***THIS DOCUMENT DELIVERED ELECTRONICALLY***

November 1, 2013

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-19B
Sacramento, California 95812-4010
Email: P65Public.comments@oehha.ca.gov

**Re: Reconsideration of Nine Chemicals Listed under Proposition 65 as Known to Cause Reproductive Toxicity. Chemicals Listed via the Labor Code Mechanism: Ethyl-tert-butyl ether (ETBE)**

Dear Ms. Oshita:

Lyondell Chemical Company, a LyondellBasell company ("Lyondell") is providing comment in response to the "Notice of Availability of Hazard Identification Materials to Support Reconsideration of Listing: Chemicals Listed via the Labor Code as Known to the State of California to Cause Reproductive Toxicity" that was published on September 20, 2013 by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA). Our comments are directed at the hazard identification materials for ethyl tert-butyl ether (ETBE; CAS RN 637-92-3).

ETBE had been listed by OEHHA in 2009 based on the 2001 ACGIH TLV documentation noting male reproductive toxicity that Lyondell disputed in comments submitted to OEHHA on July 13, 2009. In our comments we advised that the reproductive effects used as a basis for the 2001 ACGIH for TLV were questionable and that the 2001 ACGIH TLV documentation was not current on the available reproductive effects information for ETBE.

OEHHA has now determined that the 2009 basis for ETBE's listing is no longer applicable due to the March 2012 federal Hazard Communication Standard that removed reference to ACGIH TLVs as a mandatory basis for establishing that chemicals are hazardous and is reconsidering ETBE for reproductive toxicity listing through the Developmental and Reproductive Toxicant Identification Committee (DART IC). In support of their reconsideration, OEHHA has performed literature searches and data summaries that are documented in the subject report.

Lyondell welcomes OEHHA's reconsideration of ETBE and reproductive toxicity and appreciates the opportunity to comment on the hazard identification materials prepared for ETBE. Below we offer our general comments on the literature search and information provided to DART IC and then our specific comments on the hazard identification materials for ETBE.

General Comments

The literature search design and process described in Appendix A appears generally appropriate for identification of governmental and published information on the reviewed chemicals. However, proprietary reports may have been under-represented in the search. An additional database recommended for chemical data is the European Chemical Agency (ECHA) database for registered substances available at: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances. For chemicals registered for use in Europe (includes ETBE), this database includes both published and proprietary information on reproductive and developmental toxicity endpoints and hence would be an additional useful information source for OEHHA.

A criticism of the present literature search, however, is that the complete literature search results for the chemicals are not provided. Therefore, for transparency, OEHHA is urged to provide the complete results of the literature search for the reviewed chemicals in an appendix to the hazard identification materials.

The data tables prepared for the identified relevant information are useful content for the DART IC evaluation. However, OEHHA is urged to include explicit consideration of study quality issues of the reported studies. Study reliability is key to data evaluation and hence *a priori* criteria (such as Klimish scores) (Klimisch H.J., Andreae M., and Tillmann U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25(1):1-5) should be considered and reported in the data evaluation.

OEHHA has also provided DART IC the original ACGIH TLV documentation for completeness. For a historical perspective, consideration of this information may be useful. However, where ACGIH has updated their TLV documentation since the original content, OEHHA is urged to provide this information also to DART IC to provide ACGIH's more current view.

Comments on the ETBE Hazard Identification Materials

Examination of OEHHA's hazard identification materials for ETBE initially reveals that several major references that should have been included are missing. Below is a list of additional ETBE references that OEHHA should have included in the materials.

1. Gaoua, W. 2004. Ethyl tertiary butyl ether (ETBE): two-generation study (reproduction and fertility effects) by oral route (gavage) in rats. CIT under contract to TOTAL France S.A., Evreux, France; Study No. 24859 RSR. Unpublished report.

2. Japan Petroleum Energy Center. (JPEC). 2008. A prenatal developmental toxicity study of ETBE in rats. Hita Laboratory, Chemicals Evaluation and Research Institute (CERI), Japan. Study No. E09-0006. Unpublished report.

3. de Peyster, A. 2010. Ethyl t-butyl ether: Review of reproductive and developmental toxicity. Birth Defects Res. B Dev. Reprod. Toxicol. 89(3): 239−263.

4. Gaoua, W. 2003. Centre International de Toxicologie (2003). Ethyl tertiary butyl ether (ETBE), CAS No. 637-92-3: Reproduction/developmental toxicity dose-range finding/probe study by the oral (gavage) route in two strains of rat. Testing laboratory: CIT, France. Report no.: 24168 RSR. Report date: 2003-10-02. Unpublished report.

