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July 13, 2009

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-19B
Sacramento, California 95812-4010
Email: coshita@oehha.ca.gov

Re: Ethyl Tertiary Butyl Ether – Proposed Proposition 65 Listing by Labor Code Mechanism

Dear Ms. Oshita:

LyondellBasell is providing comment in response to the "Request for Comments on Chemicals Proposed for Listing by the Labor Code Mechanism (Reproductive and Developmental Toxicants)" that was published on June 12, 2009 and re-posted because of a typographical error on June 25, 2009 by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA). Our comments are directed at the proposed listing of ethyl tertiary butyl ether (ETBE; CAS RN 637-92-3) as toxic to male reproduction based on the American Conference of Government Industrial Hygienist (ACGIH) TLV (ACGIH, 2009 TLVs and BEIs).

The ACGIH listing for ETBE should not be used as basis for California Prop 65 listing for the following reasons:

1. ACGIH SHOULD NOT BE CONSIDERED AN AUTHORITATIVE BODY IN THIS MATTER

ACGIH is a private, non-governmental corporation operated as a not-for-profit enterprise. ACGIH processes do not meet the rigor and transparency requirements to be considered an authoritative body for the classification of carcinogens and reproductive toxicants. In fact the ACGIH policy and position statement on its web site (www.acgih.org) states that "*ACGIH® believes that TLVs® and BEIs® should NOT be adopted as standards without an analysis of other factors necessary to make appropriate risk management decisions.*" This is further reinforced by our second point below.

2. THE REPRODUCTIVE EFFECTS AS A BASIS FOR THE 2001 ACGIH TLV FOR ETBE IS QUESTIONABLE AND THE 2001 ACGIH TLV DOCUMENTATION IS NOT CURRENT ON THE AVAILABLE REPRODUCTIVE EFFECTS INFORMATION FOR ETBE.

In 2001, the ACGIH produced a document describing the reasoning behind setting a TLV/TWA for ETBE. The TLV value of 5 ppm was based upon a NOAEL of 500 ppm for neurological and testicular effects in a subchronic study in rats (Bond J.A. et al. 1996. ETBE: 90-day Vapor Inhalation Toxicity Study with Neurotoxicity Evaluation in F344 Rats. Report No. 95029. Chemical Industry

Institute of Toxicology, Research Triangle Park, NC. Sponsor ARCO Chemical Company) and changes in pulmonary function tests in humans exposed to 25 or 50 ppm ETBE. ACGIH, however, failed to consider the methodological limitations in this study in making their assessment. There were problems inherent with the study design of the Bond et al. study (1996) (improper fixation of the testes tissue) and these make the preliminary findings of an increased "percent of the seminiferous epithelium with spermatocyte degeneration" highly suspect. A discussion of the Bond et al. study (1996) is provided in our attached comments and a full copy of this report is available to OEHHA upon request. ACGIH's 2001 assessment of this study is therefore questionable and not justified as a basis for their TLV.

Since 2001, ETBE has been tested for effects to male reproduction and reproductive effects generally in two studies, a reproductive toxicity probe study using the same strain of rats (Gaoua, W. 2003. Ethyl Tertiary Butyl Ether (ETBE) CAS No. 637-92-3: Reproductive/Developmental Toxicity Dose-Range Finding/Probe Study by the Oral Route (Gavage) in Two Strains of Rats. CIT, Evreux, France. Laboratory Study Number 24168 RSR. Sponsor Totalfinaelf on behalf of the ETBE Producers Consortium) and a definitive two-generation reproductive toxicity study conducted in rats (Gaoua W. 2004. Ethyl Tertiary Butyl Ether (ETBE) CAS No. 637-92-3: "Two-Generation Study (Reproduction and Fertility Effects) by the Oral Route (Gavage) in Rats." CIT, Evreux, France . Laboratory Study No. 24859 RSR. Sponsor Totalfinaelf on behalf of the ETBE Producers Consortium). These studies were conducted in support of a European Union (EU) Risk Assessment that was being performed on ETBE and was submitted to EU regulatory authorities. The preliminary findings of testicular effects from the Bond et al. study (1996) were not replicated in either the probe study using the same strain of rats (Gaoua, 2003) or the definitive two-generation reproductive toxicity study (Gaoua, 2004). These studies demonstrate the lack of effects on endpoints associated with testicular structure and function in the reproductive toxicity study and confirm that ETBE is not a male reproductive toxicant. A discussion of the Gaoua studies (2003, 2004) is provided in our attached comments and full copies of the reports are available to OEHHA upon request.

