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Preliminary Materials
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Preliminary Materials for the Integrated Risk Information System (IRIS) Toxicological Review of Ethyl *tert*-Butyl Ether (ETBE)

[CASRN 637-92-3]

July 2013

NOTICE

This document is comprised of preliminary materials, consisting of a literature search strategy, evidence tables, and exposure-response arrays. This information is distributed solely for the purpose of pre-dissemination review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

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Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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PREFACE

This document presents the draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays for ethyl *tert*-butyl ether (henceforth referred to as ETBE) prepared under the auspices of EPA's Integrated Risk Information System (IRIS) Program. This material is being released for public viewing and comment prior to a public meeting, providing an opportunity for the IRIS Program to engage in early discussions with stakeholders and the public on data that may be used to identify adverse health effects and characterize exposure-response relationships.

The draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays are responsive to the National Research Council (NRC) 2011 report *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde*. The literature search strategy, which describes the processes for identifying scientific literature, screening studies for consideration, and selecting studies for inclusion in evidence tables, is responsive to NRC recommendations regarding systematic review of the scientific literature. In addition, NRC recommendations for standardized presentation of key study data are addressed in the preliminary evidence tables and preliminary exposure-response arrays.

EPA welcomes all comments on the draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays, such as remarks on the following:

- the clarity and transparency of the materials;
- the approach for identifying pertinent studies;
- the selection of studies for data extraction to preliminary evidence tables and exposure-response arrays;
- any methodological considerations that could affect the interpretation of or confidence in study results; and
- any additional studies published or nearing publication that may provide data for the evaluation of human health hazard or exposure-response relationships.

The preliminary evidence tables and exposure-response arrays should be regarded solely as representing the data on each endpoint that have been identified as a result of the draft literature search strategy. They do not reflect any conclusions as to hazard identification or dose-response assessment. After obtaining public input and conducting additional study evaluation and data integration, EPA will revise these materials to support the hazard identification and dose-response assessment in a draft Toxicological Review.

1. DRAFT LITERATURE SEARCH STRATEGY

1.1. Literature Search and Screening Strategy for ETBE

The overall literature search approach is shown graphically in Figure 1-1. The initial chemical-specific search was conducted in four online scientific databases in January, 2013, using the keywords and limits described in Table 1-1. After electronically eliminating duplicates from the citations retrieved through these databases, 658 unique citations were identified. An additional 112 citations were obtained using additional search strategies described in Table 1-2.

The resulting 758 citations were screened using the title, abstract, and/or full text for pertinence to examining the health effects of ETBE exposure. A total of 671 references were identified as not being pertinent and were excluded from further consideration (see Figure 1-1 for the exclusion categories). A total of 52 references were identified as primary sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays (see Section 1.2.1). A total of 38 references were considered pertinent, but not as primary sources of health effects data (e.g., ADME studies), and kept as additional resources for development of the Toxicological Review (see Section 1.2.2). If a reference did not provide enough material to evaluate pertinence (e.g., no abstract), it would be reserved for further possible review; no such studies were identified for ETBE (see Section 1.2.3).

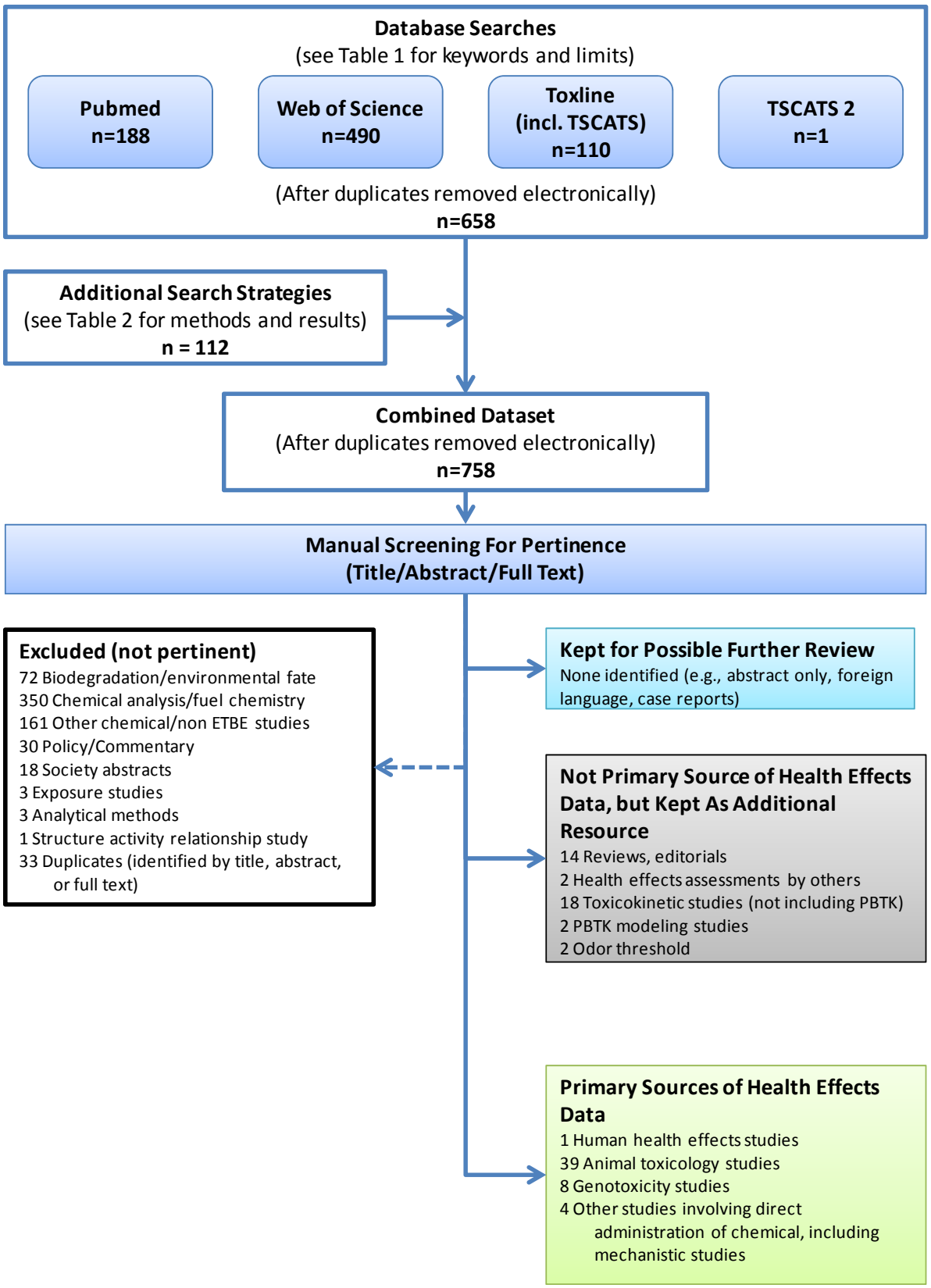


Figure 1-1. Literature search approach for ETBE.

Table 1-1. Database search strategy for ETBE

Database (Search Date)	Keywords	Limits
PubMed (01/08/2013)	*"ETBE" OR "Ethyl tert-butyl ether" OR "2-ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	None
Web of Science (01/08/2013)	*"ETBE" OR "ethyl tert-butyl ether" OR "2-ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	Lemmatization on
Toxline (includes TSCATS) (01/08/2013)	*"ETBE" OR "Ethyl tert-butyl ether" OR "2-Ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	Not PubMed
TSCATS2 (1/08/2013)	637-92-3	01/01/2004 to 01/01/2013

Table 1-2. Summary of additional search strategies for ETBE

Approach used	Source(s)	Date performed	Number of additional citations identified
Electronic backward search through Web of Science	Review article: McGregor (2007). "Ethyl tertiary-butyl ether: a toxicological review." Critical Reviews in Toxicology 37(4): 287–312.	1/2013	68 citations
	Review article: de Peyster (2010). "Ethyl t-butyl ether: Review of reproductive and developmental toxicity." Birth Defects Research, Part B: Developmental and Reproductive Toxicology 89(3): 239–263.	1/2013	26 citations
Personal communication	Japanese Petroleum Energy Center.	1/2013	18 citations

1.2. List of References Based on Search Strategy for ETBE

Citations for excluded references are not listed here, but can be found on the Health and Environmental Research Online (HERO) Web site (http://hero.epa.gov/ETBE).

1.2.1. Primary Sources of Health Effects Data

Data from citations in **bold** are displayed in Section 2. See Section 2.1 for a description of the process of selecting these studies for evidence tables and exposure-response arrays.

Human health effects studies

1) Nihlén, A; Löf, A; Johanson, G. (1998b) Controlled ethyl tert-butyl ether (ETBE) exposure of male volunteers II. Acute effects. Toxicol Sci 46(1):143–150.

Animal toxicology studies

1) Asano, Y; Ishikura, T; Kudoh, K;et al. (2011). "Prenatal developmental toxicity study of ethyl tertiary-butyl ether in rabbits." Drug and Chemical Toxicology 34(3): 311–317.

2) Banton, MI, Peachee, VL; White, KL; et al. (2011). "Oral subchronic immunotoxicity study of ethyl tertiary butyl ether in the rat." Journal of Immunotoxicology 8(4): 298–304.

3) Berger, T; Horner, CM. (2003) In vivo exposure of female rats to toxicants may affect oocyte quality. Reprod Toxicol 17(3):273–281.

4) Bond, JA; Medinsky, MA; Wolf, DC; et al. (1996a). Ethyl tertiary butyl ether (ETBE): ninety-day vapor inhalation toxicity study in CD-1 mice. Chemical Industry Institute of Toxicology under contract to ARCO Chemical Company, Research Triangle Park, NC; Laboratory Project ID 95030, 1–69. Unpublished report.

5) Bond, JA; Medinsky, MA; Wolf, DC; et al. (1996b). Ethyl tertiary butyl ether (ETBE): ninety-day vapor inhalation toxicity study with neurotoxicity evaluations in Fischer 344 rats. Chemical Industry Institute of Toxicology under contract to ARCO Chemical Company, Research Triangle Park, NC; Laboratory Project ID 95029, 1–90. Unpublished report.

6) Cohen, SM; Hard, GC; Regan, KS; et al. (2011) Pathology working group review of selected histopathologic changes in the kidneys of rats assigned to toxicology and carcinogenicity studies of ethyl tertiary butyl ether (ETBE). Research Pathology Associates under contract to Lyondell Chemical Company, Research Triangle Park, NC; 1–30. Unpublished report.

7) dePeyster, A; Stanard, B; Westover, C. (2009) Effect of ETBE on reproductive steroids in male rats and rat Leydig cell cultures. Toxicology Letters 190:74–80.

8) Dorman, DC; Struve, MF; Wong, BA; et al. (1997) Neurotoxicological evaluation of ethyl tertiary-butyl ether following subchronic (90-day) inhalation in the Fischer 344 rat. J Appl Toxicol 17(4):235–242.

9) Fujii, S; Yabe, K; Furukawa, M; et al. (2010). "A one-generation reproductive toxicity study of ethyl tertiary butyl ether in rats." Reproductive Toxicology 30(3): 414–421.

10) Gaoua, W. (2003). Ethyl *tertiary* butyl ether (ETBE), CAS No. 637-92-3: Reproductive/developmental toxicity dose-range finding/probe study by the oral route (gavage) in two strains of rat. CIT under contract for TOTAL France S.A., Evreux, France. Study No. 24168 RSR. Unpublished report.

11) Gaoua, W. (2004a). Ethyl tertiary butyl ether (ETBE): prenatal developmental toxicity study by the oral route (gavage) in rats. CIT under contract to TOTAL France S.A., Evreux, France; Study No. 24860 RSR. Unpublished report.

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

13) Hagiwara, A; Doi, Y; Imai, N; et al. (2011). "Medium-term multi-organ carcinogenesis bioassay of ethyl tertiary-butyl ether in rats." Toxicology 289(2–3): 160–166.