5. ACGIH. 2013. Ethyl tert-butyl ether (ETBE) TLV Recommendation. American Council of Governmental Industrial Hygienists (ACGIH).

6. Berger, T. and Horner, C.M. 2003. In vivo exposure of female rats to toxicants may affect oocyte quality. Reprod. Toxicol. 17:273–281 (erratum appears in Reprod. Toxicol. 2004. 18:447).

The 2010 de Peyster publication reviews all the reproductive and developmental studies conducted to date with ETBE. This review also includes the repeated exposure studies with ETBE where reproductive tissues were examined. The author identified seven reproductive and developmental toxicity studies conducted by standardized test guidelines as well as several repeated exposures studies and research papers. The conclusion of Dr. de Peyster's review is repeated here and is relevant to OEHHA's evaluation: "*ETBE does not appear to be selectively toxic to reproduction or embryofetal development in the absence of other manifestations of general toxicity. Studies using recommended methods for sample preservation and analysis have shown no targeted effect on the reproductive system. No embryofetal effects were observed in rabbits. Early postnatal rat pup deaths show no clear dose-response and have largely been attributed to total litter losses with accompanying evidence of maternal neglect or frank maternal morbidity*."

The current ACGIH documentation does not consider ETBE a testicular toxicant. The 2013 ACGIH document notes the problems with fixation of the testes found within the Medinsky et al. 1999 study (the original basis for the designation) and the lack of testicular effects in both F344 and Sprague-Dawley rats in the 2003 Gaoua study as discussed in the 2010 de Peyster publication. Although the Gaoua 2003 study was designed to be a probe experiment, it had sufficient numbers of animals and strength of study design to replicate the Medinsky et al. 1999 study, without the problematic issue of using an incorrect fixative for the testes. The lack of testicular effects resulted in a NOEL for reproductive endpoints of 1,000 mg/kg bw/day in both the F344 and Sprague-Dawley strains of rats.

The inclusion of the Gaoua 2003 probe study with F334 and Sprague-Dawley rats, the rat two-generation study (Gaoua, 2004), an additional developmental toxicity study in rats (JPEC, 2008), the Berger and Horner (2003) ovarian study and the review by de Peyster (2010) would add significant new information to the toxicity database for ETBE and enlighten the interpretation of the current database by OEHHA officials as well as the DART IC. Inclusion of the 2013 ACGIH TLV Recommendation document demonstrates that ETBE is no longer considered a reproductive toxicant by that authoritative body.

Comments on the Current Tables Included in the ETBE Hazard Identification Materials

The comments on the Table presenting the results of Medinsky et al. 1999 focus on the major design flaw in this study. The testes in this study were fixed in 10% neutral buffered formalin (NBF) rather than using Bouin's or Davidson's fixative for this tissue. The use of the Bouin's or Davidson's solutions for fixing the rat testes is recommended/proscribed in several OECD and EPA Guidelines as well as by pathologists recognized with special expertise in testicular histopathology (Creasy, D. M. 2003. Evaluation of testicular toxicology: A synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology. Birth Defects Res (B) 68:408–415). In addition, as described by Hess, R.A. and B. J. Moore. 1993. Histological methods for evaluation of the testis. In: Methods in Toxicology, vol. 3A: Male Reproductive Toxicology. Chapin RE, Heindel JJ, Tyson CA, Witschi HR, editors. New York: Academic Press. p. 52–85, fixation of testes in 10% NBF results in tissue artifacts (shrinkage, distortion, intracellular vacuoles and displacement of epithelial layers into the tubular lumen) making histopathological examination of the slides from paraffin-embedded sections much more suspect. Since this was the study that gave rise to the subsequent investigations of testicular effects due to ETBE, the shortcomings of the study should appear prominently in the Table in order to allow members of the DART IC to understand the complete history of this issue.

Comments specific to the Table entry for the Medinsky et al. 1999 rat study include the following: With the exception of the testes, the remaining tissues of relevance were only examined histologically from the high exposure (5000 ppm) and control group animals. The 25% decrease in body weight gain in the male and female animals from the 1750 and 5000 ppm groups was restricted to week 1 of the study. The significant decrease in body weight noted in the 5000 ppm female rats was found at termination and the male animals from this group were not affected. Of importance, degeneration of spermatocytes was observed in all male rats that were examined, further supporting the reasoning that there were problems with the fixative used. There was no effect of ETBE exposure on the percent of seminiferous tubules examined for stage of spermatogenesis.

The only comment pertinent to the Medinsky et al. 1999 mouse study was that the tissues of relevance (listed in the rat study portion of the study) were only examined histologically from the high exposure (5000 ppm) and control group animals.