Thus, although in 2001, ACGIH did establish a TLV for ETBE based in part on reproductive effects from an early study, their assessment is no longer current and should not be used today as a basis for the TLV. According to California Code of Regulations, Title 27, Article 3 (Science Advisory Board: Carcinogen Identification Committee and Developmental and Reproductive Toxicant (DART) Identification Committee) Section 25306(h) "*The lead agency shall find that a chemical does not satisfy the definition of "as causing reproductive toxicity" if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (g), paragraph (1) or subsection (g), paragraph (2)".*

Our comments offer ample evidence that ETBE is not toxic to male reproduction and as such should not be regulated or the public inappropriately warned about a hazard that is not scientifically justified. OEHHA should receive and take into account reliable, scientific information that bears on the listing decision and not accept from a non-authoritative body information that has questionable scientific basis and is not up to date. We believe it is important to provide OEHHA with a full

understanding of these technical issues, as well as to provide a more current evaluation of the available data on ETBE that has been developed since ACGIH's review of ETBE (see attachment). We therefore respectfully request that OEHHA reconsider listing ETBE as toxic to male reproduction.

LyondellBasell appreciates your consideration of our comments. If you have any questions, please contact me at +31 10 275-5512 or marcy.banton@lyondellbasell to discuss.

Regards,

Marcy I. Banton, DVM, PhD, DABVT
Manager, Toxicology and Risk Assessment
LyondellBasell Industries

Attachment

July 13, 2009 LyondellBasell Industries Letter to California OEHHA Regarding Ethyl Tertiary Butyl Ether – Proposed Proposition 65 Listing by Labor Code Mechanism

Attachment

DISCUSSION OF THE WEIGHT OF EVIDENCE OF THE ABILITY OF ETBE TO CAUSE TESTICULAR TOXICITY

REVIEW OF ACGIH TLV DOCUMENT FOR ETBE

In 2001, the American Council of Government Industrial Hygienists (ACGIH) produced a document describing the reasoning behind setting a TLV/TWA for ethyl tertiary butyl ether (ETBE). The TLV value of 5 ppm was based upon a NOAEL of 500 ppm for neurological and testicular effects in a subchronic study in rats (Bond et al., 1996) and changes in pulmonary function tests in humans exposed to 25 or 50 ppm ETBE (ACGIH, 2001). The TLV documentation also notes that there was a 4-week study in rats with no testicular effects. The use of testicular lesions by ACGIH for setting the TLV/TWA for ETBE has been used by the Proposition 65 Group in California to designate ETBE as a reproductive toxicant under the "Labor Code" mechanism.

REVIEW OF THE BOND ET AL. 1996 STUDY

In 1996, Bond and colleagues reported the results of a 90-day vapor inhalation study conducted with ETBE. An increased incidence of degeneration of the seminiferous epithelium was reported following exposure to either 1750 or 5000 ppm of ETBE (6 hrs/day, 5 days/week). All of the male rats in each group (11/group) were described as having degeneration of the testes with the number with "sloughed epithelium" being 7, 3, 3, and 7 for the 0, 500, 1750 and 5000 ppm groups, respectively (Table 25 of the report). In Table 26 of the report, the percent of seminiferous tubules with spermatocyte degeneration was 2, 2, 8, and 13% and lumenal debris was 2, 1, 3, and 1% for the 0, 500, 1750 and 5000 ppm groups, respectively. There were no differences between the exposed groups and the control group in terms of percent of seminiferous tubules in specified developmental stages (Table 27). The standard deviation and ranges for the spermatocyte degeneration for the 0, 500, 1750 and 5000 ppm groups were 1, 2, 4, and 11 (standard deviations) and 1-4, 1-7, 3-16, and 0-39% (ranges of percent animals affected).

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

fixatives such as Bouin's easily penetrate this fibrous capsule. The fixation artifacts that are reported to occur with testes fixed with NBF are sloughing of the seminiferous epithelium and cells within the lumen (luminal debris), the exact findings reported in the ETBE study.

