External Peer Review of Two 2004 Study Reports (Study No. 24860-RSR and Study No. 24859-RSR) on Ethyl Tertiary Butyl Ether (ETBE) Performed by CIT (Centre International de Toxicologie) Under Contract for TOTAL France

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ERG selected reviewers according to selection criteria provided by EPA. EPA confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled EPA's selection criteria. Reviewers conducted the review according to a charge prepared by EPA and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to EPA.

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PEER REVIEW COMMENTS FROM

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Note: Dr. Li conducted this review as a private consultant and not as a representative of the California EPA.

Review of Study Number 24860 RSR, "Prenatal developmental toxicity study by the oral route (gavage) in rats: Test item: ethyl tertiary butyl ether (ETBE) CAS No. 637-92-3"

CHARGE QUESTIONS

1. **Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, gavage exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?**

This study was designed and conducted in compliance with OECD guideline No. 414EC (2001), U.S. EPA guideline OPPTS 870.3700 (1998), and the requirements for teratogenicity study in rodent and non-rodent of EC Commission Directive 87/302/EEC (1987). This reviewer sees no significant issue with the test system or test article employed, endpoints recorded, terminal procedures, statistical analyses, and quality assurance. While administration by oral gavage *per se* is a common method of administration, it may be of concern in this study for two reasons:

1. A significant number of treated animals (approximately 0, 21%, 54%, and 71% of dams in the control, low-dose, mid-dose, and high-dose groups, respectively) had ptyalism (excess salivation). This observation strongly indicates that ETBE caused severe irritation in the stomach of many dosed animals under the experimental condition. Unknown amount of ETBE administered may be excreted directly via excess salivation before the chemical entered into the systemic circulation.
2. Contamination of drinking water with ETBE might cause concern. Therefore, evidence on the developmental toxicity of ETBE following exposure via drinking water is critically needed. It should be noted that ETBE has very low odor and taste thresholds in water (13 and 47 ug/L, respectively). Even a negligible amount or volume of ETBE may make drinking water unpalatable. This might be the reason why the study was done via gavage, not in drinking water or feed.

2. **Are their physiological/toxicological endpoints that should have been assessed that were not part of the investigation?**

Not really. The study was designed and conducted by carefully following several regulatory guidelines. However, because of excess salivation, it might be good to include measurement of blood concentrations of ETBE or its metabolites to verify that high doses indeed produce high internal doses.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

This study and its findings are highly relevant. The data on the developmental toxicity endpoints from individual animals were correctly summarized and interpreted.

With regard to data on the maternal toxicity, the study report appropriately summarized the data about ptyalism in Section 3.3.2, page 21. Table 2 on page 30 presented the number of animals with ptyalism on individual days. Ptyalism occurred to some animals repeatedly on multiple days. For example, a total of 17 animals in the high-dose group had ptyalism during the treatment, but Table 2 listed a total of 41 animals, since many animals had ptyalism more than once. Therefore, the data presented in table 2 is confusing.

Ptyalism was used as an endpoint for clinical signs of toxicity in this study and in the literature (e.g., Schoenig et al. Evaluation of the chronic toxicity and oncogenicity of N,N-diethyl-m-toluamide (DEET). Toxicol Sci. 1999; 47(1):99-109). Therefore, it is not convincing to say that high incidence of ptyalism is not an adverse effect.

4. Is the summary on pages 9-10 and the conclusions on page 25 of report #24860-RSR (prenatal development toxicity study) supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding ethyl tertiary butyl ether?

Excluding the data on ptyalism, the summary on pages 9-10 and the conclusions on page 25 are supported by the data. However, this reviewer believes that the high incidence of ptyalism should be considered as signs for maternal toxicity. Should this endpoint be used as critical endpoint (in addition to the body weight gain), 250 mg/kg may be considered as effective dose, assuming the increase in the incidence (21% vs. 0% in the control) is statistically significant.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

This study was properly planned, conducted, and reported in general. Inclusion of toxicokinetic studies can significantly clarify the issue about high incidence of ptyalism caused by the dosing method (gavage).

Review of Study Numbers 24859 RSR, "Two-generation study (reproduction and fertility effects) by oral route (gavage) in rats: Test item: ethyl tertiary butyl ether (ETBE) CAS No. 637-92-3"

CHARGE QUESTIONS

1. Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, gavage exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

This study was designed and conducted in compliance with a number of guidelines of the OECD, U.S. EPA, Japan, etc. This reviewer found no significant issue with the test system or test article employed, endpoints recorded, terminal procedures, statistical analyses, and quality assurance. While administration by oral gavage *per se* is a common method of administration, it may be of concern in this study for two reasons:

1. A significant number of treated animals in the F0 and F1 generations, especially in males, had ptyalism (excess salivation) in a does-dependent manner. This observation strongly indicates that ETBE caused severe irritation in the stomach of a large number of dosed animals under the experimental condition. Unknown amount of ETBE administered may be excreted directly via excess salivation before the chemical entered into the systemic circulation. While there is no data on the severity of ptyalism in indivudal animals, the higher incidence in the mid- and high-dose groups, as compared to that of the low-dose group, may suggest the internal doses in the mid- and high-dose groups could be lower than one would expect. Conclusions based on dose-response curves may then be compromised because of lack of true dose curves.
2. Contamination of drinking water with ETBE might cause concern. Therefore, evidence on the developmental toxicity of ETBE following exposure via drinking water is critically needed. It should be noted that ETBE has very low odor and taste thresholds in water (13 and 47 ug/L, respectively). Even a negligible amount or volume of ETBE may make drinking water unpalatable. This might be the reason why the study was done via gavage, not in drinking water or feed.

2. Are their physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

This study used a traditional and well recognized design for two-generation toxicity study in rodents. Additional endpoints, such as, neurobehavioral testing for developmental neurotoxicity and anogenital

distance,for developmental reproductive toxicity, were also included. However, because of excess salivation that may resulting in significantly reduced internal doses, inclusion of toxicokinetic studies will significantly enhance the confidence in making conclusions on the toxicity and the associated risks. In addition, because of the concern for the histopathological changes in the F1 males (see answers to Question 3 below), it is important to continue the treatment in the F2 pups until they reach the adulthood and conduct the microscopic examination of these animals.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

This study and its findings are highly relevant. The data on the developmental and reproductive toxicity endpoints from individual animals were in general correctly summarized and interpreted. However, interpretation of the data on ptyalism was not convincing. The histopathological data of the testis from the F1 males was not presented clearly and interpreted convincingly. If these two endpoints are considered as critical effect for the general and male reproductive toxicity, respectively, 250 mg/kg may be considered as LOAEL.

Ptyalism Ptyalism was used as an endpoint for clinical signs of toxicity in this study and in the literature (e.g., Schoenig et al. Evaluation of the chronic toxicity and oncogenicity of N,N-diethyl-m-toluamide (DEET). Toxicol Sci. 1999; 47(1):99-109). Therefore, it is not convincing to say that high incidence of ptyalism is not an adverse effect.

Histopathological data on the testis of F1 males This dataset was summarized in Section 3.6.2.3, page 74, and Table 67, page 281. Individual data were reported in Appendix 67, pages 1389-1442. Several morphological endpoints were used to characterize the structural integrity of the testis.

a. Sertoli cell-only seminiferous tubules or seminiferous tubules lined by Sertoli cells only: this abnormal morphological change occurred in 0, 3, 1, and 3 F1 males in the 0, 250-, 500-, and 1,000-mg/kg/day groups, respectively. The testis from each of these animals also had siginificantly (grade 4-5, except for one animal in the high-dose group) reduced germ cells (tailed and round spermatids, spermatocytes, and spermatogonia), indicating testicular dysgenesis in these animals. The incidence is relatively low, but it rarely occurs in control or normal rats. The biological significance of testicular dysgenesis following oral exposure to ETBE, as observed in this study, should not be dismissed without careful consideration.
b. Degenerated/necrotic cells sloughed in lumen: Sloughing of germ cells into the lumen is not common in normal rats, but is one of the common pathological changes associated with

chemical-induced testicular damages. It is usually present with germ cell degeneration and/or cytoplasmic vacuolization in the seminiferous epithelium. It could also been found in the normal testis that are not fixed well during the histopathological processing. In this study, germ cell sloughing was found in 9 animals in the control group (N=24), with severity of Grade 1 (minimal). The incidence is unusually high, and none of these animals had degenerating germ cells or cytoplasmic vacuolization in the seminiferous epithelium (vacuolated seminiferous tubules or vacuolated Sertoli cells). The reason(s) for this high incidence is unknown. Poor tissue fixation and processing cannot be excluded from consideration without other supporting evidence.

There were 25, 24, and 25 F1 males in the low-, mid-, and high-dose groups, respectively. Animals that had Seroli cell-only (SC-only) seminiferous tubules (ST, 3, 1, and 3 from the three dosed groups, respectively) wouldn't have germ cell sloughing. Therefore, these animals should not be included in the analysis for germ cell sloughing. The table below demonstrates the incidence of germ cell sloughing in the presence of degenerating germ cells or vacuolization in the epithelia.

Doses (mg/kg-day)	**0**	**250**	**500**	**1,000**
Total No. of animals	24	25	24	25
No. of animals having the SC-only STs	0	3	1	3*
No. of animals having seminiferous epithelium	24	22	23	23*
No. (%) of animals having germ cell sloughing	9 (37.5%)	4 (18.2%)	8 (34.8%)	6 (26.1%)
No. of animals having germ cell sloughing with degenerating germ cells in the STs	0	1	0	0
No. (%) of animals having germ cell sloughing with vacuolization in the STs	0 (0%)	2 (9.1%)	5 (21.7%)	3 (13.0%)

Note: Different stages of spermatogenic cycle were present in one animal (No. B2-9395) in the high-dose group, though Grade 1 SC-only STs was found in this animal.

Based on the numbers presented in the table above, it is apparent that the incidence of germ cell sloughing in the presence of vacuolization of the seminiferous epithelium was remarkably increased in the F1 males. The incidence in the high-dose group is relatively lower than that in the low- and mid-dose groups. However, ptyalism occurred more frequently in the high-dose

group, compared to that in the low- and mid-dose groups, suggesting that the internal dose of ETBE in the high dose may not be as high as the study design aimed to achieve. Therefore, lack of clear dose-response relationship is not a convincing reason to reject the biological significance of the increased incidence of germ cell sloughing. More importantly, this histopathological change following oral gavage administration is consistent with similar observations in the testis of Fischer 344 rats following 90-day inhalation exposure (based on the summary from an EU risk assessment on ETBE; see the reported download on October 26, 2008 from http://ecb.jrc.ec.europa.eu/documentation).

4. Is the summary on pages 9-10 and the conclusions on page 25 of report #24860-RSR (prenatal development toxicity study) supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding ethyl tertiary butyl ether?

Summary is presented on pages 16-21 in this report of two-generation reproductive toxicity study. The conclusions in the summary on page 21 are the same as that on page 76. Excluding the data and corresponding interpretations on ptyalism and histopathological changes in F1 males (see answers to Question 3 above), the summary and the conclusions are supported by the data. However, if these two endpoints are considered as critical, identification of NOAEL and LOAEL needs to be re-considered.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

This study was overall properly planned, conducted, and reported. Additional experiments and analysis are suggested in the answers to Question 1-3.

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Prenatal Developmental Toxicity Study by the Oral Route (Gavage) on Ethyl Tertiary Butyl Ether (ETBE), Report # 24860 RSR

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1. **Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, gavage exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?**

A total of 4 groups of 24 female rats each were given the control substance (corn oil) or the test substance (ETBE) by gavage at doses of 0, 250, 500 or 1000 mg/kg/day from Gestation Day (GD) 5 to 19. Clinical signs and mortality were checked daily and body weight and food consumption were measured at designated intervals. On GD 20, dams were sacrificed and macroscopically examined. Fetuses were removed by hysterectomy and the weight of the gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses were recorded. Fetuses were weighed, sexed and externally examined. Half of the fetuses from each litter were subjected to soft tissue examination and the other half to skeletal staining.

At the highest dose level, ETBE significantly lowered maternal body weight gain and net body weight gain over the treatment period. There were no adverse effects on any fetal parameter measured in the high dose group. In the low and mid dose groups, there were no treatment-related effects on dams, gestational parameters or fetal measurements. The No observed Adverse Effect Level (NOAEL) for maternal toxicity was 500 mg/kg/day, and the NOAEL for embryofetal development was 1000 mg/kg/day.