Suggested additions to the De Peyster et al. 2009 table entry include adding additional information under the "Results" and "Comments" column (noted in bold font for this and the other citations). In the "Results" column for "Systemic Toxicity", it should be noted that "**there was no evidence of androgen insufficiency in accessory organ weights and no testicular pathology in a subset of animals (n = 5) at 1800 mg/kg bw**". In the "Results" column for "Reproductive Toxicity", the first sentence should be expanded to "Testosterone reduced to 66% of control at 1800 mg/kg (NS) **with high variability. At 1200 mg/kg, group mean plasma testosterone level was not different from control**." Finally, within the "Comment" column, the following summary should be added "**ETBE altered reproductive steroid levels in peripheral blood 1 hr after treatment but only at extremely high doses that inhibited body weight gain**."

Suggested additions to the Asono et al. 2011 table entry include adding additional information under the "Results" and "Comments" column. An error was noted in the "Endpoints Assessed" column ("Live fetuses were weighed, **sexed** and observed macroscopically for organ abnormalities and skeletal malformations."). In the "Results" column for "Systemic Toxicity", it should be noted that "**the body weight gain in the dams from GD 6 to 28 was decreased to about 50% of the control, but this difference was not statistically significant (not unusual in rabbits**)". The statement in the same column regarding "No differences in food consumption" is misleading. In fact, there was a temporal decrease in food consumption at 1000 mg/kg/day (statistically significant from GD 8-14) that should be documented. Within the "Reproductive Toxicity" column, the following change is suggested "No significant differences in the index of external malformations or incidence of skeletal **or visceral** malformations or variations."

Within the "Comments" column, the following additions are suggested: "**NOAEL (maternal toxicity): 300 mg/kg/day based on decrease in food consumption and an approximately 50% reduction in overall BWG on GD 6-28 in high dose group; NOAEL (reproductive function): 1000 mg/kg/day; NOAEL (developmental toxicity): 1000 mg/kg/day; NOAEL (fetotoxicity): 1000 mg/kg /day. No significant differences in indices of embryo-fetal deaths, number of live fetuses, sex ratio, body weight of live fetuses, or uterus weight**."

Additional information is available from the Fujii, et al. 2010 publication that would enhance the table entry for that study. Suggested additions under the "Endpoints Assessed", "Results" and "Comments" column include the following: Under the "Endpoints Assessed" column, the first paragraph can be expanded to "F0: **Mortality, clinical signs,** BW and food consumption; **estrous cycle; copulation, fertility, and gestation indices; gestation period; nursing and lactation behavior.** At necropsy, recorded the number of implantation sites, **gross and histopathology, and select organ weights.**" Also add the following to the end of the second paragraph "**Also recorded viability indices, number of pups born, sex ratio, pup body weights and reflexes.**"

Under the "Reproductive Toxicity" column (under "Results"), the following is suggested to be added to the end of the first paragraph "**All rats delivered between 22-23 days post-mating, considered normal for the facility, no labor was unusually prolonged. There was a significant increase in the number of implantations in 1000 mg/kg group (within normal range of historical controls).**" The second paragraph then requires the following addition "No differences were found in any of the **other** studied parameters for the F1 generation." Under the column "Offspring" (under "Reproductive Toxicity" column), the first paragraph should be modified to: "No significant difference in the number of pups delivered, **pup body weights, sex ratio, viability indices, physical development or reflexes, or sexual maturity**." Finally, within the "Comments" column, the following additions are suggested: "**NOAEL (P-male): 300 mg/kg/day based on significantly increased absolute and relative liver and adrenal weights at 1000 mg/kg/ day. NOAEL (P-female): 300 mg/kg/day based on significantly increased absolute and relative liver weights. NOAEL (F1): 300 mg/kg/day based on slightly reduced pup viability during early lactation at 1000 mg/kg/day.**"

There are no suggested additions to the table entry for the Gaoua 2004 developmental toxicity study.

Additionally, we have taken the liberty of preparing table entries for the four studies that OEHHA did not include in the table for ETBE. These Table entries can be found in the Attachment.

Overall, considering all the available information for ETBE and reproductive toxicity, there is strong evidence that ETBE is not toxic to reproduction and as such should not be regulated or the public inappropriately warned about a hazard that is not scientifically justified.

Lyondell appreciates your consideration of our comments. If you have any questions, please contact me at 713-309-7192 or marcy.banton@lyondellbasell to discuss.