14) IIT Research Institute (Illinois Institute of Technology Research Institute). (1989a). Acute dermal toxicity study of ethyl-tert-butyl ether (ETBE) in rabbits. IIT Research Institute, Life Sciences Research under contract to Amoco Corporation, Chicago, IL; Study No. 1495. Unpublished report.

15) IIT Research Institute (Illinois Institute of Technology Research Institute). (1989b). Acute inhalation toxicity study of ethyl-t-butyl ether (ETBE) in rats. IIT Research Institute, Life Sciences Research under contract to Amoco Corporation, Chicago, IL; Study No. 1496. Unpublished report.

16) IIT Research Institute (Illinois Institute of Technology Research Institute). (1991). Four-week inhalation toxicity study of ethyl tert-butyl ether (ETBE) in rats. IIT Research Institute, Life Sciences Research under contract to Amoco Corporation, Chicago, IL; Study No. 1544. Unpublished report.

17) Japan Petroleum Energy Center (JPEC).(2008a). 28-day ETBE repeated dose full-body inhalation toxicity test in rats (preliminary test). Mitsubishi Chemical Safety Institute Ltd. March, 2008. Study No. B061828. Unpublished report.

18) Japan Petroleum Energy Center (JPEC). (2008b). A 90-day repeat dose toxicity study of ETBE by whole-body inhalation exposure in rats. Mitsubishi Chemical Safety Institute Ltd. March, 2008. Study No. B061829. Unpublished report.

19) Japan Petroleum Energy Center (JPEC). (2008c). A 180-day repeat dose oral toxicity study of ETBE in rats. Hita Laboratory, Chemicals Evaluation and Research Institute (CERI), Japan. March, 2008. Study No. D19-0002. Unpublished report.

20) Japan Petroleum Energy Center (JPEC).(2008d). Medium-term multi-organ carcinogenesis bioassay of 2-ethoxy-2-methylpropane in rat. Unpublished report.

21) Japan Petroleum Energy Center (JPEC). (2008e). A one-generation reproduction study of ETBE in rats. Safety Research Institute for Chemical Compounds. Study No. SR07060. Unpublished report.

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

23) Japan Petroleum Energy Center (JPEC). (2008i). Serum levels of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) in rats following 4-weeks administration of 2-ethoxy-2-methylpropane. DIMS Institute of Medical Science, Inc., Ichinomiya, Japan. March 26, 2008. Study No. 0760. Unpublished report.

24) Japan Petroleum Energy Center (JPEC). (2008j). Study for effects on embryo-fetal development in rabbits treated orally with ETBE. Kannami Laboratory, Bozo Research Center Inc., 1308-125 Kuwahara-Sambonmatsu,Kannami-cho, Tagata-gun, Shizuoka 419-0101, Japan. January 31, 2008. Study No. R-965. Unpublished report.

25) Japan Petroleum Energy Center (JPEC). (2010a). Carcinogenicity test of 2-ethoxy-2-methylpropane in rats (drinking water study). Japan Industrial Safety and Health Association, Japan Bioassay Research Center. March 25, 2010. Study No. 0691. Unpublished report.

26) Japan Petroleum Energy Center (JPEC). (2010b). Carcinogenicity test of 2-ethoxy-2-methylpropane in rats (inhalation study). Japan Industrial Safety and Health Association, Japan Bioassay Research Center. March 25, 2010. Study No. 0686. Unpublished report.

27) Li, Q, M Kobayashi, H Inagaki, et al. (2011). "Effects of subchronic inhalation exposure to ethyl tertiary butyl ether on splenocytes in mice." International Journal of Immunopathology and Pharmacology 24(4): 837–847.

28) Maltoni, C; Belpoggi, F; Soffritti, M; et al. (1999). Comprehensive long-term experimental project of carcinogenicity bioassays on gasoline oxygenated additives: plan and first report of results from study of ethyl-tertiary-butyl-ether (ETBE). Eur J Oncol 4:493–508.

29) Millennium Bioresearch Research Laboratories (MB Research Laboratories, Inc.). (1988a). Acute dermal toxicity in rabbits/LD50 in rabbits. MB Research Laboratories, Inc. under contract to ARCO Chemical Company, Spinnerstown, PA; Laboratory Project ID MB 88-9107 B. Unpublished report.

30) Millennium Bioresearch Research Laboratories (MB Research Laboratories, Inc.). (1988b). Eye irritation in rabbits. MB Research Laboratories, Inc. under contract to ARCO Chemical Company, Spinnerstown, PA; Laboratory Project ID MB 88-9107 D. Unpublished report.

31) Millennium Bioresearch Research Laboratories (MB Research Laboratories, Inc.). (1988c). Primary dermal irritation in rabbits. MB Research Laboratories, Inc. under contract to ARCO Chemical Company, Spinnerstown, PA; Laboratory Project ID MB 88-9107 C. Unpublished report.

32) Millennium Bioresearch Research Laboratories (MB Research Laboratories, Inc.). (1988d). Single dose oral toxicity in rats/LD50 in rats. MB Research Laboratories, Inc. under contract to ARCO Chemical Company, Spinnerstown, PA; Laboratory Project ID MB 88-9137 A. Unpublished report.

33) Medinsky, MA; Wolf, DC; Cattley, RC; et al. (1999). Effects of a thirteen-week inhalation exposure to ethyl tertiary butyl ether on Fischer-344 rats and CD-1 mice. Toxicol Sci 51(1):108–118.

34) Suzuki, M; Yamazaki, K; Kano, K; et al. (2012). "No carcinogenicity of ethyl tertiary-butyl ether by 2-year oral administration in rats." J Toxicol Sci 37(6): 1239–1246.

35) Utah Biomedical Testing Laboratory (UBTL Inc.). (1994). Twenty-eight (28) day dermal toxicity study in rats administered test article F-266. UBTL, Inc. under contract to ARCO Chemical Company, Salt Lake City, UT; UBTL Study No. 66894; Protocol No. ATX-92-0114, 1–174. Unpublished report.

36) Weng, ZQ; Suda, M; Ohtani, K; et al. (2011). "Aldh2 knockout mice were more sensitive to DNA damage in leukocytes due to ethyl tertiary butyl ether exposure." Industrial Health 49(3): 396–399.

37) Weng, Z; Suda, M; Ohtani, K; et al. (2012). "Differential genotoxic effects of subchronic exposure to ethyl tertiary butyl ether in the livers of Aldh2 knockout and wild-type mice." Archives of Toxicology 86(4): 675–682.

38) White, KL. (2002). Immunological evaluation of gasoline ETBE vapor condensate in female Sprague-Dawley rats using the plaque forming cell assay. ImmunoTox, Inc. under contract to Huntingdon Life Sciences, Richmond, VA; Project No. ITI 901. Unpublished report.

39) White, RD; Daughtrey, WC; Wells, MS. (1995). Health effects of inhaled tertiary amyl methyl ether and ethyl tertiary butyl ether. Toxicol Lett 82–83:719–724.

Genotoxicity studies

1) Japan Petroleum Energy Center (JPEC). (2007a). Micronucleus test of 2-ethoxy-2-methylpropane (ETBE) using bone marrow of rats administered ETBE by gavage. Japan Industrial Safety and Health Association. Japan Bioassay Research Center. Study No. 7049. June 29, 2007. Unpublished report.

2) Japan Petroleum Energy Center (JPEC). (2007b). Micronucleus test of 2-ethoxy-2-methylpropane (ETBE) using bone marrow of rats administered ETBE intraperitoneally. Japan Industrial Safety and Health Association. Japan Bioassay Research Center. Study No. 7048. June 29, 2007. Unpublished report.

3) Japan Petroleum Energy Center (JPEC). (2007c). Micronucleus test of ETBE using bone marrow of rats of the "13-week toxicity study of 2-ethoxy-2-methylpropane in F344 rats (drinking water study) [preliminary carcinogenicity study]." Japan Industrial Safety and Health Association. Japan Bioassay Research Center. Study No. 7046. June 29, 2007. Unpublished report.

4) Japan Petroleum Energy Center (JPEC). (2007d). Micronucleus test of ETBE using bone marrow of rats of the "13-week toxicity study of 2-ethoxy-2-methylpropane in F344 rats (inhalation study) [preliminary carcinogenicity study]." Japan Industrial Safety and Health Association. Japan Bioassay Research Center. Study No. 7047. June 29, 2007. Unpublished report.

5) Vergnes, JS. (1995). Ethyl tertiary butyl ether: in vitro chromosome aberrations assay in Chinese hamster ovary cells. Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company, Export, PA; Laboratory Project ID 94N1425. Unpublished report.

6) Vergnes, JS; Kubena, MF. (1995a). Ethyl tertiary butyl ether: bone marrow micronucleus test in mice. Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company, Export, PA; Laboratory Project ID 94N1426. Unpublished report.

7) Vergnes, JS; Kubena, MF. (1995b). Ethyl tertiary butyl ether: mutagenic potential in the CHO/HGPRT forward mutation assay. Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company, Export, PA; Laboratory Project ID 94N1424. Unpublished report.

8) Zeiger, E; Anderson, B; Haworth, S; et al. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19(Suppl 21):2–141.

Other studies involving direct administration of ETBE, including mechanistic studies

1) Japan Petroleum Energy Center (JPEC). (2012). Investigation of the Mechanisms of Ethyl tertiary-butyl ether (ETBE) carcinogenicity in the liver of F344 rats- Transmission Electron Microscopic Examination. Japan Industrial Safety and Health Association, Japan Bioassay Research Center. September 7, 2012. Study No. 12138. Unpublished report.

2) Martin, JV; Bilgin, NM; Iba, MM. (2002). Influence of oxygenated fuel additives and their metabolites on the binding of a convulsant ligand of the gamma-aminobutyric acid(A) (GABA(A) receptor in rat brain membrane preparations. Toxicol Lett 129(3):219–226.

3) Martin, JV; Iyer, SV; McIlroy, PJ; et al. (2004). Influence of oxygenated fuel additives and their metabolites on gamma-aminobutyric acidA (GABAA) receptor function in rat brain synaptoneurosomes. Toxicol Lett 147(3):209–217.

4) Yamaki, K; Yoshino, S. (2009). Inhibition of IgE-induced mast cell activation by ethyl tertiary-butyl ether, a bioethanol-derived fuel oxygenate. J Pharm Pharmacol 61:1243–1248.

1.2.2. Not Primary Source of Health Effects Data, but Kept as Additional Resources

Reviews, editorials

1) Ahmed, FE. (2001). Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. Toxicol Lett 123(2–3): 89–113.

2) BIBRA Working Group. (2000). Ethyl tert-butyl ether. Bibra toxicology advice & consulting. Surrey, United Kingdom.

3) Burbacher, TM. (1993). Neurotoxic effects of gasoline and gasoline constituents. Environ Health Perspect 101: 133–141.

4) Caprino, L and Togna, G. (1998). Potential health effects of gasoline and its constituents: A review of current literature (1990–1997) on toxicological data. Environ Health Perspect 106(3): 115–125.

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

6) Dekant, W; Bernauer, U; Rosner, E; et al. (2001b). Toxicokinetics of ethers used as fuel oxygenates. Toxicol Lett 124(1–3): 37–45.

7) Hard, GC; RH Bruner; SM Cohen; et al. (2011). Renal histopathology in toxicity and carcinogenicity studies with tert-butyl alcohol administered in drinking water to F344 rats: A pathology working group review and re-evaluation. Regul Toxicol Pharmacol 59(3): 430–436.

8) Hard, GC; Johnson, KJ; Cohen, SM; et al. (2009). A comparison of rat chronic progressive nephropathy with human renal disease-implications for human risk assessment. Crit Rev Toxicol 39(4): 332–346.

9) Hard, GC and Khan, KN. (2004). A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol Pathology 32(2): 171–180.

10) Johanson, G; Loef, A; Nihlén, A; et al. (1997). Toxicokinetics of ethers in humans—A comparison of MTBE, ETBE, and TAME.

11) McGregor, D. (2007). Ethyl tertiary-butyl ether: a toxicological review. Crit Rev Toxicol 37(4): 287–312.

12) McGregor, D. (2010). "Tertiary-Butanol: A toxicological review." Crit Rev Toxicol 40(8): 697–727.