The experimental design for this study was in compliance with OECD guidelines No. 414, US/EPA guidelines OPPTS 870.3700, and the EC commission Directive 87/302/EEC. The experimental design, test system and test article employed were all appropriate for a developmental toxicity study following these guidelines. The endpoints recorded, terminal procedures, statistical analyses and quality assurance were also appropriate for such a guideline-driven study.

2. **Are their physiological/toxicological endpoints that should have been assessed that were not part of the investigation?**

The investigators included all appropriate physiological/toxicological endpoints for a guideline-driven developmental toxicity study. They exceeded guidelines by including double staining of fetal skeletons to detect alterations in developing bone as well as cartilage, which provides a far more accurate appraisal of skeletal development.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

This is a clean and well-conducted study carried out by an experienced lab. There was only one protocol amendment that was fairly innocuous. The data are consistent and credible, and the toxicological results are relevant and credible. Individual animal data were presented in exhaustive detail, allowing construction of the fate of each dam and fetus in the study. The final conclusions drawn by the investigators are supported by the data. This study should be considered definitive in assessing the developmental toxicity of ETBE.

4. Is the summary on pages 9-10 and the conclusions on page 25 of report #24860-RSR (prenatal development toxicity study) supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding ethyl tertiary butyl ether?

The summary on page 9 and the conclusions on page 25 of the report are supported by the data. The use of historical control data to interpret significant and non-significant trends in fetal skeletal variations in the high dose group is appropriate. Given the high variability in fetal skeletal variation measurements, historical control data provide a better metric for true toxicologic effects. The use of double staining of the skeleton to detect changes in ossification as well as cartilage development has also provided useful information in this study. For the skeletal variations found to be elevated in the high dose group, cartilage was generally present which suggests a slight delay in ossification rather than a permanent alteration in formation of the 4^{th} metacarpal bone**.**

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

The investigation was properly planned, conducted, analyzed and reported. There are no additional procedures, observations or analyses that would have added to the quality of this investigation. This is a definitive and high quality study of the developmental toxicity of ETBE.

Two Generation Study (Reproduction and Fertility Effects) by the Oral Route (Gavage) of Ethyl Tertiary Butyl Ether (ETBE), Report # 24859 RSR

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1. **Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, gavage exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?**

The objective of the study was to evaluate the potential effects of ETBE on the integrity and functioning of the male and female reproductive systems, including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation and weaning, and on the growth and development of the offspring over two generations. Four groups of 25 male and female rats received the vehicle (corn oil) or ETBE at 250, 500 or 1000 mg/kg/day by gavage. For the F_0 generation, treatment continued for 10 weeks before mating, during a 2 week mating period, until sacrifice following weaning of pups (total of 18 weeks). For the F_1 generation, treatment began after weaning on postnatal day (PND) 22 under the same conditions during growth, mating, pregnancy and lactation until weaning of the F_2 generation (total of 10 weeks). The F2 generation was treated under the same conditions until they reached sexual maturity (approximately 7 weeks of age).

For the F_0 generation, clinical signs and mortality were checked daily and food consumption and body weight measured at designated intervals. The estrous cycle was monitored 3 weeks before mating and during the mating period. After mating, the F_0 females were allowed to deliver normally and rear their offspring. Pregnancy and litter parameters were recorded and anogenital distance (AGD) was measured on PND 1. On PND 4, the size of each litter was adjusted to so that eight pups per litter (4 males and 4 females) were retained. At PND 22, one male and female pup per litter were selected to constitute the F_1 generation. F_0 parents were sacrificed after weaning of their offspring

For the F_1 generation, in addition to measurements described for the F0 generation, time to acquisition of sexual milestones (balanopreputial separation and age at vaginal opening) were recorded. Neurobehavioral tests were conducted to assess auditory startle, visual functions and spontaneous motor activity. Estrous cyclicity was monitored followed by mating. F_1 females delivered their litters normally and reared their offspring. The same measurements were made as described for the F_0 generation. On PND 22, one male and female pup per litter were selected to constitute the F_2 generation. Time to acquisition of sexual milestones was recorded as described for the F1 generation. The F2 generation was retained until sexual maturation was reached at approximately 5 weeks of age.

After weaning of their respective progeny, F_0 and F_1 parental animals were sacrificed and selected organs were weighed along with the brain, spleen, thymus and thyroid. Epididymal sperm count,

motility and morphology as well as testicular sperm count were evaluated in males at the time of terminal sacrifice. A macroscopic examination was performed on all F_0 and F_1 parental animals, on F2 animals at sexual maturity and on three weaned (non-selected) pups per sex per litter. Any pups that died during the lactation period or were otherwise not selected for the subsequent generation also had a macroscopic examination. A microscopic examination was performed on any macroscopic lesions, the reproductive organs, adrenals and pituitary glands form all F_0 and F_1 parents from the control and high dose groups together with testes from middle and low dose groups.

The investigators concluded that at the high dose of 1000 mg/kg/day, F_0 females had significantly lower body weight gain at the end of the dosing period, and that liver and kidney weights were significantly elevated in males, accompanied by microscopic findings of hepatocellular hypertrophy and acidophilic granules, respectively. Liver weights were significantly elevated in F_1 males at the high dose. Two pups born to mothers from the F_1 generation had gross external malformations (absence of tail with anal atresia in one pup); the incidence of these anomalies was considered to be within the laboratory or external historical control databases.

At the mid dose of 500 mg/kg/day, lower body weight gain was noted at the end of the dosing period in F_0 males as well as significantly increased kidney weights. Liver and kidney weights were significantly increased in F_1 parental males, but could not be confirmed by microscopic examinations, as these were not conducted in the mid or low dose groups. At the low dose of 250 mg/kg/day, no relevant findings were observed in the F_0, F_1 and F_2 generations. The investigators concluded that the no observable adverse effect level (NOAEL) for systemic toxicity in the adult generations was 250 mg/kg/day, and the NOAEL for fertility, gonadal function, reproductive performance, parturition and lactation and development of offspring to weaning or sexual maturation was 1000 mg/kg/day.
The experimental design for this study was in compliance with OECD guidelines (1997), US/EPA guidelines OPPTS 870.3700 (1989), and the Japanese Ministry guidelines (1984). The experimental design, test system and test article employed were appropriate for a two-generation reproduction and fertility study following these guidelines. The endpoints recorded, terminal procedures, statistical analyses and quality assurance were also appropriate for such a guideline-driven study. The study exceeded guidelines in the inclusion of measurements of sexual maturation and neurobehavioral tests, anogenital distance, epididymal and testicular sperm measurements, and extensive microscopic examination of test animals at terminal sacrifice.

2. Are their physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

See page 71. There was a 10% and 19% increase in relative kidney weights in males from the F_1 generation in the low and mid dose groups, respectively, accompanied by a 58% increase in kidney weights in males from the high dose group that was associated with microscopic evidence of the cortical tubular epithelium. There was no microscopic evaluation of kidneys in the low and mid dose groups despite the fact that macroscopic changes were noted in these kidneys. Microscopic evaluation of kidneys in the low and mid dose groups may have reduced the NOAEL for systemic toxicity in parental generations.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

See page 54. The statement is made that there was a slightly higher post-implantation loss at 250 and 500 mg/kg/day that was not present in the high dose group. In fact, data in Table 18 indicate that the prevalence of post-implantation loss (%) was 4.3, 5.7, 4.9 and 6.5 in the control, low and mid and high dose groups. The table (18) and the appendices (22 and 23) do not have data indicating the extent of post-implantation loss, and it is not clear that any statistical analyses were conducted to determine if the elevation in the high dose group was conducted. The levels of post-implantation loss appear to be within normal ranges, but the data in Table 18 are inaccurate, as is the conclusion that there was no elevation in post-implantation loss in the high dose group.

See page 62. It is stated that the number of F_1 pups that died in the mid and high dose during the lactation period was slightly higher than controls during the first 4 days of lactation. In fact, the number of pups that died during the first 4 days of lactation was significantly elevated in the high dose group (7, 15, 9 and 20 for the control to low, mid and high dose groups). The viability index on PND 1-4 was 97.6, 94.8, 97.0 and 92.9 for the control, low mid and high dose groups. It is not clear whether any statistical analyses were conducted on this viability index. The investigators state that the lactation index (survival of animals from PND 4 to 21) was not altered by treatment, but this does not address the issue that there was a significant elevation in pup mortality from PND 1-4 in the high dose group. The elevation of pup mortality during PND 1-4 in the high dose group should be considered a treatment-related effect.

See page 63. It is stated that in the high dose group there were 2 pups from two different litters with external abnormalities. These consisted of acaudia in both pups and anal atresia in one pup. The investigators concluded that these were most probably spontaneous and not clearly related to treatment. The incidence of acaudia (0.7%) in this study was not within the range of the laboratories

reference range (minimum = 0, maximum 0.05%) but was said to be close to the MARTA historical control database of 0.31%. The prevalence of acaudia in this study was more than double that of the MARTA historical control database and the finding of acaudia in the high dose group should be considered treatment related. There was not summary table for clinical signs and gross external abnormalities in the F_1 pups, and there should be to put this finding into a clearer perspective. The pups that died during PND 1-4 could not be evaluated because of autolysis, and the possibility exists that they also had major malformations that were missed in the study.
See page 64. There was an 11.5% decrease in pup body weight from PND 1-4 in the high dose group that was not statistically significant but clearly above the decrease seen in the lower dose groups. It was concluded that this effect was transient and non-significant, but was possibly related to treatment. The increase in body weight gain, postnatal loss and major malformations in the high dose group are consistent with developmental toxicity in the high dose group.

4. Is the summary and the conclusions supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding ethyl tertiary butyl ether?

The summary on pages 16-20 and the conclusions on page 21- 22 of the report do not mention the increased pup mortality and major malformations in F1 pups in the high dose group. The NOAEL for systemic toxicity to adults was stated to be 250 mg/kg/day, despite uncertainties in the occurrence of microscopic abnormalities in kidneys from the low and mid dose groups. I generally support this NOAEL despite this uncertainty. The NOAEL for reproductive and developmental toxicity was stated to be 1000 mg/kg/day; I do not support this NOAEL. The increased pup mortality on PND 1-4, decreased body weight and the occurrence of major malformations above the laboratory and historical control database indicate that the NOAEL for reproductive and developmental toxicity should be 500 mg/kg/day.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

See above.

PEER REVIEW COMMENTS FROM

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Peer Review of Ethyl Tertiary Butyl Ether (ETBE) Reproductive Toxicity Studies

Prenatal Developmental Toxicity Study

1. Experimental design.

The study design employed met the requirements for the OECD 414 and OPPTS 870.3700 guidelines. All end points required by these guidelines were contained in the study. The Sprague-Dawley rat is a standard species and strain used for the prenatal developmental study. In addition, the ETBE was appropriately characterized and found to be of high purity (>98%). The terminal procedures as described in the report were appropriate to meet the objectives of the study. I did not find any major Quality Assurance problems with the study. I am surprised that the route of administration for this study was oral (gavage). Because this compound is a fuel additive with a low boiling point, the inhalation route would appear to be a more appropriate route of administration. The solubility of ETBE in water is limited; therefore, the authors needed to use corn oil as the vehicle. The authors rationale for the oral route was that uptake from the gastrointestinal tract was expected to be rapid and complete but did not provide any supporting toxicokinetic data. While the OECD 414 guideline states that the oral route is usually used, the route of administration should mimic the most likely route of human exposure.

I do not believe that all of the statistical approaches used in this report were appropriate. The authors repeatedly failed to use the litter as the experimental unit to interpret litter data. For example, % pre-implantation loss, % post-implantation loss, % early resorptions, % late resorptions and % male fetuses used the fetus as the experimental unit rather than the litter. As a result, litters with more fetuses were weighted more heavily in the statistical analyses than litters with fewer fetuses. The appropriate analyses would have calculated values for each litter and then used these values for the statistical analyses. With the later approach, it is also possible to calculate a variance term (e.g. standard deviation).