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

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

Given the unfortunate use of NBF as the fixative for the testes, the limited group size and lack of measures of testes function suggests that the Bond study should at best, be considered a screen for possible affects in the testes. It is important to note that there were no other indications of testicular effects (i.e. no effects on testes weight, no effects on any accessory sex organs and no gross findings). The lack of effect on the accessory sex organs suggests that the endocrine function of the testes was normal. The authors of the report appear to understand and appreciate the limitations of this study design for describing effects in the testes with the following statement appearing in the Discussion Section of the report: "The impact of the response of the testes to ETBE would best be measured by sperm count, motility assessment and breeding studies" (Page 78 of the report).

It is not possible from the Bond et al. study report (1996) to ascertain if the testicular staging exercise was added after the in-life portion of the study was completed or exactly how the "percent of the seminiferous epithelium with spermatocyte degeneration" data was collected and analyzed statistically. The wide range of values suggests that these effects may have been limited to a few animals in the mid- and high exposure groups.

STUDIES CONDUCTED AFTER THE BOND ET AL. STUDY AND ACGIH TLV COMPLETION

Prior to the conduct of a two-generation reproductive toxicity study with ETBE, it was necessary to conduct a probe study to assist in setting dose levels for the definitive study. Selection of the appropriate strain of rat was also critical as the Bond et al. study (1996) was conducted with Fischer 344 (F344) rats, an inbred strain commonly used in chronic bioassays. However, the test guidelines from both the US EPA and OECD specifically state that rat strains of low fecundity are not to be used. The F344 strain has low fecundity

compared to the outbred rat strains such as Sprague-Dawley (SD) and Wistar and therefore was not a candidate for use in the two-generation reproductive toxicity study with ETBE.

However, since the original preliminary testes finding was reported in the F344 rat strain, it was necessary to prove that the outbred strain used (either SD or Wistar) was of comparable or greater sensitivity to the toxicity of ETBE as the F344 rat strain. For this reason, the probe study was expanded to include a side-by-side comparison of the possible testicular toxicity in SD and F344 male rats.

Male SD and F344 rats (12/group) were treated by oral gavage with ETBE at dose levels of 0, 30, 100, 300 or 1000 mg/kg bwt/day for a total of twelve weeks (7 days/week) (Gauou, 2003). Treatment started when the male rats were six weeks old (approximately at the onset of puberty). Examination of the male rats at necropsy included many endpoints specific to the testes. These included testes and epididymal weights (individually; absolute and relative to body weights), epididymal sperm analysis (sperm motility, morphology and count), testicular sperm analysis (left testis; homogenization resistant spermatid head count), preservation of right testes and right epididymides in Bouin's fixative and preservation of prostate and seminal vesicles in 10% buffered formalin. Histopathological examination of the prostate and seminal vesicles followed routine microscopic preparation (H&E staining) while the testes and epididymides were stained with hematoxylin and PAS. All of these endpoints were collected on all of the male SD and F344 rats from all exposure groups.

In addition, the SD rats were used in a mating trial with SD female rats as part of the probe to assist in setting dose levels for the definitive two-generation reproductive toxicity study.

No effects from ETBE exposure were noted in either the F344 or SD strains of male rats. Extensive histopathological examination of the testes from all the male rats from both strains failed to find any effects due to ETBE. As is normally reported for both of these strains, there was minimal desquamated spermatocytes and minimal degeneration of seminiferous tubules in occasional animals from the control and treated groups. The incidence and severity of these common, spontaneous lesions were normal for rats of this age and of these two strains. Measures of sperm function (motility, morphology, and count) were comparable between the control and treated groups for these two strains.

In conclusion, there were no effects of ETBE exposure on any measure of testicular function or any effects on male secondary sex organs in this probe study. The findings in this study in terms of possible effects on male F344 rats are more definitive that the Bond et al study (1996) since the animals were treated continuously (7 days/week vs. 5 days/week), longer (12 vs. 9 weeks), and the measures of testicular function collected are more sensitive to possible adverse effects than those used in the Bond et al study (1996). In addition, the correct fixative (Bouin's) was used in this study vs. the use of an incorrect fixative (10% neutral buffered formalin) in the Bond et al. study (1996).