13) U.S. EPA. (1995a). Letter Summarizing Review of Methyl-tert Butyl Ether and Ethyl Tert-butyl Ether for Possible Relationship Based on Structure to Rodent Carcinogenicity, W/cvr Ltr Dtd 02/13/95. Washington, DC. Office of Toxic Substances.

14) U.S. EPA. (1995b). Toxicity and health hazard summary of tert-butyl ethyl ether with cover letter dated 01/10/95. Washington, DC.

Health effects assessments by others

1) American Conference of Governmental Industrial Hygienists (ACGIH). (2001). Documentation of the threshold limit values and biological exposure indices for ethyl tert-butyl ether Vol:7th Ed (pp. 5). Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

2) Duncan, B. (2008). "Attention: TSCA 8(e) Coordinator. RE: Ethyl tertiary butyl ether (CASRN: 637-92-3): Results from a Single Generation Reproduction in Rodents (Oral-Rat) and a Soil Biodegradation Study as part of The ETBE Utilization Study Working Group Testing Program and Risk Assessment."

Toxicokinetic studies (excluding physiologically-based toxicokinetic [PBTK] modeling studies)

1) Amberg, A; Rosner, E; and Dekant, W. (2000). Biotransformation and kinetics of excretion of ethyl tert-butyl ether in rats and humans. Toxicol Sci 53:194–201.

2) Bernauer, U; Amberg, A; Scheutzow, D; et al. (1998). Biotransformation of 12C- and 2-13C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem Res Toxicol 11(6):651–658.

3) Borghoff, SJ. (1996). Ethyl tertiary butyl ether (ETBE) a pilot/methods development pharmacokinetic study in male F344 rats and male CD-1 mice after a single nose-only inhalation exposure. Chemical Industry Institute of Toxicology under contract to ARCO Chemical Company, Research Triangle Park, NC; Laboratory Protocol Number CIIT-95025. Unpublished report.

4) Dekant, W; Bernauer, U; Rosner, E; et al. (2001a). Biotransformation of MTBE, ETBE, and TAME after inhalation or ingestion in rats and humans. Health Eff Inst Res Rep 102:29–71.

5) Hong, JY; Wang, YY; Bondoc, FY; et al. (1997). Rat olfactory mucosa displays a high activity in metabolizing methyl tert-butyl ether and other gasoline ethers. Fundam Appl Toxicol 40(2):205–210.

6) Hong, JY; Wang, YY; Bondoc, FY; et al. (1999a). Metabolism of methyl tert-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: identification of CYP2A6 as a major catalyst. Toxicol Appl Pharmacol 160(1):43–48.

7) Hong, JY; Wang, YY; Bondoc, FY; et al. (1999b). Metabolism of methyl tert-butyl ether and other gasoline ethers in mouse liver microsomes lacking cytochrome P450 2E1. Toxicol Lett 105(1):83–88.

8) Hong, JY; Wang, YY; Mohr, SN; et al. (2001). Human cytochrome P450 isozymes in metabolism and health effects of gasoline ethers. Res Rep Health Eff Inst (102):7–27.

9) Japan Petroleum Energy Center (JPEC). (2008f). Pharmacokinetic study in rats treated with [14C] ETBE repeatedly for 14 days. Kumamoto Laboratory, Mitsubishi Chemical Safety, Institute Ltd., 1285 Kurisaki-machi, Uto-shi, Kumamoto 869-0425, Japan. March, 2008. Study No. P070497. Unpublished report.

10) Japan Petroleum Energy Center (JPEC). (2008g). Pharmacokinetic study in rats treated with single dose of [14C] ETBE. Kumamoto Laboratory, Mitsubishi Chemical Safety, Institute Ltd., 1285 Kurisaki-machi, Uto-shi, Kumamoto 869-0425, Japan. March, 2008. Study No. P070496. Unpublished report.

11) Kaneko, T; Wang, PY; Sato, A. (2000). Partition coefficients for gasoline additives and their metabolites. J Occup Health 42(2):86–87.

12) Le Gal, A; Dreano, Y; Gervasi, PG; et al. (2001). Human cytochrome P450 2A6 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels. Toxicol Lett 124(1–3):47–58.

13) Nihlén, A; Löf, A; Johanson, G. (1995). Liquid/air partition coefficients of methyl and ethyl t-butyl ethers, t-amyl methyl ether, and t-butyl alcohol. J Expo Anal Environ Epidemiol 5(4):573–582.

14) Nihlén, A; Löf, A; Johanson, G. (1998a). Controlled ethyl tert-butyl ether (ETBE) exposure of male volunteers I. Toxicokinetics. Toxicol Sci 46(1):1–10.

15) Sun, JD and Beskitt, JL. (1995a). Ethyl tertiary butyl ether (ETBE): pharmacokinetics after single and repeated inhalation exposures in mice. Bush Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company, Export, PA; Laboratory Project ID 94N1455. Unpublished report.

16) Sun, JD and Beskitt, JL. (1995b). Ethyl tertiary butyl ether (ETBE): pharmacokinetics after single and repeated inhalation exposures in rats. Bush Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company, Export, PA; Laboratory Project ID 94N1454. Unpublished report.

17) Turini, A; Amato, G; Longo, V; et al. (1998). Oxidation of methyl- and ethyl- tertiary-butyl ethers in rat liver microsomes: role of the cytochrome P450 isoforms. Arch Toxicol 72(4):207–214.

18) Zhang, YP; Macina, OT; Rosenkranz, HS; et al. (1997). Prediction of the metabolism and toxicological profiles of gasoline oxygenates. Inhal Toxicol 9(3):237–254.

PBTK modeling studies

1) Nihlén, A and Johanson, G. (1999). Physiologically based toxicokinetic modeling of inhaled ethyl tertiary-butyl ether in humans. Toxicol Sci 51(2):184–194.

2) Pierce, C.H. (2004). Toxicokinetics of ethyl tertiary-butyl ether (ETBE) and methyl tertiary-butyl ether (MTBE) in men and women. NIOSH report.

Odor threshold

1) van Wezel, A; Puijker, L; et al. (2009). "Odour and flavour thresholds of gasoline additives (MTBE, ETBE and TAME) and their occurrence in Dutch drinking water collection areas." Chemosphere 76(5): 672–676.

2) Vetrano, KM. (1993). Final report to ARCO Chemical Company on the odor and taste threshold studies performed with methyl tertiary-butyl ether (MTBE) and ethyl tertiary-butyl ether (ETBE). TRC Environmental Corporation under contract to ARCO Chemical Company, Windsor, CT; Project no. 13442-M31. Unpublished report.

1.2.3. Kept for Possible Further Review

None identified.

2. PRELIMINARY EVIDENCE TABLES AND PRELIMINARY EXPOSURE-RESPONSE ARRAYS

2.1. Data Extraction: Preparation of Preliminary Evidence Tables and Preliminary Exposure-Response Arrays

The 52 references identified as primary sources of health effects data were considered for data extraction to evidence tables and exposure-response arrays. References were first collated with respect to exposure route, exposure duration, and type of endpoint, to identify those most pertinent for evaluating the human health effects from chronic oral or inhalation exposure to ETBE. As a result, data from 19 studies with one or more of the following characteristics were not extracted into evidence tables or exposure-response arrays:

- The study involved dermal exposure;
- The study only involved acute or short-term exposures (less than 90 days/13 weeks), and it was not conducted in the context of immunotoxicity, neurotoxicity, developmental, or reproductive toxicity;
- The data in the study only included endpoints related to possible mechanisms of toxicity; and
- The study's endpoints did not exhibit responses in any of the 52 available references.

Data from the 33 remaining references were prepared in preliminary evidence tables. No studies were excluded based on study quality considerations, so as to allow for public input on methodological considerations that could affect the interpretation of or confidence in each study's results. With regard to noncancer effects, health effect endpoints that were consistently affected in chronic or subchronic studies were included in the evidence tables. All data demonstrating carcinogenic effects were included. Supporting data that provide mechanistic information for each selected endpoint were also included. For each included endpoint, all studies reporting data on that endpoint are included regardless of the reported level or statistical significance of the response. Several references are grouped together as "related" references because they represent pilot (e.g., range-finding), unpublished (e.g., technical report), and/or published (e.g., journal article) versions of the same study. The tables for non-carcinogenic effects appear first and are arranged in the order from the health effect with the most data to health effect with the least data. The evidence tables for carcinogenic and genotoxic effects follow. For each endpoint, the studies are presented beginning with chronic studies followed by subchronic exposures. The information in the

preliminary evidence tables is displayed graphically in preliminary exposure-response arrays. In these preliminary arrays, the doses are labeled based only on statistical significance as determined by the study's authors, without consideration of biological significance.

2.2. Kidney Effects

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to ETBE

Reference and study design	Results					
Kidney Weight						
Suzuki et al. (2012) Rat, F344, male and female, 50 /sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Absolute kidney weight (*percent change compared to control*)					
	M	0	28	121	542	
		-	−4%	5%	18%*	
	F	0	46	171	560	
		-	3%	10%*	14%*	
	Relative kidney weight (*percent change compared to control*)					
	M	0	28	121	542	
		-	0.1%	13%*	32%*	
	F	0	46	171	560	
		-	14%*	23%*	37%*	
JPEC (2008c) Rats, Sprague Dawley Male and female, 50/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	Absolute kidney weight (*percent change compared to control*)					
	M	0	5	25	100	400
		-	0.6%	6%	5%	25%*
	F	0	5	25	100	400
		-	0.5%	0%	7%	10%*
	Relative kidney weight (*percent change compared to control*)					
	M	0	5	25	100	400
		-	8%	6%	12%*	21%*
	F	0	5	25	100	400
		-	7%	4%	11%*	15%*
Hagiwara et al. (2011) Rats, F344, male, 12/group 0, 1,000 mg/kg-day Gavage 23 weeks **Related reference**: JPEC (2008d) (unpublished study)	Absolute kidney weight (*percent change compared to control*)					
	19%*					
	Relative kidney weight (*percent change compared to control*)					
	25%*					
Gaoua (2004b) Rats, Sprague Dawley, Male and female 0, 250, 500, 1,000 mg/kg-day Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Absolute kidney weight (*percent change compared to control*)					
	M	0	250	500	1,000	
		-	11%*	15%*	21%*	
	F	0	250	500	1,000	
		-	−0.9%	2%	5%	
	Relative kidney weight (*percent change compared to control*)					
	M	0	250	500	1,000	
		-	11%*	18%*	28%*	
	F	0	250	500	1,000	
		-	9%	5%	3%	

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results				
Fujii et al. (2010)	Absolute kidney weight (*percent change compared to control*)				
Rats, Sprague Dawley,	M	0	100	300	1,000
male and female, 24/sex/group		-	5%	8%	18%*
0, 100, 300, 1,000 mg/kg-day	F	0	100	300	1,000
Gavage		-	−2%	0.0	7%*
16 weeks (males),	Relative kidney weight (*percent change compared to control*)				
17 weeks (females)	M	0	100	300	1,000
		-	8%*	12%*	26%*
Related reference: JPEC (2008e)	F	0	100	300	1,000
(unpublished study)		-	−3%	−0.9%	2%
Histopathology					
Suzuki et al. (2012)	Incidence of chronic nephropathy				
Rat, F344, male and female,	M	0	28	121	542
50 /sex/group		49/50	43/50	45/50	48/50
0, 625, 2,500, 10,000 ppm	F	0	46	171	560
(0, 28, 121, 542 mg/kg-d in males;		41/50	37/50	37/50	39/50
0, 46, 171, 560 mg/kg-d in females)[a]	Average severity of chronic nephropathy[b]				
Drinking water	M	0	28	121	542
104 weeks		2.1	1.7	1.8	2.3
Related reference: JPEC (2010a)	F	0	46	171	560
(unpublished study)		1.0	0.9	1.1	1.2
	Incidence of hyaline droplets				
	M	0	28	121	542
			Not examined		
	F	0	46	171	560
			Not examined		
	Incidence of atypical tubule hyperplasia				
	M	0	28	121	542
		0/50	0/50	0/50	1/50
	F	0	46	171	560
		0/50	0/50	0/50	2/50
	Incidence of papillary necrosis				
	M	0	28	121	542
		0/50	1/50	0/50	2/50
	F	0	46	171	560
		0/50	1/50	1/50	2/50
	Incidence of papillary mineralization				
	M	0	28	121	542
		0/50	0/50	16/50*	42/50*
	F	0	46	171	560
		0/50	0/50	1/50	3/50