2. Additional endpoints that should have been assessed.

The authors should have provided data to support the use of the oral route of administration either as part of this study or referencing previously conducted studies. Because the most likely route of human exposure is inhalation, bridging data demonstrating that the oral route provides similar exposure in terms of area under the curve (AUC) and maximum concentration (C_{max}) is missing from this report.

While the guidelines require that only half of the fetuses be examined for visceral findings and half of the fetuses for skeletal findings, had the authors used the fresh dissection technique for the visceral examination, then all of the fetuses could have been examined for both visceral and skeletal findings. This approach would have greatly increased the power of the study. This would have been especially helpful for the interpretation of the missing ribs in the 1000 mg/kg/day group.

3. Strength, credibility and relevance of the toxicological results.

In general, I agree with the interpretation of the maternal data with the following exception. In Section 3.3.3, the authors state that there is a transient reduction in body weight gain during the first 4 days of dosing in the 1000 mg/kg/day group. I disagree. Body weight gain in this group appears to be slightly less than in the control group throughout the dosing period resulting in a statistically significant lower mean body weight gain and net body weight gain over the entire dosing period.

I would have liked to see a better attempt to address the rib findings observed in the 250 and 1000 mg/kg/day groups. Additional use of the laboratory historical control data would have been useful here. In my laboratory, the most common rat skeletal malformations are vertebral and rib findings. They are often seen in combination within the same fetus as occurred in one fetus from the present study in the 1000 mg/kg/day group. I believe that a strong case can be made for a lack of an ETBE-related effect on the fused ribs observed in the 250 mg/kg/day group because no similar finding was observed in the 500 and 1000 mg/kg/day groups. In addition, as previously stated, this is a common skeletal malformation in rats. However, missing a pair of ribs in 2 fetuses from 2 different litters in the 1000 mg/kg/day group is very difficult to ignore. In my laboratory we have seen one or more pairs of ribs missing in only 4 of 49,922 control fetuses examined. Unless the authors can show that this finding occurs more frequently in their laboratory or they provide some additional information, I do not think they can simply ignore this finding in 2 of 136 fetuses because the incidence was low. Malformations observed in a low incidence are often of toxicologic significance.

4. Are summary and conclusions supported by the data?

I agree with the conclusions on p.8 that there was maternal toxicity in the 1000 mg/kg/day group as evidenced by a lower body weight gain over the entire treatment period. I would have pointed out that the change was not of sufficient magnitude to result in a statistically significant lower mean body weight at

this dose. I also agree that there was no maternal or developmental toxicity at dose levels of 250 and 500 mg/kg/day.

Based on the information presented, I do not believe that the absent ribs in the 1000 mg/kg/day group can be ignored. While I do not believe that any of the other findings in the 1000 mg/kg/day group fetuses are related to maternal ETBE treatment, I believe that the NOAEL for prenatal developmental toxicity should be 500 mg/kg/day.

5. Was the investigation properly, planned, conducted and reported?

As I previously stated, I believe the study was properly planned in terms of the study design. However, there are some flaws in the study conduct and the data interpretation, such as the lack of use of litter-based statistics and not providing any evidence as to why the missing 13^{th} ribs in the 1000 mg/kg/day group should not be considered to be developmental toxicity. An additional concern is whether the study should have been conducted via the inhalatory route instead of the oral route (gavage).

Two-Generation Reproductive Toxicity Study

1. Experimental design.

The study design employed met the requirements for the OECD 416 and OPPTS 870.3800 guidelines. All end points required by these guidelines were contained in the study. The Sprague-Dawley rat is a standard species and strain used for the multi-generation study. In addition, the ETBE was appropriately characterized and found to be of high purity (>98%). The terminal procedures as described in the report were appropriate to meet the objectives of the study. I am surprised that the route of administration for this study was oral (gavage). Because this compound is a fuel additive with a low boiling point, the inhalation route would appear to be a more appropriate route of administration. The solubility of ETBE in water is limited; therefore, the authors needed to use corn oil as the vehicle. The authors rationale for the oral route was that uptake from the gastrointestinal tract was expected to be rapid and complete but did not provide any supporting toxicokinetic data. While the OECD 416 guideline states that the oral route is preferred, the route of administration should mimic the most likely route of human exposure.

I do not believe that all of the statistical approaches used in this report were appropriate. The authors repeatedly failed to use the litter as the experimental unit to interpret litter data. For example, % post-implantation loss, % males on post-partum day 1 and % postnatal survival used the pup as the experimental unit rather than the litter. As a result, litters with more pups were weighted more heavily in the statistical analyses than litters with fewer pups. The appropriate analyses would have calculated values for each litter and then used these values for the statistical analyses. With the later approach, it is also possible to calculate a variance term (e.g. standard deviation). In addition, there was no attempt to statistically analyze the estrous cycle data. I have no idea how the authors could make a claim that there were no effects on estrous cyclicity. An even greater problem with the estrous cycle data is that it appears that the individuals determining the stage of estrus were not properly trained. I will address this in more detail in item no.3.

While the study was reviewed by the Quality Assurance Department, there are numerous errors in the report. The most striking error is on the text table in Section 3.6.2.1.1. Large differences are shown in the ETBE groups relative to the control group in the text table for uterine weight. However, these weight differences are not corroborated by Table 64. In addition, the text table in Section 3.6.1.1.1 does not indicate that the kidney weights (absolute and relative) in the 250 mg/kg/day group males were statistically significant relative to the control group as shown on Table 25.

2. Additional endpoints that should have been assessed.

The authors should have provided data to support the use of the oral route of administration either as part of this study or referencing previously conducted studies. Because the most likely route of human exposure is inhalation, bridging data demonstrating that the oral route provides similar exposure in terms of area under the curve (AUC) and maximum concentration (C_{max}) is missing from this report. Otherwise, I believe that the end points included in this study were sufficient to meet the study objectives.

3. Strength, credibility and relevance of the toxicological results.

I have several problems with the data interpretation and study results. The authors lend too much credence to % differences in body weight gain. Over a large period of time this may be appropriate, but over a short time span the results can be deceiving. For example, in Section 3.2.1.3 the authors state that a non-dose-related statistically significant lower mean body weight gain in the 500 and 1000 mg/kg/day group F0 males during study days 85-113 is related to treatment. However, when absolute body weight is assessed in the 500 mg/kg/day group males, the difference following 113 day of dosing is only 1.5% (590 g vs. 599 g; see Table 5, p.115). Therefore, the lower weight gain during study days 85-113 is so minimal that I do not believe it is due to the compound. In addition, a similar lower weight gain was not observed in the F1 males in the 500 mg/kg/day group. In the 1000 mg/kg/day group, the lower weight gain is also of questionable significance because it did not occur in a dose-related manner and was not reproduced in the F1 males at this dose. Furthermore, if the authors believe that the lower weight gain in the F0 males in the 1000 mg/kg/day group is of toxicologic significance (mean weight was 3.7% lower than in the control group on study day 113), then the authors should also consider the 5.5% lower mean body weight in the F0 females in the 1000 mg/kg/day group at the end of the pre-mating period (study day 71; Table 7) of toxicologic significance.

The laboratory performing this study does not understand how to assess vaginal cytology (estrous cycle data). The estrous cycle consists of 4 stages – diestrus (D), proestrus (P), estrus (E) and metestrus (M). While some stages of the cycle may not always be identified when virginal cytology is assessed only once per day, it is not possible to have cycle patterns as reported in Appendices 21 and 55. For example on p.417 F0 control female B29602 is a cycle pattern of EDPPEPPPPPDDDDDDDDDMDM. Based on the endocrinology of the estrous cycle, a rat cannot proceed from E to P. The animal must go from E to M or D. In addition, I have never seen in animal that was in P for 5 consecutive days or seen this in the

literature. Finally, an animal cannot go from D to M without first going through P and E. The cycles for all of the animals are similar to this. If there was a problem with the ability of the control animals to mate, then maybe the cycles could have been disrupted, but the fertility data is clearly normal. Because of the poor data for the control group, there is no way to assess if the estrous cycles were disrupted in the ETBE groups.

In Section 3.2.3, the authors state that post-implantation loss was higher in the 250 and 500 mg/kg/day F0 females due to litters found dead or sacrificed prematurely. This makes no sense. First, the post-implantation loss presented in the text table of this section shows that post-implantation loss is higher in the 1000 mg/kg/day group than in either the 250 and 500 mg/kg/day groups. Secondly, post-implantation loss has nothing to do with postnatal survival. This parameter is the difference between the number of pups born and the number of implantation sites. While I agree that post-implantation loss is unaffected in this study, I question how well the authors understand the end points they are interpreting.

There were 1 and 3 total litter losses in the F0 females in the 250 and 500 mg/kg/day groups. In addition, there was 1 total litter loss in the 250 and 1000 mg/kg/day groups in the F1 generation. Three total litter losses in a group is very concerning to me. The authors simply state that this is not of concern because no total litter losses were observed in the 1000 mg/kg/day group for the F0 generation. I have never seen 3 total litter losses in a control group and the authors do not present any historical control data to indicate how frequently this occurs in their laboratory. If this is not an ETBE-related effect, then I question the husbandry practices of the laboratory. A stronger case needs to be made for the statistically significant increase in pup deaths in the 250 and 500 mg/kg/day groups in the F0 generation. The strongest arguments for a lack of effect in the 500 mg/kg/day group are an absence of effect in the 1000 mg/kg/day group in the F0 generation and an absence of effect in the 500 mg/kg/day group F1 females in the F1 generation.

In Section 3.3.2.1 and 3.3.2.3, the tests for auditory function and locomotor activity were crude. The auditory function test was very subjective and insensitive. There are several manufacturers of automated startle response testing equipment that provide force and time to response data that would be much better tests of auditory function. For motor activity, the test should have been conducted over a longer period of time (approximately 1 hour is typical) to assess exploratory behavior and habituation instead of only a 10-minute session.

In Section 3.3.5.1 the authors claim that the higher number of pup deaths in the 250 mg/kg/day group F2 pups was due to a female with a particularly high litter size. This is not supported by the data. Female number B29739 delivered 11 pups (see p.1096). The number of pups born to this dam was not even equivalent to the mean litter size in this group. The mean number of pups delivered in this group was 13.7. The authors further state a lack of dose response to explain why the significant increase in pup deaths in the 1000 mg/kg/day group is not ETBE-related. A do not see how this claim can be made when the greatest number of pup deaths were in the 1000 mg/kg/day group (the highest dose level tested). A much better argument is that 16 of the 20 pup deaths occurred in only 1 litter (B29799).

In Section 3.3.5.3, I disagree that F_2 pup body weight gain during day 1-4 post-partum is related to treatment in the 1000 mg/kg/day group. Again, using % change in weight gain is misleading. On day 4 post-partum, mean absolute pup weight in the 1000 mg/kg/day group is only 2.2% lower than in the control group (see Table 58). A treatment-related change of this small magnitude cannot be detected.

In Section 3.5, I question the ability of the authors to measure sperm motility. Based on the study methods, it appears motility was determined using light microscopy. Industry standard is to use a computer-assisted sperm analyzer. However, other methods such as video taping and using a frame-by-frame playback can also yield good results. All males in the F0 250 mg/kg/day group had 100% motility. This is not possible. The methods used in this study are very insensitive. In addition, although required by the OPPTS guidelines, progressive motility was not determined (likely due to the insensitivity of the methods used).

4. Are summary and conclusions supported by the data?

I agree with the majority of the conclusions drawn in this study. However, on several occasions the authors used flawed logic to draw their conclusions and did not appropriately use a weight-of-evidence approach. In terms of parental toxicity, the only findings that I believe to be relevant are the liver and kidney weight effects and microscopic findings in these organs. The authors state that the NOAEL for systemic toxicity is 250 mg/kg/day based on body weight and organ weight changes. They completely ignore the statistically significant lower kidney weights in the 250 mg/kg/day F0 and F1 males.

With regards to the NOAEL for fertility, gonadal function, reproductive performance, parturition and lactation in the parental generations and development of the offspring to weaning or sexual maturity, I agree that the NOAEL should be 1000 mg/kg/day. However, as mentioned above, it is not possible to

interpret the estrous cycle and sperm motility data. In addition, the 3 total litter losses in the 500 mg/kg/day group F_0 raises concern for the conduct of the study.