In 2004, Gaoua reported a two-generation reproductive toxicity study with ETBE conducted using the oral gavage route of administration. The study was designed to comply with OECD Guideline 416, EPA Guideline OPPTS 870.3800 and EC Commission Directive 87/302/EEC. The dose levels selected were 0, 250, 500, and 1000 mg/kg/bwt/day (continuous seven days/week exposure). Endpoints of testicular toxicity that were collected included mating and fertility, testes weights of all F0 and F1 adult males, epididymal sperm count, motility and

morphology, testicular homogenization resistant head count, daily sperm production (calculated), gross pathological examination of testes, and histopathological examination of Bouin-fixed testes (using hematoxylin/PAS staining). The histopathological examination included all of the male rats from all four exposure groups. The histopathological examination included a detailed exam to evaluate retained spermatids, missing germ cell layers or types, multinucleated giant cells and/or sloughing of spermatogenic cells in the lumen. The detailed examination of the testes was expanded beyond what is currently required in the test guideline to fully explore the possibility of finding effects similar to those reported in the Bond et al. 90-day inhalation study (2009).

There were no effects on any testicular parameters that were related to exposure to ETBE. The background incidence of testicular lesions was distributed within all of the exposure groups, including the control animals and the type of lesion were that normally noted in this strain of rat at this age. There was no effect of ETBE on sperm function, count, motility or morphology.

COMPARISON OF THE BOND ET AL. AND GAOUA STUDIES

Of the two studies discussed above, the two-generation reproductive toxicity study is clearly the more robust study for determining effects in the testes. In addition, the two-generation study used group sizes of 25 males/group, adding significant statistical power to the study design for detecting adverse effects. The two-generation study did not have the significant methodological shortcomings regarding fixation of testicular tissue found in the Bond study and explored for possible testes effects in greater detail, depth and scope. For this reason, the two-generation reproductive toxicity study should be considered the definitive study for determining possible effects of ETBE on the testes. The two-generation study with ETBE did not find any effects on reproduction even at dose levels as high as 1000 mg/kg bw/day and therefore ETBE should not be considered a reproductive toxicant. It is significant that there was marked systemic toxicity in the adult male and female rats in the two-generation reproductive toxicity study, indicating that a maximally tolerated dose level was included in the study design.

Comparison Of the Internal Dose Levels From the Inhalation and Oral Gavage Studies

The oral gavage study used does levels of 0, 250, 500 and 1000 mg/kg bwt/day. Based on the pharmacokinetic studies available with ETBE, it can be safely assumed that 100% of the administered dose was absorbed across the gut into the systemic circulation.

According to McGregor (2007), the inhalation exposures of 0, 500, 1750 or 5000 ppm from the Bond study would have provided an internal systemic exposure of 0, 159, 555, or 1590 mg/kg bwt/day. The Gaoua (2004) study also calculated equivalent systemic exposures but used a higher retention fraction and thus suggested that the values would be approximately 60% higher than what McGregor calculated. All of these calculations are simple approximations given the absorption and metabolism of ETBE following oral and inhalation exposures. What is evident is that there was considerable overlap in exposure levels between the Bond et al. (1996) subchronic study and the Gaoua (2004) two-generation reproductive toxicity study. If the findings of the subchronic study were real, they should have been replicated in the reproductive toxicity study.

However, the Bond et al. study (1996) also only had exposures five of the seven days of the week. The effects on the testes would be expected to be cumulative over time with increasing exposures. Therefore, these dose levels need to be adjusted by a factor of 5/7 or 0.71. The dose levels on a daily basis (averaged over the entire week) would then be 0, 114, 396, or 1136 mg/kg bwt/day. This correction factor brings the internal dose levels from the subchronic study and the reproductive toxicity study even closer in terms of comparable dose ranges used.

CONCLUSION

The preliminary findings of testicular effects from the ETBE subchronic inhalation study (Bond et al. 1996) were not replicated in a reproductive toxicity probe study using the same strain of rats (Gaoua, 2003) or in a definitive two-generation reproductive toxicity study (Gaoua, 2004). The problems inherent with the study design of the Bond study (improper fixation of the testes tissue) makes the preliminary findings of an increased "percent of the seminiferous epithelium with spermatocyte degeneration" highly suspect. The lack of effects on endpoints associated with testicular structure and function in the reproductive toxicity study confirm that ETBE is not a male reproductive toxicant. The ACGIH TLV/TWA document should be updated to include the definitive two-generation reproductive toxicity study and ETBE should not be listed as a Proposition 65 reproductive toxicant since it does not meet the criteria for listing. An outdated ACGIH document should not be the justification for a Proposition 65 listing simply because it is based on incomplete analysis of the research studies available.

REFERENCES

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