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results					
Cohen et al. (2011) Reanalysis of the renal sections from Suzuki et al. (2012)	Incidence of chronic nephropathy					
	M	0 49/50	28 Not examined		121 Not examined	542 50/50
	F	0 45/50	46 41/50		171 46/50	560 46/50
	Average severity of chronic nephropathy					
	M	0 2.1	28 Not examined		121 Not examined	542 2.7
	F	0 1.1	46 1.0		171 1.2	560 1.4
JPEC (2008c) Rats, Sprague Dawley Male and female, 50/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	Incidence of hyaline droplets					
	M	0 0/15	5 0/15	25 0/15	100 4/15*	400 10/15*
	F	0 0/15	5	25 Not examined	100	400 0/15
	Incidence of hyaline droplets positive for α2u-globulin					
	M	0 Not reported	5	25 Not examined	100 2/2	400 1/1
	F	0	5	25 Not examined	100	400
	Incidence of papillary mineralization					
	M	0 0/15	5 0/15	25 0/15	100 1/15	400 0/15
	F	0 0/15	5	25 Not examined	100	400 0/15
Urinalysis						
Suzuki et al. (2012) Rat, F344, male and female, 50 /sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Incidence of proteinuria					
	M	0 39/39	28 37/37		121 34/34	542 35/35
	F	0 37/37	46 37/37		171 38/38	560 38/38
	Average severity of proteinuria[b]					
	M	0 3.0	28 3.1		121 3.1	542 3.1
	F	0 2.8	46 3.0		171 3.0	560 3.1

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results					
JPEC (2008c) Rats, Sprague Dawley Male and female, 50/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	Incidence of proteinuria					
	M	0 10/10	5 10/10	25 10/10	100 10/10	400 10/10
	F	0 8/10	5 9/10	25 7/10	100 9/10	400 7/10
	Average severity of proteinuria[b]					
	M	0 1.5	5 1.6	25 1.6	100 1.3	400 1.5
	F	0 1.2	5 1.3	25 1.0	100 1.3	400 1.0
	Incidence of urinary casts					
	M	0 0/10	5 Not examined	25 0/10	100	400
	F	0 0/10	5 Not examined	25 0/10	100	400

[a]Conversion performed by study authors.
[b]Calculated by EPA Σ(grade x #of affected animals)/total # of animals exposed.
[*]Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

Table 2-2. Evidence pertaining to kidney effects in animals following inhalation exposure to ETBE

Reference and study design	Results				
Kidney Weight					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Absolute kidney weight (*percent change compared to control*)				
	M	0 -	2,090 8%*	6,270 17%*	20,900 23%*
	F	0 -	2,090 5%	6,270 6%*	20,900 18%*
	Relative kidney weight (*percent change compared to control*)				
	M	0 -	2,090 19%*	6,270 26%*	20,900 66%*
	F	0 -	2,090 11%*	6,270 16%*	20,900 51%*
JPEC (2008b) Rats, Sprague Dawley Male and female, 10–16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks {} = subset with 28 day recovery after 13 week exposure	Absolute kidney weight (*percent change compared to control*)					
	M	0 -	627 10%	2,090 11%	6,270 18%*	20,900 15%* {19%}
	F	0 -	627 0.2%	2,090 −0.9%	6,270 4%	20,900 7% {8%}
	Relative kidney weight (*percent change compared to control*)					
	M	0 -	627 10%	2,090 9%	6,270 20%*	20,900 24%* {15%*}
	F	0 -	627 8%	2,090 7%	6,270 13%*	20,900 20%* {5%}
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Absolute kidney weight (*percent change compared to control*)				
	M	0 -	2,090 7%	7,320 10%*	20,900 19%*
	F	0 -	2,090 5%	7,320 12%*	20,900 21%*
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group, 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996a) (unpublished study)	Absolute kidney weight (percent change compared to control)				
	M	0 -	2,090 9%	7,320 10%	20,900 5%
	F	0 -	2,090 −0.2%	7,320 6%	20,900 4%

Table 2-2. Evidence pertaining to kidney effects in animals following inhalation exposure to ETBE (continued)

Reference and study design	Results				
Histopathology					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Incidence of chronic nephropathy				
	M	0	2,090	6,270	20,900
		49/50	50/50	49/50	50/50
	F	0	2,090	6,270	20,900
		32/50	38/50	41/50	40/50
	Average severity of nephropathy				
	M	0	2,090	6,270	20,900
		2.4	2.6	2.7	3.1*
	F	0	2,090	6,270	20,900
		0.9	1.3	1.3	1.6*
	Incidence of hyaline droplets				
	M	0	2,090	6,270	20,900
		Not examined			
	F	0	2,090	6,270	20,900
		Not examined			
	Incidence of papilla mineralization				
	M	0	2,090	6,270	20,900
		0/50	0/50	1/50	6/50*
	F	0	2,090	6,270	20,900
		Not examined			
	Incidence of atypical tubule hyperplasia				
	M	0	2,090	6,270	20,900
		Not examined			
	F	0	2,090	6,270	20,900
		Not examined			
JPEC (2008b) Rats, Sprague Dawley Male and female, 10–16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks	Incidence of hyaline droplets, proximal tubule				
	M	0	627	2,090	6,270	20,900
		0/10	3/10	8/10*	8/10*	8/10*
	F	0	627	2,090	6,270	20,900
		Not observed				
	Incidence of hyaline droplets positive for α2u-globulin					
	M	0	627	2,090	6,270	20,900
		Unspecified representative samples reported positive for α2u-globulin				
	F	0	627	2,090	6,270	20,900
		Not examined				
	Incidence of urinary casts					
	M	0	627	2,090	6,270	20,900
		0/6	0/6	0/6	0/6	0/6
	F	0	627	2,090	6,270	20,900
		0/6	0/6	0/6	0/6	0/6

Table 2-2. Evidence pertaining to kidney effects in animals following inhalation exposure to ETBE (continued)

Reference and study design	Results				
Medinsky et al. (1999)	Average severity of hyaline droplets				
Rats, F344, male and female	M	0	2,090	7,320	20,900
10/sex/group		1.8	3.0	3.2	3.8
0, 500, 1,750, 5,000 ppm	F	0	2,090	7,320	20,900
(2,090, 7,320, 20,900 mg/m^3)[a]			Not observed		
Whole body inhalation	Average proximal tubule proliferation				
6 hours/day, 5 days/week	M	0	2,090	7,320	20,900
13 weeks		0.91	2.16*	3.4*	2.47*
Related reference: Bond et al. (1996b)	F	0	2,090	7,320	20,900
		0.59	1.02	0.97	0.87
Urinalysis					
JPEC (2010b)	Incidence of proteinuria				
Rat, F344, male and female,	M	0	2,090	6,270	20,900
50 /sex/group		44/44	38/38	40/40	31/31
0, 500, 1,500, 5,000 ppm	F	0	2,090	6,270	20,900
(0, 2,090, 6,270, 20,900 mg/m^3)[a]		35/38	39/39	30/30	30/30
Whole body inhalation	Average severity of proteinuria				
6 hours/day, 5 days/week	M	0	2,090	6,270	20,900
104 weeks		3.7	3.5	3.6	3.6
	F	0	2,090	6,270	20,900
		2.8	3.1	3.3	3.4*
JPEC (2008b)	Incidence of proteinuria				
Rats, Sprague Dawley	M	0	627	2,090	6,270	20,900
Male and female, 10–16/sex/group		3/6	5/6	5/6	6/6	4/6
0, 150, 500, 1,500, 5,000 ppm	F	0	627	2,090	6,270	20,900
(0, 627, 2,090, 6,270, 20,900		1/6	1/6	1/6	2/6	2/6
mg/m^3)[a]	Average severity of proteinuria					
Whole body inhalation	M	0	627	2,090	6,270	20,900
6 hours/day, 5 days/week		0.5	1.2	1.2	1.3	1.0
13 weeks	F	0	627	2,090	6,270	20,900
		0.2	0.3	0.2	0.5	0.3

[a]4.18 mg/m^3 = 1 ppm.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

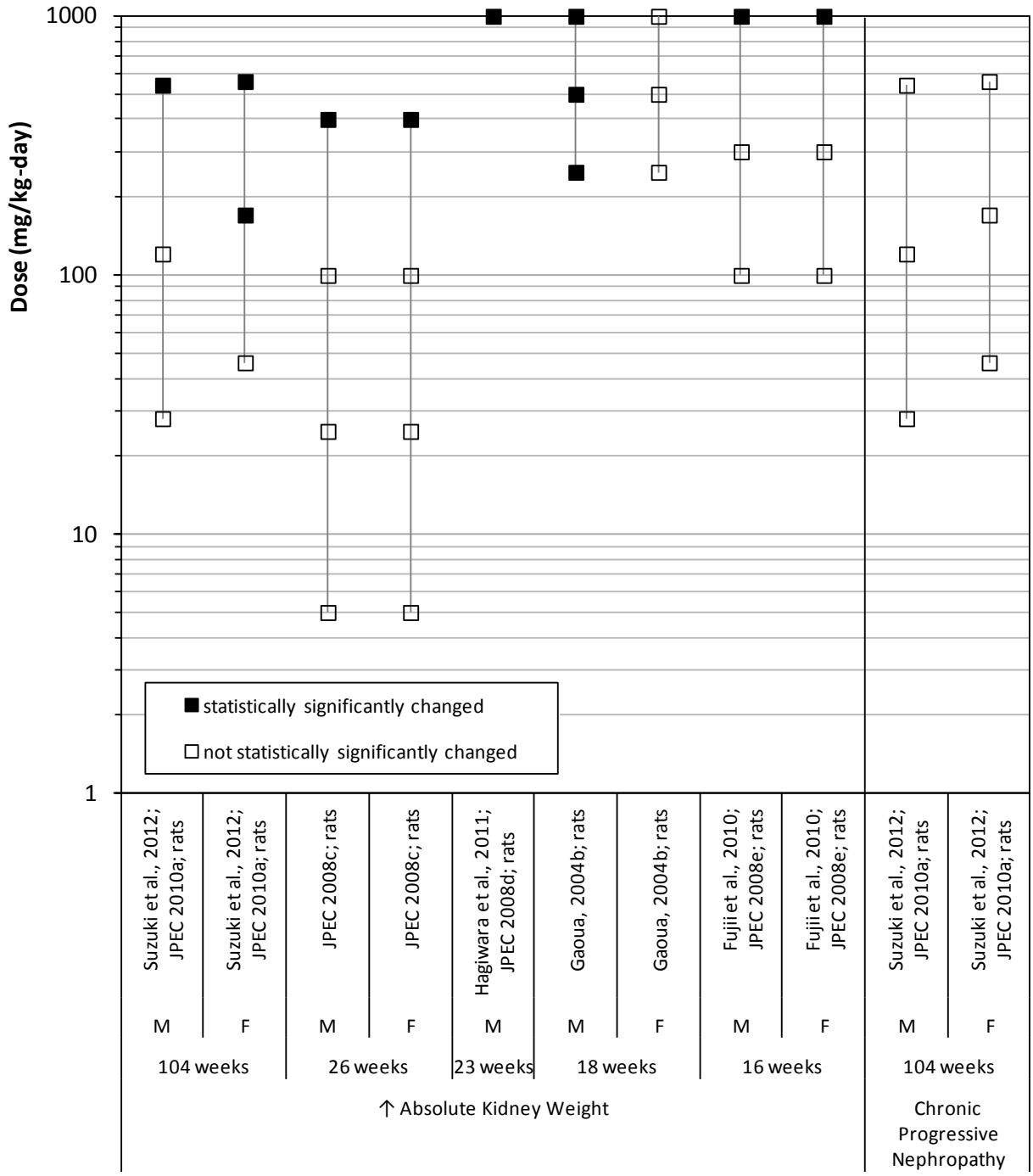


Figure 2-1. Exposure-response array of kidney effects following oral exposure to ETBE.