5. Was the investigation properly, planned, conducted and reported.

As I previously stated, I believe the study was properly planned in terms of the study design. However, there are numerous flaws in the study conduct and the approach used to interpret the data, such as the lack of use of litter-based statistics. I cannot interpret the estrous cycle and sperm motility data, and have concerns about the number of pup deaths in the 500 mg/kg/day F_0 generation, especially the amount of cannibalism. These concerns raise question about the strength of the laboratory conducting the study. With these caveats, I still believe that the NOAEL for reproductive and neonatal toxicity is 1000 mg/kg/day. My greatest concern is whether the study should have been conducted via the inhalatory route instead of the oral route (gavage).

ADDITIONAL REFERENCE SUBMITTED BY

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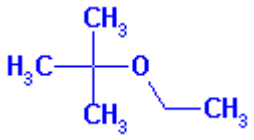
Note: Dr. Li conducted this review as a private consultant and not as a representative of the California EPA.

ECBI/83/06

In accordance with
APPENDIX 1 FORMAT FOR CLASSIFICATION AND LABELLING REPORT
Annex XIV
Proposal for Harmonised Classification and Labelling of a Chemical Substance

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance name: 2-ethoxy-2-methylpropane
EC number: 211-309-7
CAS number: 637-92-3
Registration number(s):
Molecular formula: $C_6H_{14}O$
Structural formula:



Purity: The purity of ETBE depends on production process but is typically from about 80 % up to >> 90 %.

Impurities: Ethanol and tert-butanol are the main impurities.

Proposed classification: Structurally close substance, MTBE (methyl tert- butyl ether), is classified in the 29th ATP with F; R11 Xi; R38. The classification of TAME is concluded in the Technical Committee on Classification and Labelling as F; R11 Xn; R22 R67 but not yet published in an ATP. Neither of the substances is classified dangerous for environment.

According to the information available ETBE would not be classified for environment or human health. The lowest acute aquatic toxicity value is between 10 and 100 mg/l for a marine invertebrate Mysidopsis bahia. Test results and QSAR calculations show also values above 100 mg/l for freshwater fish, algae and Daphnia. The substance is not bioaccumalative judging by the log Kow of 1.48. The substance is not readily biodegradable. This would lead to R52-53 classification. However, it seems likely when comparing the toxicity values in Tables 18 to 20 that the long term NOEC with the most sensitive species for ETBE would not be lower that the respective NOEC for TAME (3.39 mg/l). This would allow the use of the escape clause and the conclusion for the environmental classification of ETBE would be 'not classified for environment'.

ETBE toxicity profile in test animals is similar to those seen with MTBE and TAME. Classification for acute toxicity is not warranted for any route. Signs of CNS depression were reported and they justify an

additional risk phrase of R67. Although ETBE causes slight skin and eye irritation, a classification is not warrented based on the EU classification and labelling criteria. Continued skin exposure to ETBE may lead to eczema due to skin fatigue. ETBE is not sensitising.

In repeated dose toxicity, ETBE's NOAEC is 500 ppm (2.1 g/l) due to histopathological changes in testes and bone marrow and increased liver and kidney weights seen in a 90-day rat study. No classification is foreseen.

ETBE is not mutagenic. The available study on carcinogenicity is not reliable enough to allow judgement of that endpoint.

NOAEL for reproduction was 1000 mg/kg bw/day and NOAEL for adult toxicity was 250 mg/kg bw/day for increased organs weights. This would not lead to classification.

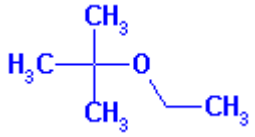
Proposal for harmonised classification: **F; R11; R67**

Proposed labelling: F
Proposed specific concentration limits (if any): None.
Proposed notes (if any): None.

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

Name: 2-ethoxy-2-methylpropane
EC Number: 211-309-7
CAS Number: 637-92-3
IUPAC Name: 2-ethoxy-2-methylpropane
Molecular Formula: $\mathbf{C_6H_{14}O}$
Structural Formula:



Molecular Weight: 102.2 g/mol
Synonyms: 1,1-dimethylethyl-ethyl-ether, tert-butyl-ethyl-ether, ethyl-tert-butyl-oxide, 2-ethoxy-2-methylpropane, ethyl-1,1-dimethylethyl-ether

1.1 Purity/Impurities/Additives

1.2 Physico-Chemical properties

Table 1 Summary of physico-chemical properties of **ETBE**

Some physico-chemical properties	**ETBE**
Water solubility: mg/l at 20 ºC,	23 700 [3] (Chatin et al. 1994) 12 000 [1] (ChemIDplus, Industry, Noureddini)
Vapour pressure: hPa at 20 ºC	165 [1] at 25 ºC (ChemIDplus)
Henry's law constant: Pa m3/mol at 20 ºC	140 [2] at 25 ºC (ChemIDplus)
Log Kow	1.48 [1 [4] at 25 ºC (IUCLID)

[1] experimental; [2] estimated; [3] Elf method; [4]FDA Technical Assistance Handbook Document

2 MANUFACTURE AND USES

Not relevant for this type of dossier.

3 CLASSIFICATION AND LABELLING

ETBE is not currently listed in Annex I.
ETBE is currently self-classificated by industry: **F, R11** (30-NOV-2003)
Source: IUCLID export file

4 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this type of dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

ETBE toxicokinetics has been evaluated only by the inhalation route. The absorption, distribution and elimination of ETBE resembles to that of MTBE (methyl tert-butyl ether). The half-life is shorter in rats compared to humans and ETBE is more efficiently metabolised in rodents than in humans. Upon inhalation exposure the toxicokinetics of ETBE appear to have quite some similarity to that of MTBE. Data on fate after oral and dermal exposure are not available, but the uptake of ETBE from the gastrointestinal tract is expected to be rapid and complete. For MTBE and TAME oral, inhalation and dermal absorptions are set at 100, 40-50 and 17-30%, respectively. Similar absorption figures could be adopted for ETBE. The metabolism of ETBE and MTBE is quite similar and same metabolites are found in humans and in rats, only quantitative differences were evident.

5.2 Acute toxicity (oral, inhalation, dermal)

Table 2 Acute toxicity studies

Animal species & strain	Number of animals per dose level	Doses, route of administration, vehicle	LD_{50} (mg/kg bw)	Purity of ETBE	Guideline	Reference year
Rat, OFA	Male, 5 Female, 5	Oral**, 25, 200, 2000 mg/kg bw, 1% CMC**	**> 2000**	Not stated	OECD 401, GLP	Institut Pasteur de Lille, 1992b
Rat, SD	5 male 5 female	**Oral**, undiluted 2.67 ml/kg, equivalent to 2003 mg/kg	**> 2003**	95.8%	Limit test, OECD 401, GLP	Pharmakon Europe, 1994e
Rat, Wistar	2 males and 2 females per group	500, 100, 2500, 5000 mg/kg bw, oral undiluted	**> 5000**	Not stated.	GLP (in-house protocol)	MB Research Laboratories, 1988a (Unpublished report)
Rat, Crj: CD(SD)	5 males and 5 females	**Inhalation** 4 hours, 5.99 mg/l, vehicle: filtered air	**> 5.88 mg/**l	99%	OECD 403, Limit test, GLP	IIT Research Institute, 1989b
Rabbit, NZW	5 males and 5 females	**Dermal**, undiluted, 2000 mg/kg bwt, occlusion	**> 2000**	Not stated	comparable to OECD 402, Limit test, GLP	MB Research Laboratories, 1988d
Rabbit, NZW	5 males and 5 females	**Dermal**, undiluted, 2000 mg/kg bwt, occlusion	**> 2000**	99%	comparable to OECD 402, Limit test, GLP	IIT Research Institute, 1989a

Conclusion

No classification is proposed for acute toxicity.

5.3 Irritation

5.3.1 Skin

ETBE was not irritating under semiocclusive dressing (Table 3). During 24 hours (Centre International de Toxicologie, 1992a) or 48 hours (Pharmakon Europe, 1994d), slight erythema and/or oedema was observed (mean scores around 1). There was no erythema or oedema present at 72 hours (Centre International de Toxicologie, 1992a) or only slight erythema (score 0.33) (Pharmakon Europe, 1994d). One animal showed slight desquamation at the application site on day 7 (Pharmakon Europe, 1994d).

Table 3 Skin irritation studies

Animal species & strain	Number of animals	Doses	Result	Purity of ETBE	Guideline GLP	Reference
Rabbit, NZW	6 males	100%, 4 hours, semiocclusive	**Not irritating**	Purity not stated	OECD 404 GLP	Centre International de Toxicologie, 1992a
Rabbit, NZW	2 males, 4 females	Undiluted, 4 hours, occlusive	**Moderately irritating** to intact and abraded skin, PDII 3.08	Purity not stated	Comparable to OECD 404 GLP	MB Research Laboratories, 1988b
Rabbit, NZW	3 males	100%, 4 hours, semiocclusive	**Not irritating**	95.8%	OECD 404 GLP	Pharmakon Europe, 1994d

Conclusion

No classification is proposed for skin irritation.

5.3.2 Eye

ETBE was not irritating to the rabbit eye (Table 4). Chemosis (mean score = 0.8) present at 1 hr post treatment had resolved by the 24 hr observation period (Centre International de Toxicologie, 1992b). Scores for discharge, iris effects, corneal opacity and surface opacity were zero at all time points included in the investigation (Centre International de Toxicologie, 1992b).

Mean individual scores for chemosis, redness, congestion and opacity were less than 1 at 24, 48 or 72 hours, except the score for redness was 1.67 at 48 hours (Pharmakon Europe, 1994c). Residual redness in one animal (score = 1) and residual congestion in another (score = 1) had resolved completely by day 7 (Pharmakon Europe, 1994c).

In the unwashed eyes corneal opacity (grade 2) was noted in 1 out of 6 eyes on days 1-3, fully resolved by day 7 (MB Research Laboratories, 1988c). Minor iris involvement (score 1) was also present (same animal) at day 1 only. Conjunctival redness (highest score 2 on days 1 and 2) was present in all animals and was resolved by day 7. Conjunctivae swelling (chemosis, scores 0-2) was present in all animals, resolved by day 7. Scores for 3 washed eyes were generally slightly lower. Overall mean irritation Draize score (maximum possible 110) were 12.2 on day 1, 8.3 on day 2, 4.0 on day 3 and 0 on days 7 and 14.

Table 4 Eye irritation studies

Animal species & strain	Sex, number of animals	Doses	Result	Purity of ETBE	Guideline GLP	Reference
Rabbit, NZW	6 males	100%, 1 ml, 24 hours	**Not irritating**	Purity not stated	OECD 405 GLP	Centre International de Toxicologie, 1992
Rabbit, NZW	3 males	100%, 1 ml, 24 hours	**Not irritating**	95.8%	OECD 405 GLP	Pharmakon Europe, 1994c
Rabbit, NZW	9 animals (females and males	undiluted, 1 ml, 24 hours, rinsed after	**Moderately irritating**	Purity not stated, pure liquid	OECD 405 GLP	MB Research Laboratories, 1988c

Conclusion

No classification is proposed for eye irritation.

5.3.3 Respiratory tract

No data was available to assess respiratory tract irritation.

No classification is proposed for respiratory tract irritation.

5.4 Corrosivity

ETBE is not considered skin or eye irritant or corrosive.

No classification is proposed for corrosion.

5.5 Sensitisation

In a guinea pig maximization test, 30 guinea pigs (Hartley) (controls: 5 males + 5 female, test:10 males + 10 females) were used to study the sensitizing potential of ETBE (purity 95.8%, batch no. 93050601) (Pharmakon Europe, 1994b). For induction, ETBE concentration was 10% intracutaneously and for challenge occlusive epicutaneous application of 100% ETBE was used. As a vehicle sterile liquid paraffin was used. The study was done according to OECD guideline 406 “Skin sensitisation“ and with GLP. Visual examination of the challenge site revealed no evidence of any redness or oedema in any of the control or test animals (all scores = 0). Under the conditions of the test, ETBE did not provoke any sign of cutaneous sensitisation in the guinea pig

Conclusion

No classification is proposed for sensitisation.