Regards,

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

Attachment

## Attachment

## Ethyl-tert-Butyl Ether (ETBE): Studies Recommended for Inclusion in Hazard Identification Materials

| Reference | Experimental Parameters | | | | | Endpoints Assessed Parents/ Offspring | Results (Effects/NOEL/LOEL) | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Chemical (Source/ Purity/ Preparation) | Animal Model (Species/ Strain/Sex/ Age) N | Study Design | Exposure (Route/Period/ Frequency/ Vehicle) | Doses/ Concen-trations | | Parents | Offspring | |
| Aso (JPEC) 2008 | ETBE Tokyo Chemical Industry Co., Ltd. Lot 74EE 99.3% pure | Crl:CD(SD) male rats, 13 weeks at start of mating; female rats were 12 weeks at start of mating<br><br>21-22 pregnant dams/group | Developmental toxicity study<br><br>Animals treated daily, sacrificed at day 20 post mating.<br><br>Macroscopic examination of dams and fetuses. Litter parameters recorded. | Gavage, once/day ; from day 5 to 19 after mating<br><br>Olive oil vehicle | 0 (vehicle), 100, 300 or 1000 mg/kg/ day | Clinical signs and mortality. Body weight and food consumption. Litter parameters: weight of gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed, sexed. Half of the fetuses from each treatment group were subjected to a detailed examination of soft tissue, while the remainder underwent a detailed skeletal examination. | Except for transient salivation immediately after dosing, no adverse effects were noted in the dams.<br><br>NOAEL (maternal toxicity):<br>1000 mg/kg day | No treatment-related effects on gestational parameters orfetuses<br><br>NOAEL (developmental toxicity):<br>1000 mg/kg/day<br><br>NOAEL (fetotoxicity):<br>1000 mg/kg /day | |

## Ethyl-tert-Butyl Ether (ETBE): Studies Recommended for Inclusion in Hazard Identification Materials (continued)

| Reference | Experimental Parameters | | | | | Endpoints Assessed Parents/ Offspring | Results (Effects/NOEL/LOEL) | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Chemical (Source/ Purity/ Preparation) | Animal Model (Species/ Strain/Sex/ Age) N | Study Design | Exposure (Route/Period/ Frequency/ Vehicle) | Doses/ Concen-trations | | | | |
| Gaoua 2004<br><br>2-gen | ETBE (TOTAL France S.A., Paris-la-Defense, France), batch Nos. S02-08-159-13 (S02-08-159-I3, S02-08-159-I3/2) and 308 596<br><br>Purity >98% | Sprague-Dawley male and female rats, 6 weeks old at start of treatment of F0 generation<br><br>25/sex for each group in the F0, F1 and F2 generations | 2-Generation Reproduction and Fertility Study<br><br>Animals treated daily; after weaning of F1 generation, F0 generation sacrificed; 25 F1 pups/sex were selected and treated daily.At weaning of F2 generation, F1 generation sacrificed and 25 F2 pups/sex were selected and treated until sexual maturity.<br><br>Macroscopic and microscopic exam of males, females in each generation. Microscopic exam of ovaries and testes | Gavage, once/day<br><br>F0: 10 wk before mating, during mating and until sacrifice (after weaning of pups) for males; 10 week before mating, during mating, pregnancy and lactation (until day 21 PP for females<br><br>F1: same conditions<br><br>F2: At weaning, males and females were dosed until sexual maturity<br><br>Corn oil vehicle | 0 (vehicle), 250, 500 or 1000 mg/kg/ day | Clinical signs and mortality; body weights and food consumption; estrous cycle monitored; pregnancy and litter parameters were recorded.<br><br>Pups: survival, clinical signs, body weight, sex ratio, litter size, reflex development, time to acquisition of sexual milestones, neurobehavior-al tests, anogenital distance measured<br><br>Epididymal and sperm parameters examined<br><br>Exams also conducted on non-selected pups | For all generations, a dose-dependent increase in salivation was observed.<br><br>NOAEL (adult toxicity): 250 mg/kg/day (male/female) based on stat. signif. reduction in BWG in F0 males, absolute and relative kidney weight increased in F0 and F1 males, and absolute and relative liver weights in F1 males<br><br>NOAEL (reproduction): 1000 mg/kg day (male/female). Treatment had no effect on mating, fertility, gestation, fecundity, delivery, lactation or sperm parameters. No effects were observed on the progeny from delivery until weaning and there was no effect on sexual maturity. | | |