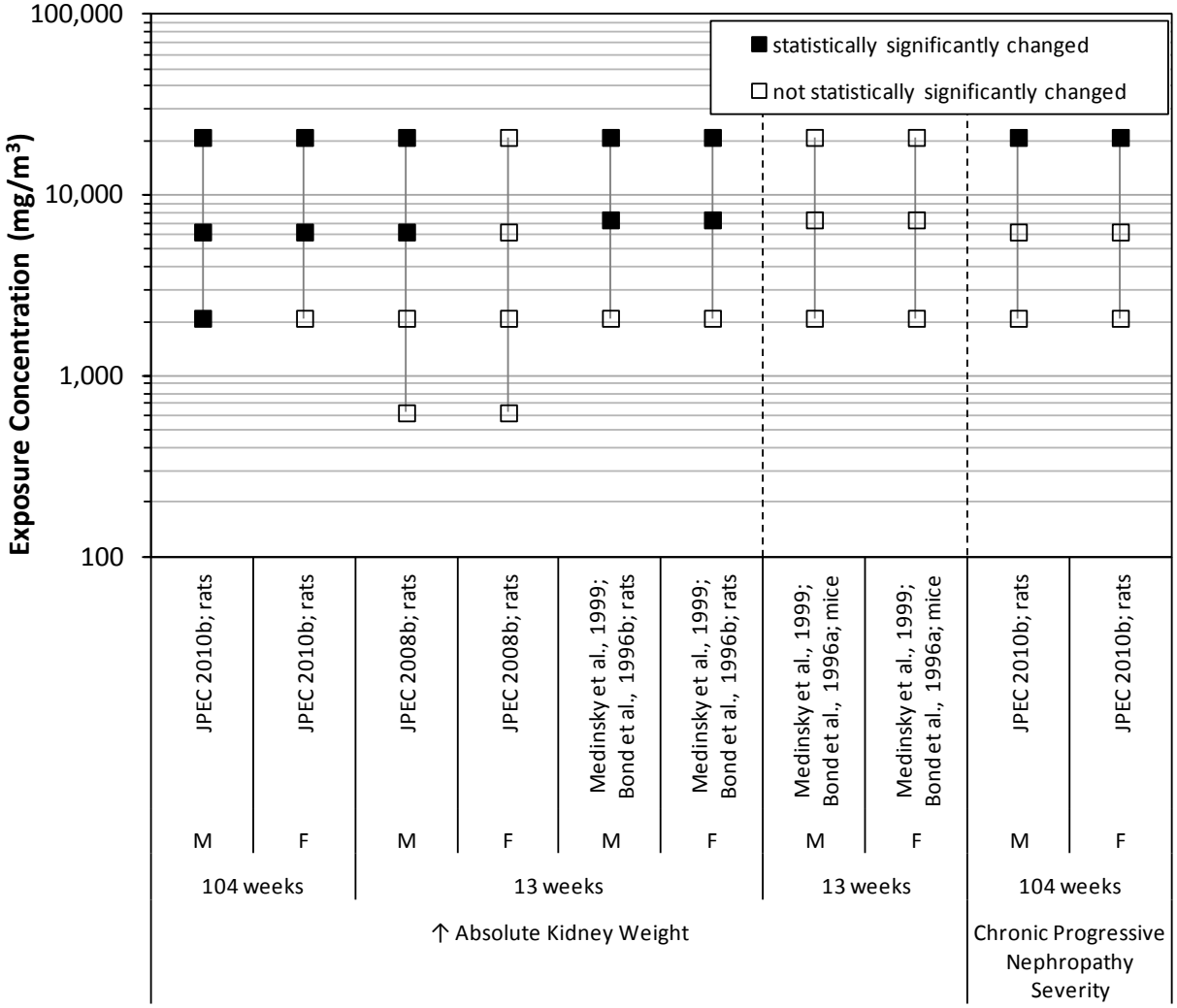


Figure 2-2. Exposure-response array of kidney effects following inhalation exposure to ETBE.

2.3. Liver Effects

Table 2-3. Evidence pertaining to liver effects in animals following oral exposure to ETBE

Reference and study design	Results				
Liver Weight					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Absolute liver weight (*percent change compared to control*)				
	M	0	28	121	542
		-	−11%*	−4%	2%
	F	0	46	171	560
		-	−6%	−2%	−10%
	Relative liver weight (*percent change compared to control*)				
	M	0	28	121	542
		-	−8%	3%*	12%*
	F	0	46	171	560
		-	4%	9%	8%
JPEC (2008c) Rats, Sprague Dawley Male and female, 15/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	Absolute liver weight (*percent change compared to control*)				
	M	0	5	25	100	400
		-	−2%	7%	4%	19%
	F	0	5	25	100	400
		-	−4%	−1%	2%	9%
	Relative liver weight (*percent change compared to control*)					
	M	0	5	25	100	400
		-	5%	7%	9%	17%*
	F	0	5	25	100	400
		-	1%	1%	4%	12%*
Hagiwara et al. (2011) F344 Rats, male, 12/group Gavage 0, 1,000 mg/kg-day 23 weeks **Related reference**: JPEC (2008d) (unpublished study)	Absolute liver weight (*percent change compared to control*)				
	21%*				
	Relative liver weight (*percent change compared to control*)				
	27%*				
Gaoua (2004b) Rats, Sprague Dawley, Male and female, 25/sex/group 0, 250, 500, 1,000 mg/kg-day Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Absolute liver weight (*percent change compared to control*)				
	M	0	250	500	1,000
		-	2%	2%	17%*
	F	0	250	500	1,000
		-	−1%	4%	6%
	Relative liver weight (*percent change compared to control*)				
	M	0	250	500	1,000
		-	3%	6%	24%*
	F	0	250	500	1,000
		-	10%	8%	4%

Table 2-3. Evidence pertaining to liver effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results					
Fujii et al. (2010) Rats, Sprague Dawley, male and female, 24/sex/group 0, 100, 300, 1,000 mg/kg-day Gavage 16 weeks (males), 17 weeks (females) **Related reference**: JPEC (2008e) (unpublished study)	Absolute liver weight (*percent change compared to control*)					
	M	0	100	300	1,000	
		-	−3%	1%	13%*	
	F	0	100	300	1,000	
		-	−1%	4%	14%*	
	Relative liver weight (*percent change compared to control*)					
	M	0	100	300	1,000	
		-	1%	3%	21%*	
	F	0	100	300	1,000	
		-	−2%	2%	8%*	
Serum Liver Enzymes						
Suzuki et al. (2012) Rat, F344, male and female, 50 /sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking Water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	AST, ALT, and ALP Enzymes in Liver *(% change compared to control)*					
	M	0	28	121	542	
	AST	-	−21%	−3%	−1%	
	ALT	-	−17%	2%	−4%	
	ALP	-	−5%	3%	0.3%	
	F	0	46	171	560	
	AST	-	−19%	−17%	−46%*	
	ALT	-	−10%	−15%	−26%	
	ALP	-	−16%	2%	−15	
JPEC (2008c) Rats, Sprague Dawley Male and female, 15/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	AST, ALT, and ALP Enzymes in Liver *(% change compared to control)*					
	M	0	5	25	100	400
	AST	-	16%	19%	20%	23%
	ALT	-	10%	48%	13%	36%
	ALP	-	2%	12%	−8%	27%
	F	0	5	25	100	400
	AST	-	10%	13%	20%	4%
	ALT	-	11%	21%	46%	21%
	ALP	-	6%	−21%	−18%	−19%
Centrilobular Hypertrophy						
Suzuki et al. (2012) Rat, F344, male and female, 50 /sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking Water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Centrilobular hypertrophy					
	Not observed					

Table 2-3. Evidence pertaining to liver effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results					
JPEC (2008c)	Incidence of centrilobular hypertrophy					
Rats, Sprague Dawley Male and female, 15/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	M	0 0/15	5 0/15	25 0/15	100 0/15	400 6/15*
	F	0 0/15	5 0/15	25 0/15	100 0/15	400 6/15*
Gaoua (2004b)	Incidence of centrilobular hypertrophy					
Rats, Sprague-Dawley, Male and female, 25/sex/group Gavage, (F0 generation) 0, 250, 500, 1,000 mg/kg-day 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	M	0 0/25	250 0/25	500 0/25	1,000 3/25	
	F	0 0/25	250 0/25	500 0/25	1,000 0/25	

[a]Conversion performed by study authors.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

Table 2-4. Evidence pertaining to liver effects in animals following inhalation exposure to ETBE

Reference and study design	Results				
Liver Weight					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Absolute liver weight (*percent change compared to control*)[2]				
	M	0	2,090	6,270	20,900
		-	0.9%	11%*	10%
	F	0	2,090	6,270	20,900
		-	−4%	−8%	0.5%
	Relative liver weight (*percent change compared to control*)				
	M	0	2,090	6,270	20,900
		-	9%*	19%*	49%*
	F	0	2,090	6,270	20,900
		-	3%	1%*	30%*
JPEC (2008b) Rats, Sprague Dawley Male and female, 10−16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks {} = subset with 28 day recovery after 13 week exposure	Absolute liver weight (*percent change compared to control*)				
	M	0	627	2,090	6,270	20,900
		-	5%	6%	4%	2% {13%}
	F	0	627	2,090	6,270	20,900
		-	−3%	−8%	−2%	5% {11%}
	Relative liver weight (*percent change compared to control*)					
	M	0	627	2,090	6,270	20,900
		-	5%	5%	6%	10% {9%*}
	F	0	627	2,090	6,270	20,900
		-	4%	−1%	6%	18%* {7%}
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Absolute liver weight (*percent change compared to control*)				
	M	0	2,090	7,320	20,900
		-	6%	14%*	32%*
	F	0	2,090	7,320	20,900
		-	2%	9%	26%*
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996a) (unpublished study)	Absolute liver weight (percent change compared to control)				
	M	0	2,090	7,320	20,900
		-	4%	13%*	18%*
	F	0	2,090	7,320	20,900
		-	2%	19%*	33%*