5.6 Repeated dose toxicity

5.6.1 Oral studies

No data was available. **No classification is proposed for repeated toxicity via oral route.**

5.6.2 Dermal studies

No data was available **No classification is proposed for repeated toxicity via respiratory route**

5.6.3 Inhalation studies

28-day inhalation study in rats

Survival, body weight and the majority of clinical chemistry and haematological parameters were unremarkable in male and female F344 rats exposed up to 4000 ppm (17 mg/l) ETBE by inhalation for up to 28 days (IIT Research Institute, 1991; White et al., 1995). An increase in relative liver weight in females exposed to 2000 ppm with no accompanying clinical chemistry or histopathological changes appears of doubtful toxicological relevance. Overall the results are consistent with a sub-acute NOAEC for ETBE in the rat of 2000 ppm, based upon CNS depression (sedation and ataxia) seen in animals exposed to higher exposure levels (IIT Research Institute, 1991; White et al., 1995). The author's abstract conclude that repeated exposure to 4000 ppm ETBE produced transient signs of CNS depression and associated changes in body temperature and possibly hind limb splay (Ryan et al., 1991, abstract).

Signs of general sedation and reduced motor activity were noted in rats exposed to 4000 ppm ETBE vapour, with some animals exhibiting mild to moderate ataxia (IIT Research Institute, 1991; Ryan et al., 1991; White et al., 1995). After 3 hours exposure no startle response was evident in the majority of high exposure animals. All treated animals appeared to be in 'sleeping position' (muscle relaxation not evident) during exposure but were normal 15 minutes post-exposure. Other treatment-related clinical signs included salivation, redness around nose/mouth/face and discoloured paws/forelimbs. There were no statistically significant effects of treatment on bw or bw gain or differences in clinical signs, mobility/gait, sensory perception and reflex responses (IIT Research Institute, 1991; White et al., 1995).

Absolute liver kidney and adrenal weights were significantly increased in males at 4000 ppm (Table 5). Absolute and relative liver weight increased also in females at 4000 ppm and only relative at 2000 ppm. No histopathological changes were associated with increased organ weight findings (IIT Research Institute, 1991; Ryan et al., 1991; White et al., 1995).

Table 5 Main findings after 28-day inhalation exposure in rats (IIT Research Institute, 1991; White et al., 1995)

Parameter	0 ppm	500 ppm	2000 ppm	4000 ppm
CNS effects				↑
Organ weights				
Liver, absolute				↑*(16% M, 10% F)
Liver, relative			↑*(10% F)	↑*(16% M, 12% F)
Kidney absolute				↑*(12% M)
Adrenal absolute				↑*(14% M)
White blood cell count			↑*(61% F)	↑*(81% F)

* $p > 0.05$
M=males, F=females

Body temperature was decreased by approx. 0.5 degrees C ($P<0.05$) in 4000 ppm males on day 5 only. Hind limb splay at day 20 was significantly increased (27%; $P<0.05$) when results for high exposure males and females were combined and compared with the controls. However, combined values at day

20 (7.51±1.98) are essentially identical to combined day 0 control and high exposure animal values (8.36±1.91 and 7.45±1.94) suggesting a marginal biological significance (IIT Research Institute, 1991; White et al., 1995).

White blood cell counts were significantly increased in females at 2000 ppm and 4000 ppm without any histopathological changes in bone marrow or evidence of inflammation that could account for these observations. Lung foci, present in all groups were the only macroscopic observation of note (IIT Research Institute, 1991; White et al., 1995)**.** Lymphoid and plasma cell hyperplasia in mandibular lymph nodes, and lymphoid hyperplasia and haemorrhage in the respiratory lymph nodes was commonly seen but not treatment related. (IIT Research Institute, 1991; White et al., 1995)

90-day inhalation study in rats

Survival, body weight and the majority of clinical chemistry and haematological parameters were unremarkable in male and female F344 rats exposed up to 5000 ppm ETBE by inhalation for up to 13 weeks (Table 6) (Bond et al., 1996b; Medinsky et al., 1999). Transient ataxia was the only clinical sign noted (high dose males only, post-exposure only).

Table 6 Main findings in 13-week inhalation exposure study in rats (Bond et al., 1996b; Medinsky et al., 1999)

Parameter	**0 ppm**	**500 ppm**	**1750 ppm**	**5000 ppm**
CNS effects (ataxia)				↑ (M)
Body weight Body weight gain			 ↓* (approx. 25% M&F)b)	↓* (3-6% M, 2-5% F) a) ↓* (approx. 25% M&F) b)
Organ weights Liver, absolute Kidney absolute Adrenal absolute Heart absolute			 ↑*(22-32% M) ↑*(10-19% M, 12-21% F)	 ↑*(22-32% M, 26% F) ↑*(10-19% M, 12-21% F) ↑*(34% M) ↑*(12% F)
Platelet count, wks 6 and 13 MCHC, wks 6 and 13 MCV, wks 6 and 13			 ↓*(2.5% M) c)	↑*(14-16% M) ↓*(2-4% M) ↑*(1% F)
Serum chloride, wks 6, 13 Total protein, wks 6, 13 Phosphorus, wk 6 Bilirubin, wk 6			↓*(3-6% M) ↑*(9-11% M)	↓*(3-6% M) ↑*(9-11% M) ↓*(17% F) ↓*(39% F)

* $p > 0.05$

M=males, F=females

a) during study weeks 1-3
b) during the first week of treatment
c) at study termination only

Kidneys and testes from males and femoral bone marrow from females were the only tissues to exhibit treatment related microscopic lesions. The source references contain only incidence data, and generally no information on severity (Table 7).

Degenerative changes in testicular seminiferous tubules were present in males exposed to ≥ 1750 ppm, while concurrently exposed females responded with increased congestion of femoral bone marrow (Table 7) (Bond et al., 1996b; Medinsky et al., 1999). An increased incidence of renal nephropathy, cell proliferation and accumulation of protein droplets in proximal tubules from all exposed males was associated with positive immunoreactivity toward a2u-globulin, suggesting the findings were specific

to the male rat. Overall these results are consistent with a subchronic NOAEC for ETBE in the rat of 500 ppm, based upon histopathological changes in testes and bone marrow at 1750 ppm (Bond et al., 1996b; Medinsky et al., 1999).

The report notes in those testes with tubular degeneration, aberrant spermatids appeared most common in seminiferous tubules in stages IX-XIII (no further details) (Bond et al., 1996b; Medinsky et al., 1999).

Table 7 Microscopic lesions in kidneys, testes and bone marrow in rats after subchronic inhalation exposure to ETBE (Bond et al., 1996b; Medinsky et al., 1999)

Organ	Observations	LOAEL	Sex affected	Dose response	Remarks
Kidney	Nephropathy (occurrence of regenerative foci)	500 ppm	males	4/11 (36%), 10/11 (91%) 11/11 (100%), 11/11 (100%)	No NOAEC
Testes	Degeneration of the seminiferous tubules a)	1750 ppm	males	2(1), 2(2), 8(4), 13(11)	NOAEC 500 ppm
Bone marrow	Congestion	1750	females	0/10, 0/11, 5/11 (45%), 11/11 (100%	NOAEC 500 ppm

Results of neurotoxicology substudy (Bond et al., 1996b; Dorman et al., 1997)
There were no gross macroscopic changes in brain or nervous tissue. The FOB evaluation found no evidence of sensorimotor dysfunction, neuromuscular dysfunction, ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration or abnormal pupil reflex.

Decreased mean hind limb grip strength and increase in mean hind limb splay were observed in low exposure group males at first exposure, with a statistically significant increase in mean forelimb grip strength in high exposure males on day 10. A statistically significant decrease in mean forelimb grip strength was observed in low dose females following 65 exposures. The absence of any consistent dose-related trend suggests these observations are of doubtful toxicological significance. There was no treatment-related effect on overall motor activity during any observation period.

No gross, functional or microscopic abnormalities were observed in male and female rats exposed up to 5000 ppm ETBE vapour for up to 13 wk.

90-day inhalation study in mice

Survival, body weight, clinical chemistry determinations and haematological parameters were unremarkable in male and female CD-1 mice exposed to 500, 1750 or 5000 ppm ETBE vapour for up to 13 weeks (Bond et al., 1996a; Medinsky et al., 1999). Absolute liver weights were increased in animals exposed to 1750 ppm and above, while an increased incidence of centrilobular hypertrophy was present in high dose animals of both sexes (Table 8). Hepatic cell proliferation (a common non-adverse change seen in mouse liver after exposure to mitogenic substances) was increased following 1-13 weeks exposure to 1750 ppm and above. The NOAEC is 1750 ppm, based on the occurrence of increased liver weight and mild histopathological changes present in animals exposed to 5000 ppm (Bond et al., 1996b; Medinsky et al., 1999). Transient ataxia was occasionally observed post-exposure

in high dose animals of both sexes. Absolute body weight and body weight gain were unaffected by treatment (Bond et al., 1996b; Medinsky et al., 1999).

Haemoglobin and haematocrit values were increased significantly in male mice at 1750 ppm only, possibly unrelated to treatment (12; Medinsky et al., 1999). No treatment related gross abnormalities were present in either sex at scheduled necropsy (Bond et al., 1996b; Medinsky et al., 1999). Absolute liver weights were increased significantly in male and female mice at ≥ 1750 ppm.

Table 8 Microscopic lesions in organs in mice after subchronic inhalation exposure to ETBE (Bond et al., 1996b; Medinsky et al., 1999)

Organ	Observations	NOAEC/ LOAEC	Dose response
Liver	Centrilobular hypertrophy	1750/5000 ppm	males: 0/15, 0/15, 2/15, 8/10* (P<0.05, Fischer's exact test) females: 0/13, 2/15, 1/15, 9/14*(P<0.05, Fischer's exact test)
Kidney	Nephropathy	- /500 ppm for males, 1750/5000 ppm for females	Males: 4/15 (27%), 7/15 (47%), 8/15 (53%), 4/10 (40%) Females: 4/13 (31%), 6/15 (40%), 6/15 (40%), 9/14 (64%)

The observed centrilobular hypertrophy was characterised by enlargement of hepatocytes in the centrilobular to midzonal areas, with homogeneously eosinophilic cytoplasm. The liver of male and female mice exhibited a treatment and concentration dependent increase in cell proliferation. Labelling index (LI) remained elevated in males following 4 wk treatment but returned to control levels by wk 13. In females, LI was comparable to control values at wk 4, but elevated at wk 13 (males unaffected). The study report notes that a transient increase in hepatocyte LI is a common response observed in mice following exposure to mitogenic substances, with a more exaggerated response typically seen in females (Bond et al., 1996b; Medinsky et al., 1999).

The minimal renal nephropathy was characterised by thickened tubular basement membranes, lymphocytic interstitial infiltrates, fibrosis and/or regenerative, basophilic tubules. There was no NOAEC for males. These findings were not considered indicative of a definitive effect of ETBE on mouse kidney (Bond et al., 1996b; Medinsky et al., 1999).

5.6.4 Summary and discussion of repeated dose toxicity

Toxicity profiles for ETBE, MTBE (methyl tert-butyl ether) and TAME (tert-amyl methyl ether) appear to have clear overlap: comparable effects were noticed in liver, kidney and adrenals for all compounds, though of different potency. Based on available inhalation studies, a NOAEC for ETBE appeared to be 500 ppm, based on effects after 90 days exposure in testis and bone marrow in females at 1750 ppm; these effects were not observed with MTBE and TAME. The NOAEC for MTBE was 800 ppm based on mild liver effects in 90-day rat study and for TAME 250 ppm based on organ weight increases in a 90-day rat study, respectively. No repeated oral toxicity studies for ETBE were available. The NOAEL of 250 mg/kg derived from a two generation study (see below) is proposed for repeated dose toxicity for ETBE (based on organ and body weight effects at 500 mg/kg). The NOAELs for MTBE and TAME were 300 mg/kg (based on liver effects in a 90-day rat study) and 125 mg/kg (based on increased adrenal weight in 28-day study), respectively.

Conclusion

Due to clear signs of CNS-depression in at least one inhalation study, the application of R67 is indicated.

However, no classification is proposed for repeated dose toxicity via the respiratory route.

5.7 Mutagenicity

5.7.1 In vitro data

ETBE was not mutagenic to Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 both in the absence and presence of S9 fraction (Table 9). A HGPRT assay and a chromosomal aberration test were negative.

