Female Rats. WIL-14069. September 2, 2008.

Yang, YS; Ahn, TH; Lee, JC; Moon, CJ; Kim, SH; Park, SC; Chung, YH; Kim, HY; Kim, JC. (2007). Effects of Tert-Butyl Acetate on Maternal Toxicity and Embryo-Fetal Development in Sprauge-Dawley Rats. Birth Defects Research (Part B) 80:374-382.

Yuan, Y; Wang, HF; Sun, HF; Du, HF; Xu, LH; Llu, YF; Ding, XF; Fu, DP; Liu, KX. (2007) Adduction of DNA with MTBE and TBA in Mice Studied by Accelerator Mass Spectrometry. Environmental Toxicology 630-635.

Zeiger, E; Anderson, B; Haworth, S; Lawlor, T; Mortelmans, K; Speck, W. (1987). *Salmonella* Mutagenicity Tests: III. Results from the Testing of 255 Chemicals. Environmental Mutagenesis 9(Sup 9):1-110.