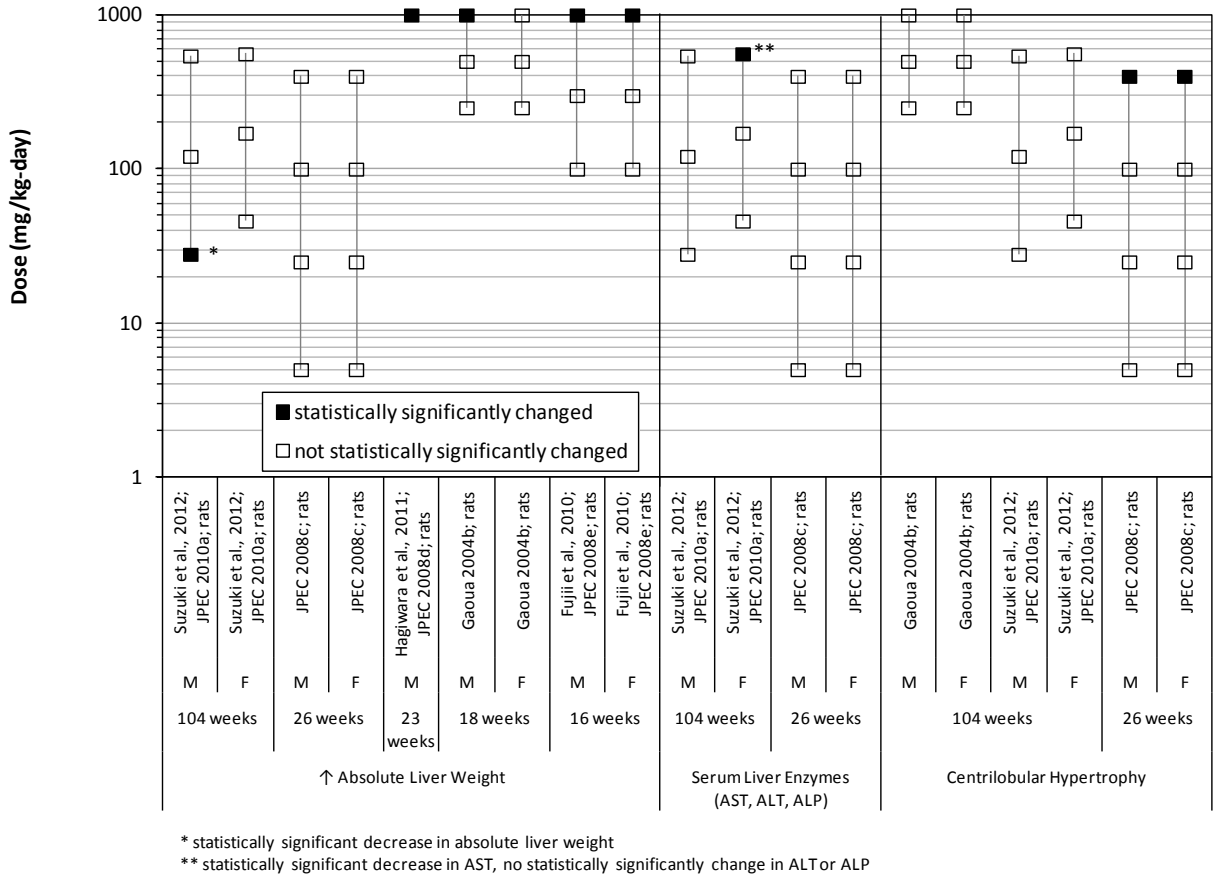
Table 2-4. Evidence pertaining to liver effects in animals following inhalation exposure to ETBE (continued)

Reference and study design	Results					
Serum Liver Enzymes						
JPEC (2010b) Rats, F344, male and female, 50/sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	AST, ALT, and ALP Enzymes in Liver (percent change compared to control)					
	M	0	2,090	6,270	20,900	
	AST	-	29%	−16%	−2%*	
	ALT	-	53%	−3%	24%	
	ALP	-	0 %	−21%*	−5%	
	F	0	2,090	6,270	20,900	
	AST	-	22%	10%	18%*	
	ALT	-	2%	−5%	4%*	
	ALP	-	12%	−4%	4%	
Centrilobular Hypertrophy						
JPEC (2010b) Rats, F344, male and female, 50/sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m³)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Centrilobular hypertrophy					
	Not observed					
JPEC (2008b) Rats, Sprague Dawley Male and female, 10−16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks {} = subset with 28 day recovery after 13 week exposure	Incidence of centrilobular hypertrophy					
	M	0	627	2,090	6,270	20,900
		0/10	0/10	0/10	0/10	4/10* {0/6}
	F	0	627	2,090	6,270	20,900
		0/10	0/10	0/10	0/10	6/10* {0/6}
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Centrilobular hypertrophy					
	Not observed					

Table 2-4. Evidence pertaining to liver effects in animals following inhalation exposure to ETBE (continued)

Reference and study design	Results				
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Incidence of centrilobular hypertrophy				
	M	0 0/15	500 0/15	1,750 2/15	5,000 8/10*
	F	0 0/13	500 2/15	1,750 1/15	5,000 9/14*
Weng et al. (2012) C57BL/6 mice, male and female 5/sex /group 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,315, 20,900 mg/m³)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks	Incidence of centrilobular hypertrophy				
	M	0 1/5	2,090 0/5	7,315 0/5	20,900 5/5*
	F	0 0/5	2,090 0/5	7,315 1/5	20,900 5/5*

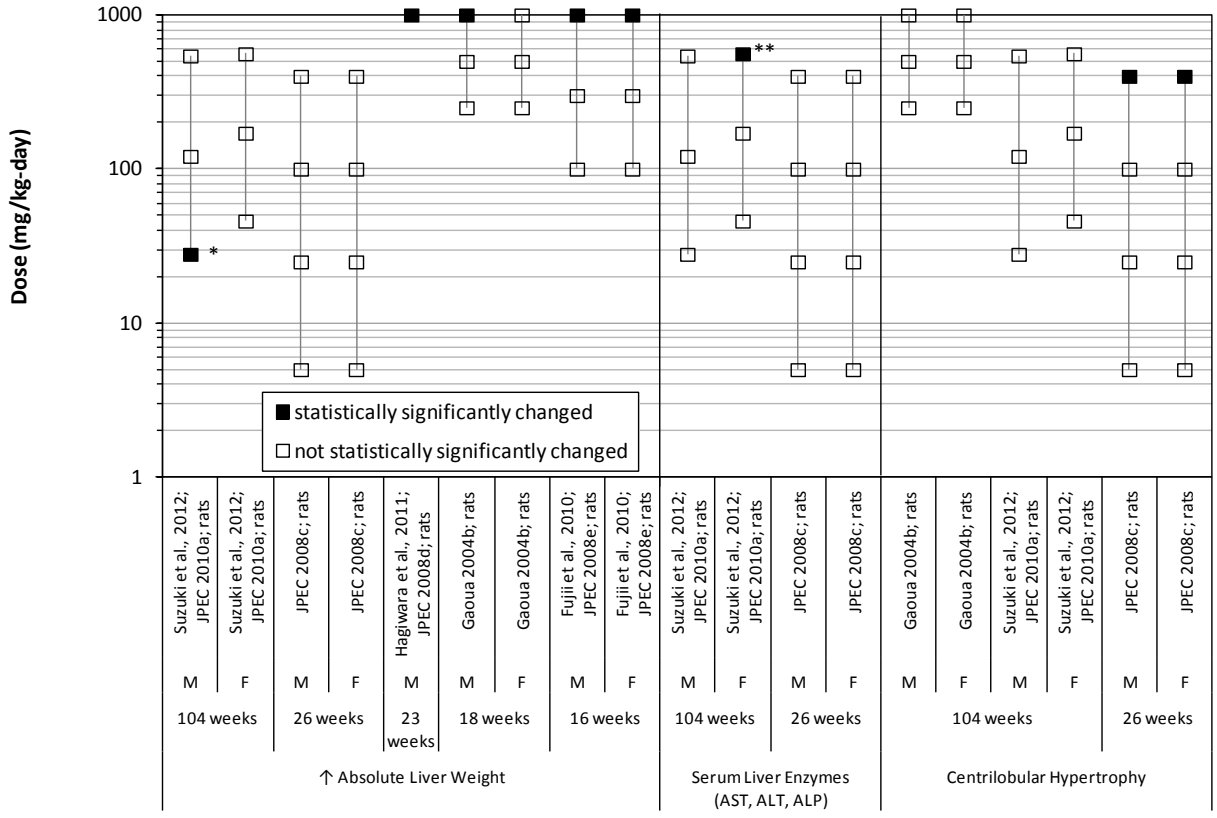
[a]4.18 mg/m^3 = 1 ppm.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.



* statistically significant decrease in absolute liver weight
** statistically significant decrease in AST, no statistically significantly change in ALT or ALP

1

2 **Figure 2-3. Exposure-response array of liver effects following oral exposure to**
3 **ETBE.**



* statistically significant decrease in absolute liver weight
** statistically significant decrease in AST, no statistically significantly change in ALT or ALP

Figure 2-4. Exposure-response array of liver effects following inhalation exposure to ETBE.

2.4. Reproductive Effects

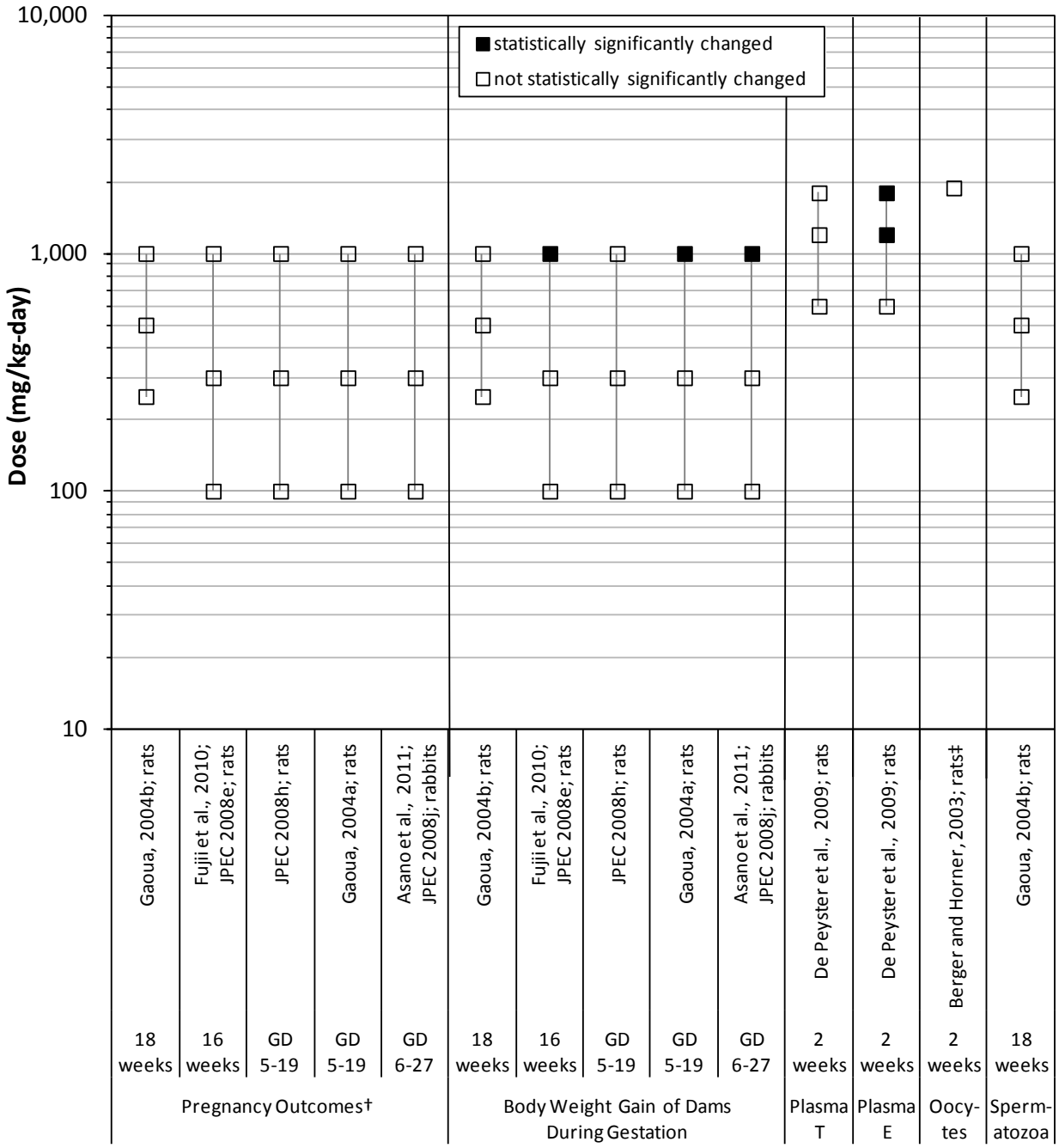
Table 2-5. Evidence pertaining to reproductive effects in animals following oral exposure to ETBE