## Ethyl-tert-Butyl Ether (ETBE): Studies Recommended for Inclusion in Hazard Identification Materials (continued)

| Reference | Experimental Parameters | | | | | Endpoints Assessed Parents/ Offspring | Results (Effects/NOEL/LOEL) | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Chemical (Source/ Purity/ Preparation) | Animal Model (Species/ Strain/Sex/ Age) N | Study Design | Exposure (Route/Period/ Frequency/ Vehicle) | Doses/ Concen-trations | | | | |
| Berger and Horner 2003 | ETBE (Aldrich Chemical CO. Milwaukee, WI) Purity not reported. | Simonson (a SD derived strain) male and female rats. Males were at least 100 days old; females were 28-45 days old<br><br>3-4 females/ replicate; 2 trials of 3 replicates each; # of males not specified | Evaluate fertilizability of rat oocytes *in vitro* following *in vivo* exposure to ETBE<br><br>Untreated males were used as semen donors. Sperm suspensions were prepared at 7 x $10^6$ and 0.5 x $10^6$ sperms/ml.<br><br>Females were treated for 2 weeks before oocyte recovery. Oocytes were removed, processed, mixed with sperm suspension, and incubated for 20 h at 37º C, and evaluated. | Drinking water for 2 weeks before recovery of oocytes and in vitro fertilization | 0 or 0.3% | Weight gain; final weight, percentage of female ovulating; number of oocytes recovered per ovulating female; percentage of oocytes remaining after removal of zona pellucid; percentage of oocytes fertilized; penetrated sperm/oocyte | No statistically significant effects on: final weight; weight gain; percentage of females ovulating; number of oocytes per ovulating female; fragility of oocytes; fertilizability of oocytes. | | Publication concentrated heavily on evaluation of tri- and tetrachloroethylene. Only 1 data table was included for the 4 fuel oxidants studied. Data for the individual replicates for ETBE were not provided. |

## Ethyl-tert-Butyl Ether (ETBE): Studies Recommended for Inclusion in Hazard Identification Materials (continued)

| Reference | Experimental Parameters | | | | | Endpoints Assessed Parents/ Offspring | Results (Effects/NOEL/LOEL) | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Chemical (Source/ Purity/ Preparation) | Animal Model (Species/ Strain/Sex/ Age) N | Study Design | Exposure (Route/Period/ Frequency/ Vehicle) | Doses/ Concen-trations | | Parents | Offspring | |
| Gaoua, 2003, CIT Report #: 24168<br><br>Preliminary reprod. study and testicular toxicity study comparing SD and F344 strains | ETBE (TOTAL France S.A., Paris-la-Defense, France), Batch Nos.: S02-08-159-I3 and S02-08-159-13/2 Purity>98% | Fischer F344 male rats; 12/group; 6 weeks old at start of treatment<br><br>Sprague-Dawley Crl CD rats; male and female; 24 females and 12 males/group; males - 6 weeks old at start of treatment; females - 10 weeks old at start of treatment. | Reproduction/ developmental dose range-finding study in two strains of rats<br><br>Study to provide information on the effects of ETBE on the integrity and performance of the male and female reproductive systems, including gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation and on the growth and development of offspring until weaning.<br><br>Also evaluated the effects of ETBE on spermatogen-esis in 2 strains of rat. | Gavage, once/day<br><br>Fischer rats: Male rats treated daily for 12 weeks<br><br>Male SD rats: daily for 12 weeks<br><br>Female SD rats:<br>-Pre-mating (2 wk)<br>-Mating (2 wk)<br>-Pregnancy (until Day 19 post-coitum for 12 females/dose level<br>-Until end of lactation (day 21 post-partum for 12 females/dose<br><br>Olive oil vehicle | 0 (vehicle), 50, 250, 500 or 1000 mg/kg/ day | Clinical signs and mortality; body weight and food consumption; mating data (mating index, pre-coital time and fertility index); seminology; delivery and litter data; observation of the progeny during lactation period; hysterectomy data; pathology of adults.<br>Litter parameters: weight of gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed, sexed, and examined externally for malformations and variations. | No mortality; transient dose-related salivation immediately after dosing in both sexes; no effect on body weight or food consumption for males of either strain at any dose-level; ↓ in BW gain during pregnancy in HD females; no effect on pre-mating period, or mating or fertility indices; no effect on postimplantation and neonatal losses, delivery or sexual differentiation; no effect on ovulation, implantation or fecundity; no macroscopic findings in males or females at any dose level.<br><br>No clear treatment-related effects on sperm parameters or epididymal or testes weights.<br><br>NOAEL (maternal toxicity): 500 mg/kg day (significantly decreased body weight gain at 1000 mg/kg/day)<br><br>NOAEL (F344 and SD parental toxicity): 1000 mg/kg/day<br><br>NOAEL (reproduction): 1000 mg/kg/day (M&F) | No treatment-related effects on gestational parameters or fetal survival or development.<br><br>NOAEL (embryofetal toxicity): 1000 mg/kg /day | |