The structures of methyl- and ethyl-tert-butyl-ethers were analyzed by CASE, an expert system, and compared to the structural determinants previously recognized as being associated with carcinogenicity in rodents, mutagenicity in Salmonella or the induction of sister chromatid exchanges and chromosomal aberrations in cultured mammalian cells (Rosenkranz & Klopman, 1991). On the basis of this analysis the two chemicals are predicted to be neither genotoxic nor carcinogens.

Table 9 *In vitro* mutagenicity studies

Test system	Test object	Concentration	Purity of ETBE	Results	Guideline	Reference and year
Bacterial mutation assay	*Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA98, TA 100 (with independent repeat)	First test: 0, 8, 40, 200, 1000 and 5000 µg/plate with and without S-9 mix; Second test: 0, 313, 625, 1250, 2500 and 5000 µg/plate with and without S-9 mix	95.8%	Negative Cytotoxic Concentration: > 5000 µg/plate	OECD 471, Ames test, GLP	Pharmakon Europe, 1994
	Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA98, TA 100 (with independent repeat)	0, 5, 15, 50, 150 and 500 µg/plate with and without S-9 mix	purity not stated	**Negative**, Cytotoxic Concentration: >1500 µg/plate for TA 1537, TA 1538 and TA 98; > 500 µg/plate for TA 1535 and TA 100	Method: Maron and Ames, Mut Res 113, 173 - 215, near-guideline, Ames test, non-GLP	Institut Pasteur de Lille, 1992c
	Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535 (no independent repeat)	0, 100, 333, 1000, 3333, 10000 µg/plate, with and without S-mix	99%	Negative **a),** Cytotoxic Concentration: >10000 µg/plate	Briefly reported experimental investigation from US-NTP, non-GLP	Zeiger et al., 1992

Mammalian gene mutation assay in CHO cells	HGPRT assay	100, 300, 1000, 3000, 5000 µg/ml with and without metabolic activation	98% pure containing 13 ppm antioxidant	**Negative** b), Cytotoxic Concentration: >5000 µg/ml	OECD 476, GLP	Bushy Run Research Center, 1995c
Chromosomal aberration test	Metaphase analysis in CHO cells	0, 300, 1000, 3000, 5000 µg/ml, with and without metabolic activation	98% pure containing 13 ppm antioxidant	**Negative**, Cytotoxic Concentration: >5000 µg/ml	OECD 473, GLP	Bushy Run Research Center, 1995b

a) The report states that ETBE was evaluated using all strains, but results are only presented for TA 100 and TA 1535 in absence of S9.
b) The second test without S9 was repeated due to high values in a vehicle control culture and in the cultures treated with 100, 3000 and 5000 µg/ml. A negative result was obtained.

5.7.2 In vivo data

The two acceptable micronucleus tests were negative (Table 10). Under the conditions of the oral study, no increase in micronuclei was found in male and female mice 24 hr and 48 hr after oral administration of 5000 mg ETBE/kg bwt (Institut Pasteur de Lille, 1992a). All animals survived 48 hr post-dosing with no apparent signs of toxicity.

In the inhalation study, no increase in micronuclei was found in male and female mice following 5 consecutive daily exposures to 400-5000 ppm ETBE vapour (Bushy Run Research Center, 1995a).

Table 10 *In vivo* mutagenicity studies

Test system	Test object	Concentration	Purity of ETBE	Results	Guideline	Reference
Micronucleus assay	OF1 male and female mice	0, 5000 mg/kg bwt, vehicle 1% CMC, single dose, exposure period 24 and 48 hours	Purity not stated,	**Negative**	Directive 84/449/EEC, B.12 "Other effects – Mutagenicity (micronucleus test)", GLP	Institut Pasteur de Lille, 1992a
Micronucleus assay	Male and female CD-1 mice, 5 mice, per sex/dose	0, 400, 2000 or 5000 ppm inhalation, whole-body, 6 hr/d for 5 d	98% (containing 13 ppm antioxidant)	**Negative**	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test", GLP	Bushy Run Research Center, 1995a

5.7.5 Summary and discussion of mutagenicity

ETBE was negative in gene mutation tests (in prokaryotes and mammalian HGPRT), chromosomal aberration test *in vitro* and in two micronucleus tests. Both MTBE and TAME were considered not genotoxic, although some test results were positive or inconclusive. There are no valid data on carcinogenicity available for ETBE. The EU C&L group did not label MTBE for carcinogenicity based on the available data. For MTBE a NOAEC of 400 ppm and a LOAEL of 250 mg/kg was derived for inhalation and oral exposure, respectively. For TAME the available data were considered inconclusive:

it is concluded that there is no concern for some direct carcinogenic action of TAME and a LOAEL of 250 mg/kg is proposed for risk characterisation.

No classification is proposed for mutagenicity.

5.8 Carcinogenicity

5.8.1 Oral studies

One carcinogenicity study has been conducted with ETBE (Maltoni et. al, 1999). However, the study was only a preliminary one and a very brief summary of the study was available.

Materials and methods

60 Male and 60 female Sprague-Dawley rats from the CRC/RF colony were administered ETBE (>94%) in olive oil by gavage at dose levels of 0, 250 and 1000 mg/kg bw for 4 days weekly from age of 8 weeks until to the natural death of the animals. Histopathology was performed on all the macroscopically observed pathological lesions and on skin and subcutaneous tissues, brain, pituitary, Zymbal glands, salivary glands, Harderian glands, cranium, tongue, thyroid and parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and main stem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys and adrenal glands, oesophagus, stomach, intestine, bladder, prostate, uterus, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes. All organs and tissues were preserved, except for bones which were decalcified. The experiments were stated to be conducted in conformity with the principles of GLP.

Results

The last animal died at the age of 145 weeks. No major differences were observed in water or feed consumption or in body weights. There was a dose-correlated increase in mortality starting from the 40^{th} week of the study. The higher earlier mortality was observed in males at both treated groups and in females at high dose. In females, the difference in mortality disappeared on study week about 88 and in low dose males on study week 96. Survival decreased to 50% in control males on study week 88, low dose males on study week 80 and high dose males on study week 72. In females, 50% mortality was achieved between study weeks 84 and 88 in all groups. There was an increase in number of treated females with malignant tumours without any dose-response (Table 11). In treated males total oncological lesions in mouth epithelium was increased. Oncological lesions in forestomach were increased in males at 250 mg/kg bw but not at the high dose level. In uterus, the incidence of sarcoma was increased (1, 8 and 2 at 0, 250 and 1000 mg/kg bw, respectively) and there was one animal with carcinoma in the control group and two animals with carcinomas at 250 mg/kg bw. The incidence of haemolymphoreticular neoplasia was increased in both treated groups without any dose-dependence.

Table 11 Tumours observed after ETBE administration in rats

	Number and percentage (%) of animals with tumours								
Tumour	**0 mg/kg bw**			**250 mg/kg bw**			**1000 mg/kg bw**		
	M	F	T	M	F	T	M	F	T
Number of animals examined	60	60	120	60	60	120	60	60	120
Benign tumours	40 66.7	50 83.3	90 75.0	40 66.7	53 88.3	93 77.5	32 53.3	49 81.7	81 67.5
Malignant tumours	11 18.3	9 15.0	20 16.6	14 23.3	21 35.0	35 29.2	14 23.3	19 31.6	33 27.5

Mouth epithelium	6 10.0	14 23.3	20 16.7	14 23.3	16 26.7	30 25.0	15 25.0*	18 30.0	33 25.5
Forestomach	13 21.7	12 20.0	25 20.8	24 40.0	10 16.7	34 28.3	13 21.7	11 18.3	24 20.0
Uterus	-	2 3.3	-	-	10 16.7*	-	-	2 3.3	-
Haemolymphoreticular neoplasia	3 5.0	3 5.0	6 5.0	8 13.3	6 10.0	14 11.6	6 10.0	5 8.3	11 9.2

* $p \leq 0.05$, χ^2 test
M = males, F = females, T = total

From the oncological lesions in the mouth epithelium squamous cell carcinoma was observed only in treated animals (three females and one male) (Table 12). The incidence of squamous cell dysplasia was increased among males at both dose levels without dose-response. In forestomach, squamous cell carcinoma was found in 4 animals at 250 mg/kg bw only and the incidence of squamous cell dysplasia was increased only in males at 250 mg/kg bw.

Table 12 Oncological lesion in the mouth epithelium (oral cavity, tongue and lips) and forestomach

	Number and percentage (%) of animals with tumours								
Tumour	**0 mg/kg bw**			**250 mg/kg bw**			**1000 mg/kg bw**		
	M	F	T	M	F	T	M	F	T
Number of animals examined	60	60	120	60	60	120	60	60	120
Mouth epithelium Total	6 10.0	14 23.3	20 16.7	14 23.3	16 26.7	30 25.0	15 25.0*	18 30.0	33 25.5
-Squamous cell carcinoma	0	0	0	0	2 3.3	2 1.7	1 1.7	1 1.7	2 1.7
-Squamous cell dysplasia borderline with in situ carcinoma	0	2 3.3	2 1.7	0	1 1.7	1 0.8	1 1.7	2 3.3	3 2.0
-Squamous cell dysplasia	5 8.3	11 18.3	16 13.3	14 23.3	11 18.3	25 20.8	11 18.3	12 20	23 19.2
-Acanthoma	1 1.7	1 1.7	2 1.7	0	2 3.3	2 1.7	2 3.3	3 5.0	5 4.2
Forestomach Total	13 21.7	12 20.0	25 20.8	24 40.0	10 16.7	34 28.3	13 21.7	11 18.3	24 20.0
-Squamous cell carcinoma	0	0	0	3 5.0	3 5.0	6 5.0	0	0	0
-Squamous cell dysplasia	8 13.3	7 11.7	15 12.5	14 23.3	4 6.7	18 15.0	9 15.0	5 8.3	14 11.7
-Acanthoma	5 8.3	5 8.3	10 8.3	7 11.7	3 5.0	10 8.3	4 6.7	6 10.0	10 8.3

* $p \leq 0.05$, χ^2 test
M = males, F = females, T = total

Myeloid leukaemia, lymphoblastic lymphoma and lymphocytic lymphoma were observed only in treated groups without dose-response (Table 13). A total of three males and four females were affected. The incidence of lymphoimmunoblastic lymphoma was slightly higher among treated animals but

again, without a dose-response. Lymphoimmunoblastic lymphoma was most frequently seen in lungs (in 1, 4 and 5 males and 1, 2 and 1 females at 0, 250 and 1000 mg/kg bw, respectively).

Table 13 Haemolymphoreticular neoplasia and its distribution by histiocytotype

	Number and percentage (%) of animals with tumours								
Tumour	0 mg/kg bw			250 mg/kg bw			1000 mg/kg bw		
	M	F	T	M	F	T	M	F	T
Number of animals examined	60	60	120	60	60	120	60	60	120
Haemolymphoreticular neoplasia Total	3 5.0	3 5.0	3 5.0	8 13.3	6 10.0	14 11.6	6 10.0	5 8.3	11 9.2
-Myeloid leukaemia	0	0	0	2 3.3	1 1.7	3 2.5	0	0	0
-Lymphoblastic lymphoma	0	0	0	1 1.7	1 1.7	2 1.7	0	0	0
-Lymphocytic lymphoma	0	0	0	0	1 1.7	1 0.8	0	1 1.7	1 0.8
-Lymphoimmunoblastic lymphoma	2 3.3	1 1.7	3 2.5	4 6.7	3 5.0	7 5.8	5 8.3	2 3.3	7 5.8
-Histiocytic sarcoma	1 1.7	2 3.3	3 2.5	1 1.7	0	1 0.8	1 1.7	2 3.3	3 2.5

* $p \leq 0.05$, χ^2 test
M = males, F = females, T = total

5.8.2 Summary and discussion of carcinogenicity

There were some indications of the carcinogenicity of ETBE, especially in mouth epithelium and forestomach, uterus and haemolymphoreticular system. The exposure route (oral in this study) may have an impact to the induction of the neoplasia in mouth and forestomach.

The main limitations of the study are that only two dose levels were used, the study protocol allowed all animals to die before autopsy and the low survival rate on study week 104. Allowing the animals to reach natural death is quite an unusual practice in carcinogenicity studies.