Reference and study design	Results				
Reproductive effects					
Gaoua (2004b) Rat, Sprague Dawley, male and female, 25/sex/group 0, 250, 500, 1,000 mg/kg-d Gavage Approximately 18 wks in F0 (10 wks before mating, 2-wk mating period, 3-wk gestation, until weaning F1); F1 generation gavaged from weaning until weaning of F2 offspring	F0 reproductive effects (*percent change compared to control*)				
	F	0	250	500	1,000
	Viability index PND 4	-	−5%	−16%	0.1%
	Lactation index	-	−3%	2%	5%
	Body weight gain (GD0−20)	-	2%	3%	3%
	Fertility index	-	−9%	−4%	9%
	M	0	250	500	1,000
	Spermatazoa	-	2%	1%	−0.5%
	F0 reproductive effects (incidence)				
	F	0	250	500	1,000
	Post implantation loss(%)[a]	4%	6%	5%	7%
	Total litter loss PND 4	0/23	1/21	3/22	0/25
	F1 reproductive effects (*percent change compared to control*)				
	F	0	250	500	1,000
	Viability index PND 4	-	−3%	−1%	−5%
	Lactation index	-	1%	2%	2%
	Body weight gain (GD0−20)	-	−1%	−3%	−6%
	F1 reproductive effects (*incidence*)				
	F	0	250	500	1,000
	Post implantation loss(%)[b]	4%	5%	3%	7%
	Total litter loss PND 4	0/21	1/21	0/22	1/20
Fujii et al. (2010) Rat, Sprague Dawley, male and Female, 24/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 16−17 week exposure to F0 rats **Related reference**: JPEC (2008e) (unpublished study)	F0 reproductive effects (*percent change compared to control*)				
	M	0	100	300	1,000
	Fertility index	-	14%	9%	5%
	F	0	100	300	1,000
	Viability index PND 4	-	−1%	2%	−10%
	Lactation index[c]	-	−1%	−1%	−5%
	Body weight gain (GD0−20)	-	−4%	8%	12%*
	Fertility index	-	14%	9%	5%
	F0 reproductive effects (*incidence*)				
	F	0	100	300	1,000
	Post implantation loss(%)[a]	7%	14%	11%	10%
	Total litter loss PND 4	0/21	0/22	0/23	3/22
JPEC (2008h) Rat, Sprague Dawley, female 21−22litters/ group 0, 100, 300, 1,000 mg/kg-d Gavage Gestational days 5−19	Reproductive effects (*percent change compared to control*)				
	F	0	100	300	1,000
	Body weight gain (GD0−20)	-	−7%	−4%	−7%
	Reproductive effects (*incidence*)				
	F	0	100	300	1,000
	Pre-implantation loss (%)[b]	7%	9%	8%	12%
	Post-implantation loss(%)[a]	6%	7%	4%	5%

Table 2-5. Evidence pertaining to reproductive effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results				
Gaoua (2004a) Rat, Sprague Dawley, female 19–22 litters/ group 0, 250, 500, 1,000 mg/kg-d Gavage Gestational days 5–19	Reproductive effects (*percent change compared to control*)				
	F Body weight gain (GD5–20)	0 -	100 –4%	300 –3%	1,000 –17%*
	Reproductive effects (*incidence*)				
	F Pre-implantation loss (%)[b] Post-implantation loss(%)[a]	0 15% 5%	100 13% 7%	300 13% 7%	1,000 14% 8%
Asano et al. (2011) Rabbit, New Zealand white, female 22–24/ sex/ group 0, 100, 300, 1,000 mg/kg-d Gavage Gestational days 6–27 **Related reference**: JPEC (2008j) (unpublished study)	Reproductive effects (*percent change compared to control*)				
	F Body weight gain (GD0–28) Uterine weight	0 - -	100 –13% 4%	300 0% 5%	1,000 –38%* –16%
	Reproductive effects (*incidence*)				
	F Pre-implantation loss (%)[b] Post-implantation loss (%)[a]	0 20% 11%	100 15% 11%	300 11% 7%	1,000 23% 9%
de Peyster et al. (2009) Rat, F344, male 12/sex/group 0, 600, 1,200, 1,800 mg/kg-d Gavage 14 days	Plasma hormone levels (*percent change compared to control*)				
	M Testosterone Estradiol	0 - -	600 50% 29%	1,200 26% 106%*	1,800 –34% 105%*
Berger and Horner (2003) Rat, Simonson, female 3–4/sex/group 0.3% (estimated 1,887 mg/kg-day) Drinking water 2 weeks	Oocyte effects				
	F Oocytes/female Oocyte fertilized	0 30 84%		1,887 29 82%	
Gaoua (2004b) Rat, Sprague Dawley, male and female,25/sex/group 0, 250, 500, 1,000 mg/kg-d Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Number of spermatozoa in F0 (*Percent change compared to control*)				
	M -	0	250 2%	500 1%	1,000 –0.5%

[a]Post-implantation loss = (Resorptions + dead fetus/ total implantations) × 100, calculated per litter.
[b]Pre-implantation loss = (corpora lutea-implantations/corpora lutea) × 100, calculated per litter.
[c]Lactation index = (pups alive at day 21/pups at day 4) × 100; LI is the same as viability index on day 21.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.



†Pregnancy outcomes include: Viability, Lactation & Fertility Indices and Pre- & Post-Implantation Loss and Total litter loss

‡ Dose estimated from 0.3% in drinking water using drinking water rate reported by JPEC 2010a

Figure 2-5. Exposure-response array of reproductive effects following oral exposure to ETBE.

2.5. Body Weight Effects

Table 2-6. Evidence pertaining to body weight effects in animals following oral exposure to ETBE

Reference and study design	Results				
Body Weight					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Body weight (*percent change compared to control*)				
	M	0	28	121	542
		-	−5%	−7%*	−10*%
	F	0	46	171	560
		-	−10*%	−11*%	−17*%
JPEC (2008c) Rats, Sprague Dawley Male and female, 15/sex/group 0, 5, 25, 100, 400 mg/kg-day Gavage 26 weeks (180 consecutive days)	Body weight (*percent change compared to control*)				
	M	0	5	25	100	400
		-	−6%	0%	−6%	2%
	F	0	5	25	100	400
		-	−5%	−2%	−2%	−3%
Hagiwara et al. (2011) F344 Rats, male, 12/group Gavage 0, 1,000 mg/kg-day 23 weeks **Related reference**: JPEC (2008d) (unpublished study)	Body weight (*percent change compared to control*)				
	−5%*				
Gaoua (2004b) Rats, Sprague Dawley, Male and female, 25/sex/group 0, 250, 500, 1,000 mg/kg-day Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Body weight (*percent change compared to control*)				
	M	0	250	500	1,000
		-	−1%	−3%	−5%*
	F	0	250	500	1,000
		-	−7%	−2%	0%

Table 2-6. Evidence pertaining to body weight effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results				
Fujii et al. (2010)	F0 generation body weight (*percent change compared to control*)				
Rats, Sprague Dawley, male and female, 24/sex/group	M	0	100	300	1,000
0, 100, 300, 1,000 mg/kg-day		-	−4%	−4%	−7%
Gavage	F	0	100	300	1,000
16 weeks (males), 17 weeks (females)		-	1%	1%	5%
Related reference: JPEC (2008e) (unpublished study)	F1 generation body weight (*percent change compared to control*)				
	M	0	100	300	1,000
		-	2%	0%	1%
	F	0	100	300	1,000
		-	−1%	−3%	−2%

[a]Conversion performed by study authors.
[*]Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

Table 2-7. Evidence pertaining to body weight effects in animals following inhalation exposure to ETBE

Reference and study design	Results				
Body Weight					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Body weight (*percent change compared to control*)				
	M	0 -	2,090 −7%*	6,270 −7%*	20,900 −26%*
	F	0 -	2,090 −6%*	6,270 −10%*	20,900 −23%*
JPEC (2008b) Rats, Sprague Dawley Male and female, 10−16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks {} = subset with 28 day recovery after 13 week exposure	Body weight (*percent change compared to control*)				
	M	0 -	627 0%	2,090 1%	6,270 −1%	20,900 −6% {3%}
	F	0 -	627 −2%	2,090 −4%	6,270 −3%	20,900 −6% {3%}
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Body weight (*percent change compared to control*)				
	M	0 -	2,090 2%	7,320 5%	20,900 2%
	F	0 -	2,090 −3%	7,320 3%	20,900 6%*
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996a) (unpublished study)	Body weight (*percent change compared to control*)				
	M	0 -	2,090 −1%	7,320 −1%	20,900 −3%
	F	0 -	2,090 −3%	7,320 −1%	20,900 2%

[a]4.18 mg/m^3 = 1 ppm.

*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.

Percentage change compared to control = (treated value − control value) ÷ control value × 100.

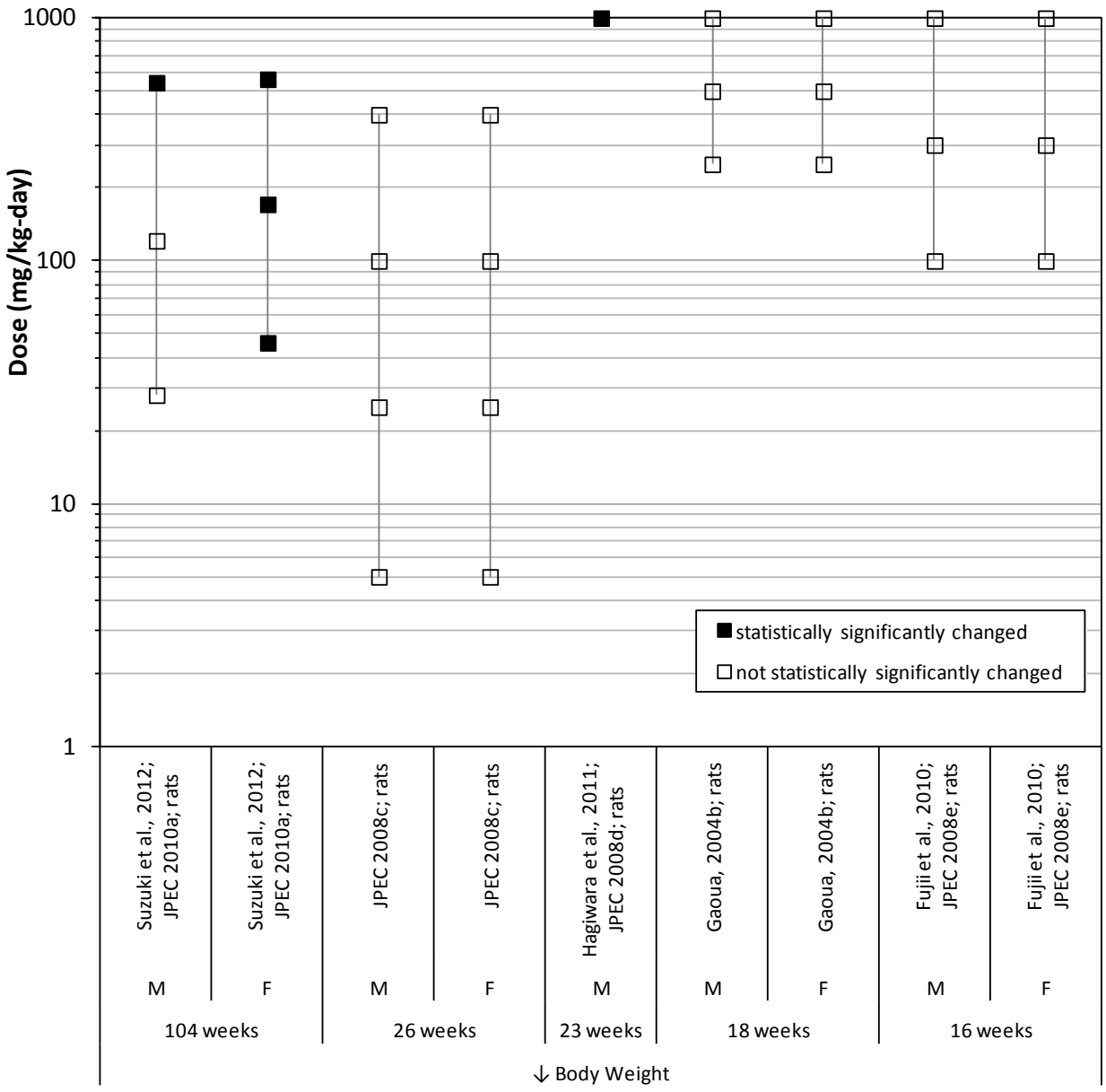
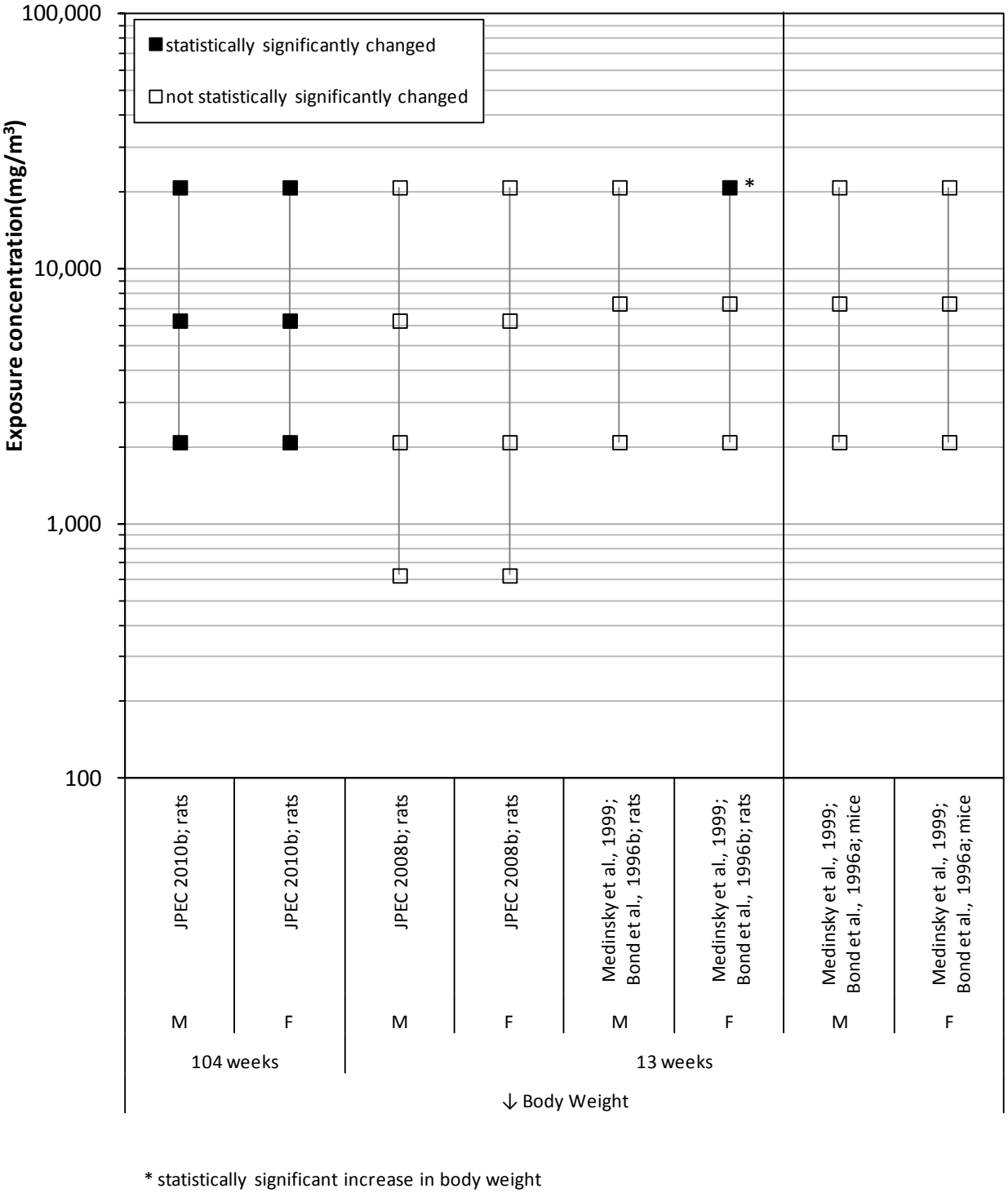