The higher earlier mortality in treated animals and the low overall survival rate in all animals hamper the study. The survival rate on week 104 (normal length of a carcinogenicity study in rats) was below 20% in males (less than 12 animals alive per group) and below 30% in females. The survival rate decreased to below 50% during study weeks 72-88 in all groups. Using the normal criteria, animals should have been killed at this stage of the study. The mortality was high also in control group, and the normal length of the carcinogenicity study (104 weeks) with 50% survival could not have been achieved in this study even in control animals. The reason for this low survival rate of SD rats in this laboratory was not given in the article. It was considered that the length of life span of most of the animals, including controls, was too short for a rat carcinogenicity study and the length of the exposure was not long enough for proper evaluation of the carcinogenicity. The general state of animal health in the laboratory was not reported.

There was no dose-response in cancer incidences which may be due to that only two doses were studied and the differences in mortality between different groups.

The early results were stated to be published in the article and thus the results are considered only preliminary and no definite conclusion can be drawn from them. The study claimed that the experiment had been conducted following with the principles of GLP. However, no GLP compliance or quality assurance statement regarding this study was available to verify authenticity of the claimed GLP status. Neither is there evidence that the laboratory in question has ever had a GLP status according to the international GLP database.

In conclusion, because of the several shortcomings in the study design, and the preliminary nature of the results, the results were not considered reliable and the carcinogenicity of ETBE could not be evaluated based on this published article.

CASE
The structures of methyl- and ethyl-tert-butyl-ethers were analyzed by CASE, an expert system, and compared to the structural determinants previously recognized as being associated with carcinogenicity in rodents, mutagenicity in Salmonella or the induction of sister chromatid exchanges and chromosomal aberrations in cultured mammalian cells (Rosenkanz & Klopman, 1991). On the basis of this analysis the two chemicals are predicted to be neither genotoxic nor carcinogens.

No classification is proposed for carcinogenicity.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Two-generation study (reproduction and fertility effects) by oral route (gavage) in rats (Gaoua W, 2004)

Guidelines: Design and conduct of the study was in compliance with OECD test guideline 416/Directive 87/302/EEC, ”two generation reproduction test”.
GLP: Yes.

Material and methods
Four groups of 25 female and 25 male Sprague-Dawley rats received the test item (Purity >98%) by daily oral administration (gavage) at 250, 500 or 1000 mg/kg bw/day as follows for the F0 generation: through pre-mating (10 weeks) and mating periods (2 weeks) and until sacrifice (after weaning of the pups for the males, and 10 weeks before mating, during mating, pregnancy and lactation periods (until day 21 post-partum) for the females. A group of 25 males and 25 females received the vehicle (corn oil) under the same experimental conditions and acted as a reference control group.

After weaning of the F1 generation, on day 22 post-partum, three groups of 25 male and 25 female rats (one or two males and females pups per litter) received the test item, under the same experimental conditions as described above, during growth, mating, pregnancy and lactation, until weaning of the F2 generation. At weaning of the F2 generation, on day 22 post-partum, three groups of 25 male and 25 female Sprague-Dawley rats received the test item under the same experimental conditions as described above until sexual maturity.

Mortality and clinical signs were checked daily. Body weights and food consumption were recorded at designated intervals. The oestrous cycle was monitored 3 weeks before mating and during the mating period (for both F0 and F1 adult females). Males and females were paired for up to 2 weeks or until mating occurred. The females were allowed to deliver normally, and rear their progeny. Pregnancy and litter parameters were recorded and anogenital distance was measured on day 1 post-partum (for F1 and F2 pups).

During the lactation period, the pups (F1 and F2 generation) were observed daily for survival and clinical signs; body weight was recorded at designated intervals; the sex ratio was recorded. On day 4 post-partum, the size of each litter was adjusted to give eight pups per litter (four males and four females). Reflex development was assessed at designated time points (F1 and F2 generation). Time to acquisition of sexual milestones (preputial separation/vaginal opening) was recorded for F1 and F2 pups. Neurobehavioral tests were conducted at designated intervals to assess auditory and visual functions for F1 animals. Spontaneous locomotor activity was also evaluated when F1 animals were between 7 and 8 weeks old. After weaning of their respective progeny, F0 and F1 parental males and females were sacrificed.

Selected organs from F0 and F1 parents were weighed together with brain, spleen, thymus and thyroid from one pup per sex per litter from each generation. Epididymal and testicular sperm parameters were evaluated in F0 and F1 males. A macroscopic post-mortem examination was performed on all F0 and F1 parent animals, on F2 animals at sexual maturity and on three weaned (non-selected) pups per sex per litter from each of the F0 and F1 females. Macroscopic lesions, reproductive organs, adrenal glands, and pituitary glands from all parental animals were sampled and preserved. Any macroscopic lesions present in the pups were also preserved. A microscopic examination was performed on any lesions, the reproductive organs, adrenals and pituitary glands from all F0 and F1 parents from the control and high dose groups together with testis from intermediate and low-dose animals. Ovaries and testis were subject to a particularly detailed histological examination.

Results

There was no treatment-related mortality in any groups. Transient excessive secretion of saliva (ptyalism) was observed dose-dependently in all dose groups (Table 14).

Statistically significant reduction in body weight gain was observed in F0 males at ≥500 mg/kg bw/day. F1 generation pup body weight gain was slightly lower during the first 4 days of lactation (12%) at 1000 mg/kg bw/day. In F0 females, slightly higher food consumption was recorded during the lactation period (10%) at 1000 mg/kg bw/day. No changes were noted in body weight and food consumption for other generation or dose groups.

Absolute and relative liver weights were increased in high dose F0 and F1 males and F1 females and appeared related to the presence of slight to moderate centrilobular hypertrophy in liver in males.

Absolute and relative kidney weights were significantly greater in high dose F0 and F1 males and F1 females. The changes in the males correlated with the presence of acidophilic globules (slight to moderate severity) in renal tissue. At 500 mg/kg bw/day, absolute and relative kidney weight increased in F0 and F1males and absolute and relative liver weights in F1 males.

Treatment had no effect on mating, fertility, gestation, fecundity or delivery or sperm parameters. Several F1 pups were found dead or sacrificed due to poor clinical condition at 250 and 500 mg/kg bw/day. At 250 and 500 mg/kg bw/day, one litter with 6 pups and three litters with totally 35 pups were found dead or killed for ethical reason. Mortality among F2 pups was increased at 250 and 1000 mg/kg bw/day, but concerned mainly one litter in each of these dose groups (12 pups at 250 mg/kg bw/day and 16 pups at 1000 mg/kg bw/day. The increased pup mortality at 250 and 1000 mg/kg bw/day among F2 pups was considered spontaneous and not treatment related. The reason for high pup mortality at 250 and 500 mg/kg bw/day among F1 pups before lactation day 4 remains obscure but was partly due to total litter losses. Pup mortality was not increased at 1000 mg/kg bw/day in F1 generation and thus the pup survival was considered not affected by the treatment. No other effects were observed on the progeny from delivery until weaning.

Table 14 Main observations in the two-generation study.

Dose level (mg/kg bw/day)	0	250	500	1000
F0 generation				
Body weight gain	-a	-	↓ **(22% males)*****b	↓ **(29% males)****b
Food consumption	-	-	-	↑(10% females)***c
Ptyalism	-	a few animals	most males, a few females	**most animals**
Liver				
- absolute weight	-	-	-	↑ (17% males)**
- relative weight	-	-	-	↑ (24% males)**
- centrilobular hypertrophy	N	N	N	3/3 (males)
Kidney				
- absolute weight	-	-	↑ (15% males)**	**↑ (21% males)****
- relative weight	-	-	↑ (18% males)**	**↑ (28% males)****
- acidophilic globules	N	N	N	5/6
Sperm analysis	-	-	-	-
Postimplantation loss	4.3	5.7	4.9	6.5
Total litter loss	0	1	3	0
F1 generation adults				
Body weight gain	-	-	-	-
Food consumption	-	-	-	-
Transient ptyalism	-	most males, some females	most animals	most animals
Liver				
- absolute weight	-	-	**↑ (14% males)***	**↑ (27% males)****
				↑ (10% females)*
- relative weight	-	-	**↑ (11% males)***	**↑ (25% males)****
				↑ (9% females)*
- enlargement	-	-	-	observed (males)
- centrilobular hypertrophy	N	N	N	2/2 (males)

Kidney - absolute weight - relative weight acidophilic globules	- - N	- - N	↑ (22% males)** ↑ (19% males)** N	**↑ (58% males)**** **↑ (11% females)**** **↑ (58% males)**** **↑ (10% females)**** 4/4 males
Sperm analysis Total litter loss	- 0	- 1	- 0	- 1
F1 generation, pups Pups/litter Pup weight in day 1 Pup weigh gain, days 1 to 4 Pup weight on day 21 **Pups which died between days 1 and 4 pp** Total number of litters with pups lost during lactation e Total number of decedent pups (%) during lactation Viability index on day 4 (%) Lactation index (%)	328/23 6.6 2.2 49.1 8 (1)e 5 18(5.5) 97.6 94.6	296/21 6.5 2.2 50.4 **21**** (8) 13 **35** (11.8) **92.9** 91.7	327/22 6.3 (-5%) 2.0 (-9%) 49.4 58*** (9) 11 **64** (19.6) **82.3** 96.1	354/25 6.8 2.4 51.5 8 (2) 4 10 (2.8) 97.7 99.5
F2 generation, pups Pups/litter Pup weight in day 1 Pup weigh gain, days 1 to 4 **Pup weight on day 21** **Pups which died between days 1 and 4 pp** Total number of decedent pups (%) during lactation Viability index on day 4 Lactation index (%) Transient ptyalism	297/21 6.7 2.6 50.6 7 11 (4%) 97.6 97.6	288/21 6.5 2.5 51.0 15 17 (5%) 94.8 98.8 a few animals	301/22 6.5 2.5 (-4%) 49.6 9 9 (3%) 97.0 100.0 some animals	290/20 6.6 2.3 (-11.5%) 50.2 **20**** 21 (8%) 92.9 99.3 half of the animals

*p <0.05, ** p <0.01, *** p <0.001 Anova + Dunnett -test.
[a] no change, N= not examined
[b] at the end of the treatment period
[c] during lactation
[e] number of litters in parenthesis, including females with total litter loss

Conclusions

NOAEL for reproduction was 1000 mg/kg bw/day and NOAEL for adult toxicity was 250 mg/kg bw/day for increased organs weights. There was no NOEL for transient ptyalism. The uptake of ETBE from the gastrointestinal tract is expected to be rapid and complete.

No classification is proposed for effects on fertility.

5.9.2 Developmental toxicity

Teratogenicity test by the oral route in the rat

Prenatal developmental toxicity study by the oral route (gavage) in rats (Gaoua W, 2004)

Guidelines: Yes. Design and conduct in compliance with OECD 414 (2001) and Directive 87/302/EEC "Teratogenicity study - rodent and non-rodent", 1987. The procedure complied with the US/EPA guideline OPPTS 870.3700, August 1998.
GLP: Yes.

Material and Methods
Groups of 24 mated female Sprague-Dawley (Crl:CD®(SD) IGS BR) rats received daily oral doses of ETBE (purity >98%) at levels of 0, 250, 500 and 1000 mg/kg bw/day administered by gavage in corn oil from gestation day 5 through 19. Individual maternal food consumption and body weights were recorded at designated intervals. On day 20 post-coitum, the uterus was weighed and the foetuses were removed from dams by caesarean section. The number and location of viable foetuses, early and later resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined grossly. All viable foetuses were weighed, sexed and examined for external malformations and variations. Approximately half of the live foetuses per litter were examined for visceral malformations and variations. The remaining live foetuses were examined for skeletal malformations and variations.

Results
There was no death in any group. Ptyalism (excess salivation) was noted in 5, 13 or 17 females from the 150, 500 and 1000 mg/kg bw/day dose groups at various times during the study. It occurred mainly during the middle part of the investigation and was present immediately post-dosing for one hour. Significant reductions of mean maternal body weight gain were observed at 1000 mg/kg bw/day (Table 15). Food consumption was unaffected by the treatment.

The percentage of early resorptions was slightly higher at 1000 mg/kg bw/day when compared to the control group (7.5% vs 4.8%). The extent of post-implantation loss was consequently minimally increased in the high-dose females (7.5%) in comparison with the controls (5.2%; non-significant).

Except for one foetus with umbilical hernia (malformation) and a bent tail (variation) at 250 mg/kg/day, no other finding was recorded in any group. In the 1000 mg/kg bw/day treated group, an absence of kidneys, ureters and adrenals was observed in a single foetus. In addition following variations were observed: dilated ureters in one foetus from the control, 250 and 1000 mg/kg bw/day groups, short uterine horns in one foetus from the 500 mg/kg bw/day group, dilated renal pelvis in one foetus from the 250 and 1000 mg/kg bw/day groups. Following skeletal malformations were recorded: misaligned sternebrae in one foetus from the control group, at 250 mg/kg bw/day, fused rib and misshapen sacral vertebra in one foetus, split sternebrae and fused ribs in one foetus, at 500 mg/kg bw/day, bilateral misshapen ileum noted in one foetus, at 1000 mg/kg bw/day, absence of one pair of ribs observed in two foetuses, one of which also had an absence of thoracic vertebra. In general, foetal skeletal findings were recorded in all groups without any dose-relationship and/or statistical significance and/or occurred at incidences consistent with historical control background data. The single exception was a significant increase in the incidence of unossified 4^{th} metacarpal at the highest dose: 43/136 foetuses (31.6%, $p<0.05$) were affected in the 1000 mg/kg bw/day group, versus 27/135 (20%9 in the control group. However, when expressed on a litter/litter basis, it was not statistically significant (32.4% vs. 21.4%) and within historical control data. Furthermore when ossification was incomplete, cartilage was generally present suggesting that this skeletal variation was due to slightly delayed ossification rather than to a persistent alteration.

There was no change in foetal viability, weights, placental weights or any other reported parameters. Changes indicate no teratogenic potential of the substance.

Table 15 Main results of the rat teratogenicity study

Parameter	Control	250 mg/kg bw/day	500 mg/kg bw/day	1000 mg/kg bw/day
Pregnant females	21	19	20	22
Maternal body weights (g)				
-day 5 post-coitum	250	250	251	249
-day 9	274	273	274	269
-day 15	317	315	315	307
-day 20 post-coitum	385	382	385	369
Maternal body weight gain (g)				
-days 5 to 9 post-coitum	24	23 (-4%)a	23 (-4%)	20 (-17%)
-days 5 to 20 post-coitum	135	132 (-2%)	134 (-1%)	120* (-11%)
-net body weight change from day 5	61.8	59.4 (-4%)	60.0 (-3%)	51.5** (-17%)
Food consumption (g/rat/day)				
-days 5 to 6	21	21	20	20
-days 15 to 18	27	27	28	26
-days 19 to 20	25	25	26	24
Number of foetuses	255	226	246	258
Live foetuses/litter	12.1	11.9	12.3	11.7
Dead foetuses	0	0	0	0
Resorptions (%)	5.2	6.6	7.2	7.5
Early resorptions (%)	4.8	5.8	6.8	7.5
Late resorptions (%)	0.4	0.8	0.4	0
Postimplantation loss (%)	5.2	6.6	7.2	7.5
Foetal body weights (g)	3.81	3.92	3.83	3.79
Male foetuses	3.92	4.03	3.94	3.91
Female foetuses	3.77	3.82	3.75	3.66
Number of foetuses (litters) with				
-external malformations	0	1(1)[b]	0	0
-external variation	0	1(1)[c]	0	0
-visceral malformations	0	0	0	1/122[d]
-visceral variations	1/120[e]	1/109[e]	1/116[f]	3/122[g]
-skeletal malformations	1/135[h]	2/117[i]	1/130[j]	2/136[k]
-skeletal variations	125/135	101/117	116/130	112/136
-unossified 4th metacarpals	27/135	21/117	24/130	43/136*

*$p<0.05$

[a] % difference from control

[b] umbilical hernia

[c] kinky tail

[d] one foetus with absent kidney, absent adrenal and absent ureter

[e] dilated ureter

[f] short uterine horn

[s] one foetus with dilated renal pelvis, one litter with enlarged kidney, one litter with dilated ureter

[h] misaligned sternebrae

[I] one foetus with misshapen sacral vertebrae and fused ribs, one foetus with split sternebrae and fused ribs

[j] misshapen ileum

[k] two foetuses with absent ribs

Conclusions

The NOAEL for maternal toxicity was 500 mg/kg bw/day (decreased maternal body weight gain) and 1000 mg/kg bw/day for foetal toxicity/teratogenicity. No treatment-related malformations were observed.

No classification is proposed for developmental toxicity.

5.9.3 Other effects
Fertilization *in vitro*

Rats (Simonson, SD derived) were exposed to ETBE via drinking water for two weeks (Berger & Horner, 2003). The concentration of ETBE was 0.3% in drinking water. Female rats (Simonson, SD derived) received either 0.3% ETBE in drinking water or drinking water alone for 2 wk preceding oocyte recovery (n=6 per group). Purity of ETBE was not specified (Aldrich Chemical Co.).

Female body weights, number of females ovulating and the number of oocytes collected was recorded. Ovulation was induced using 15 IU pregnant mare's serum gonadotropin i.p. followed 2 d later by 15 IU human chorionic gonadotrophin i.p. Animals were sacrificed 16-18 hr later, oviducts and attached ovaries removed and isolated oocytes were rinsed and the zona pellucida removed. The motility of collected sperm was evaluated before *in vitro* fertilization. Oocytes were mixed with diluted sperm (7 or 0.5 x 10^6 sperm/ml) in modified Tyrone's medium. Oocytes in all treatments within a replicate were inseminated with aliquots from the same suspension. After 20 hr incubation (37ºC, 5% CO_2) oocytes were rinsed, stained and examined for decondensed sperm heads and attached sperm.

The percentage ovulating females was 72 and 84% in control and ETBE exposed group, respectively. Number of oocytes recovered was 29-30 per ovulating females in both groups. Percentage oocytes fertilized was similar in both groups (84 and 82%) and number of penetrating sperm per oocyte was 1.84 and 1.72 in controls and ETBE groups, respectively. Similarly to ETBE, MTBE treatment did not affect the fertilization of oocytes *in vitro* (Berger & Horner, 2003). However, TAME slightly reduced the percentage of fertilized oocytes (64 vs 84%) and reduced the ratio of penetrated sperm/oocyte (1.54 vs. 1.84).

Models

The prediction of toxicological properties of ETBE and its putative predicted metabolites based on their potential biological reactivity using a SAR model (CASE/MULTICASE) has been published (Zhang et al., 1997). Based on the prediction, several metabolites of ETBE are potential sensory irritants, contact sensitisers, mutagens, developmental toxicants or carcinogens.

The reliability of some of the metabolic predictions included in publication (i.e. putative formation of ethylene oxide from ETBE) is unknown, but appears inconsistent with results from metabolism and toxicokinetic studies available. Because of the unknown validity of this modelled data, it cannot be used in assessment. .

5.9.4 Summary of reproductive toxicity

For ETBE oral two generation reproductive and developmental toxicity studies in rats are available. No reproductive toxicity effects were observed up to and including 1000 mg/kg bw/day.

No classification for reproductive toxicity is indicated.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES

Not relevant for this type of dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

Ready degradability

Not readily biodegradable (Table 16).

Table 16 Biodegradation of ETBE

Substance	Type of test	Detection	Result	Day	Method	Conc. of TS	Inoculum	Ref.
STANDARD "READY BIODEGRADABILITY" TESTS								
ETBE	Closed Respi-rometer	O_2 consump	0%	28	ISO 9408 (comparable to OECD 301F)	150 mg	30 mg/l	Fayolle, F et. al 1998
NON STANDARD BIODEGRADABILITY TESTS ADAPTED INOCULUM								
ETBE	Closed Respiro meter	O_2 consump	85%	28 d	(ISO 9408 standard, modified)	150 mg	380mg/l	Fayolle, F et. al 1998
ETBE	Non standard aerobic		60% minerali-sation	1	Special propane oxidising (28 ^oC)	20 mg/l	70-100 mg/l	Steffan et al., 1997
ETBE	aerobic mineraliz ation	O_2 uptake + GC	0.027 hr^{-1}	160 hr	petroleum refinery wastewater activated sludge	80-100 mg/l	Undefin ed mixed cultures	Cowan et al. (1996)
ETBE	aerobic mineraliz ation		full ETBE mineralizat ion in 1-2 days		Isolated strains of specific ETBE degraders	ca. 200	243 mg/l (as cell protein)	Kharoune , M, et. al 1998

Bioaccumulation

Not bioaccumulative. Log Kow 1.48 (experimental value at 25 °C). Information from IUCLID file. There are no measured bioconcentration factors for fish available.

Aquatic toxicity

(Information related to MTBE and TAME are taken from the risk assessment reports)

Acute

ETBE is very slightly toxic to Daphnia magna and algae. On the other hand ETBE is harmful to a marine invertebrate, Mysidopsis bahia, which is also the most sensitive species tested with MTBE and TAME. No valid fish result is available but it is concluded by QSARS and by comparison with structurally similar substances MTBE and TAME that the acute fish test result would not be lower than the value with Mysidopsis bahia.

Chronic

There is no information available on chronic aquatic toxicity of ETBE. The QSAR calculations in Table 20 seem to indicate that ETBE acts by a non-specific mode of action which is the case also with MTBE and TAME. Considering all information in Tables 17, 18 and 19 together, it seems likely that the chronic NOEC for ETBE, if tested with the most sensitive species Mysidopsis bahia, would be greater than 1 mg/l.

Table 17 Acute toxicity of MTBE, TAME and ETBE to fish, invertebrates and algae

	MTBE	**TAME**	**ETBE**
Acute toxicity	LC/EC50 in mg/l	LC/EC50 in mg/l	LC/EC50 in mg/l
Fish	574 (marine)	580	no valid test available [3
Daphnia magna	472	100	**110 (NOEC 56)** [1 (Wetton & McKenzie 2003)
Mysidopsis bahia, marine	136	EC50: 14	EC50: 37 NOEC: 25 (Boeri et al. 1994)
Algae	ErC50: 184 (96 h)	72 h: EbC50: 230 72 h: ErC50: 780	**72 h: EbC50: 37** [1 **72 h: ErC50: 1100** [2 (Mead 2003)

[1 new studies requested from Industry in bold [2 control growth not exponential after 48 h [3 concentrations not measured; no information of the prevention of volatility

Table 18 Chronic toxicity of MTBE, TAME and ETBE to fish, invertebrates and algae

	MTBE	**TAME**	**ETBE**
Chronic toxicity	mg/l	mg/l	mg/l
Fish	IC20: 279	-	-
Daphnia magna	NOEC: 51	-	-
Mysidopsis bahia (marine)	NOEC: 26	NOEC: 3.39	-
Algae	IC20: 103	NOEC: 77	-

Table 19 QSAR calculations on acute and chronic toxicity of MTBE, TAME and ETBE (calculated according to the ECOSAR developed by U.S. Environmental Protection Agency 2000)

	MTBE	**TAME**	**ETBE**
Acute toxicity	LC/IC50 in mg/l	LC/IC50 in mg/l	LC/IC50 in mg/l
Fish	499 14 d: 779	200 14 d: 338	233 14 d: 389
Marine fish	72	36.9	41.5
Daphnia	501 16 d: 17	208 16 d: 8.77	242 16 d: 9.85
Mysidopsis bahia (marine)	281	79.7	97.5
Algae	297	126	146
Chronic toxicity	Chronic value	Chronic value	Chronic value
Fish	55	24.0	27
Algae	17	9.78	10.8

Proposed classification

No classification for environment.

The substance is harmful to aquatic organisms and it is not readily biodegradable. This would lead to a R52-53 classification. However, it is likely, based on QSARS and comparison with structurally similar substances MTBE and TAME, that the chronic NOEC for the most sensitive species would be greater than 1, leading to no classification for environment.

7.2 Terrestrial compartment
7.3 Atmospheric compartment
7.4 Microbial activity in sewage treatment plant
7.5 Indirect exposure via the food chain/secondary poisoning
7.5.1 Effect data
This may be relevant where data on non-mammalian species are being used.
8 PBT, VPVB AND EQUIVALENT CONCERN ASSESSMENT
Not relevant for this type of dossier.
9 JUSTIFICATION FOR ACTION AT COMMUNITY LEVEL

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