Figure 2-6. Exposure-response array of body weight effects following oral exposure to ETBE.



* statistically significant increase in body weight

Figure 2-7. Exposure-response array of body weight effects following inhalation exposure to ETBE.

2.6. Other Systemic Effects

Table 2-8. Evidence pertaining to other systemic effects in animals following oral exposure to ETBE

Reference and study design	Results				
Immunological Studies					
Banton et al. (2011) Rats, Sprague-Dawley, female, 10/group 0, 250, 500, 1,000 mg/kg-day Gavage 28 consecutive days	Antibody response (*percent change compared to control*)				
	IgM antibody forming cells	0 -	250 −21%	500 42%	1,000 8%
Spleen Weight					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Absolute spleen weight (*percent change compared to control*)				
	M	0 -	28 −3%	121 19%	542 39%
	F	0 -	46 −35%	171 −0.6%	560 −50%*
	Relative spleen weight (*percent change compared to control*)				
	M	0 -	28 2%	121 28%	542 55%*
	F	0 -	46 −35%	171 3%*	560 −45%
Hagiwara et al. (2011) F344 Rats, male, 12/group Gavage 0, 1,000 mg/kg-day 23 weeks **Related reference**: JPEC (2008d) (unpublished study)	Absolute spleen weight (*percent change compared to control*)				
	−5%				
	Relative spleen weight *(percent change compared to control)*				
	0%				
Gaoua (2004b) Rats, Sprague Dawley, Male and female, 25/sex/group 0, 250, 500, 1,000 mg/kg-day Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Absolute spleen weight (*percent change compared to control*)				
	M	0 -	250 2%	500 2%	1,000 0%
	F	0 -	250 −4%	500 −2%	1,000 −3%
	Relative spleen weight (*percent change compared to control*)				
	M	0 -	250 3%	500 6%	1,000 6%
	F	0 -	250 4%	500 1%	1,000 −6%
Banton et al. (2011) Rats, Sprague-Dawley, female, 10/group 0, 250, 500, 1,000 mg/kg-day Gavage 28 consecutive days	Absolute spleen weight (*percent change compared to control*)				
		0 -	250 −3%	500 −15%	1,000 −9%

Table 2-8. Evidence pertaining to other systemic effects in animals following oral exposure to ETBE (continued)

Reference and study design	Results				
Adrenal Weight					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Absolute adrenal weight (*percent change compared to control*)				
	M	0	28	121	542
		-	5%	5%	79%
	F	0	46	171	560
		-	−7%*	6%	−8%*
	Relative adrenal weight (*percent change compared to control*)				
	M	0	28	121	542
		-	9%	9%*	105%*
	F	0	46	171	560
		-	4%	19%*	11%*
Gaoua (2004b) Rats, Sprague Dawley, Male and female, 25/sex/group 0, 250, 500, 1,000 mg/kg-day Gavage (F0 generation) 18 weeks (10 weeks before mating, during a 2-week mating period, 3-week gestation and until after weaning F1)	Absolute adrenal weight (*percent change compared to control*)				
	M	0	250	500	1,000
		-	15%*	13%*	27%*
	F	0	250	500	1,000
		-	−3%	5%	6%
	Relative adrenal weight (*percent change compared to control*)				
	M	0	250	500	1,000
		-	16%*	17%*	34%*
	F	0	250	500	1,000
		-	6%	6%	4%
Mortality					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Survival (*percent change compared to control*)				
	M	0	28	121	542
		-	−3%	−11%	−11%
	F	0	46	171	560
		-	3%	6%	6%

[a]Conversion performed by study authors.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

Table 2-9. Evidence pertaining to other systemic effects in animals following inhalation exposure to ETBE

Reference and study design	Results					
Immunological Studies						
Li et al. (2011) Mice, C57BL/6 and 129/SV male, 5–6/group 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,315, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 6 or 13 weeks	Number of CD3+, CD4+, and CD8+ splenic T cells in C57BL/6 (*percent change compared to control*)					
	6 weeks	0	25	100	400	
	CD3+	-	−14%	−13%	−24%*	
	CD4+	-	−15%	−11%	−23%	
	CD8+	-	−12%	−13%*	−23%*	
	13 weeks	0	25	100	400	
	CD3+	-	−9%	−17%*	−24%*	
	CD4+	-	11%	−28%*	−37%*	
	CD8+	-	−8%	−12%	20%	
Weng et al. (2011) Mice Aldh2-/- and wt C57BL/6 male and female, 5/group 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,315, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks	Leukocyte DNA damage in Aldh2-/- (*percent change compared to control*)					
	M	0	2,090	7,320	20,900	
		-	35%*	61%*	74%*	
	F	0	2,090	7,320	20,900	
		-	9%	34%	56%*	
Spleen Weight						
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Absolute spleen weight (*percent change compared to control*)					
	M	0	28	121	542	
		-	4%	32%	17%	
	F	0	46	171	560	
		-	5%	−39%	−43%*	
	Relative spleen weight (*percent change compared to control*)					
	M	0	28	121	542	
		-	15%	43%*	66%*	
	F	0	46	171	560	
		-	30%	−31%	−25%	
JPEC (2008b) Rats, Sprague Dawley Male and female, 10–16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks {} = subset with 28 day recovery after 13 week exposure	Absolute spleen weight (*percent change compared to control*)					
	M	0	627	2,090	6,270	20,900
		-	−0.4%	7%	−1%	−9% {10%}
	F	0	627	2,090	6,270	20,900
		-	−9%	−2%	−5%	−1% {6%}
	Relative spleen weight (*percent change compared to control*)					
	M	0	627	2,090	6,270	20,900
		-	0%	5%	1%	−2% {6%}
	F	0	627	2,090	6,270	20,900
		-	−3%	5%	1%	12% {0%}

Table 2-9. Evidence pertaining to other systemic effects in animals following inhalation exposure to ETBE(continued)

Reference and study design	Results				
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Absolute spleen weight (*percent change compared to control*)				
	M	0	2,090	7,315	20,900
		-	6%	3%	5%
	F	0	2,090	7,315	20,900
		-	−3%	3%	0%
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996a) (unpublished study)	Absolute spleen weight (*percent change compared to control*)				
	M	0	2,090	7,320	20,900
		-	−5%	0%	−15%
	F	0	2,090	7,320	20,900
		-	−11%	−2%	−11%
Adrenal Weight					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Absolute adrenal weight (*percent change compared to control*)				
	M	0	2,090	6,270	20,900
		-	−42%	−55%	−49%
	F	0	2,090	6,270	20,900
		-	−7%	−13%*	−9%
	Relative adrenal weight (*percent change compared to control*)				
	M	0	2,090	6,270	20,900
		-	−35%	−52%	−33%*
	F	0	2,090	6,270	20,900
		-	−3%	−6%	16%*
JPEC (2008b) Rats, Sprague-Dawley Male and female, 10−16/sex/group 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks	Absolute adrenal weight (*percent change compared to control*)					
	M	0	627	2,090	6,270	20,894
		-	11%	8%	5%	10%
	F	0	627	2,090	6,270	20,894
		-	−0.4%	−4%	2%	−4%
	Relative adrenal weight (*percent change compared to control*)					
	M	0	626.8	2,089	6,270	20,894
		-	11%	7%	7%	18%*
	F	0	626.8	2,089	6,270	20,894
		-	6%	2%	9%	7%

Table 2-9. Evidence pertaining to other systemic effects in animals following inhalation exposure to ETBE(continued)

Reference and study design	Results				
Medinsky et al. (1999) Rats, F344, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996b) (unpublished study)	Absolute adrenal weight (percent change compared to control)				
	M	0 -	2,090 11%	7,315 9%	20,900 34%*
	F	0 -	2,090 7%	7,315 7%	20,900 18%*
Medinsky et al. (1999) Mice, CD-1, male and female 10/sex/group 0, 500, 1,750, 5,000 ppm (2,090, 7,320, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 13 weeks **Related reference**: Bond et al. (1996a) (unpublished study)	Absolute adrenal weight (*percent change compared to control*)				
	M	0 -	2,090 0%	7,315 50%	20,900 0%
	F	0 -	2,090 −8%	7,315 8%	20,900 −8%
Mortality					
JPEC (2010b) Rat, F344, male and female, 50 /sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Survival rate				
	M	0 88%	2,090 76%	6,270 80%	20,900 60%*
	F	0 76%	2,090 78%	6,270 60%*	20,900 60%*

[a]4.18 mg/m^3 = 1 ppm.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

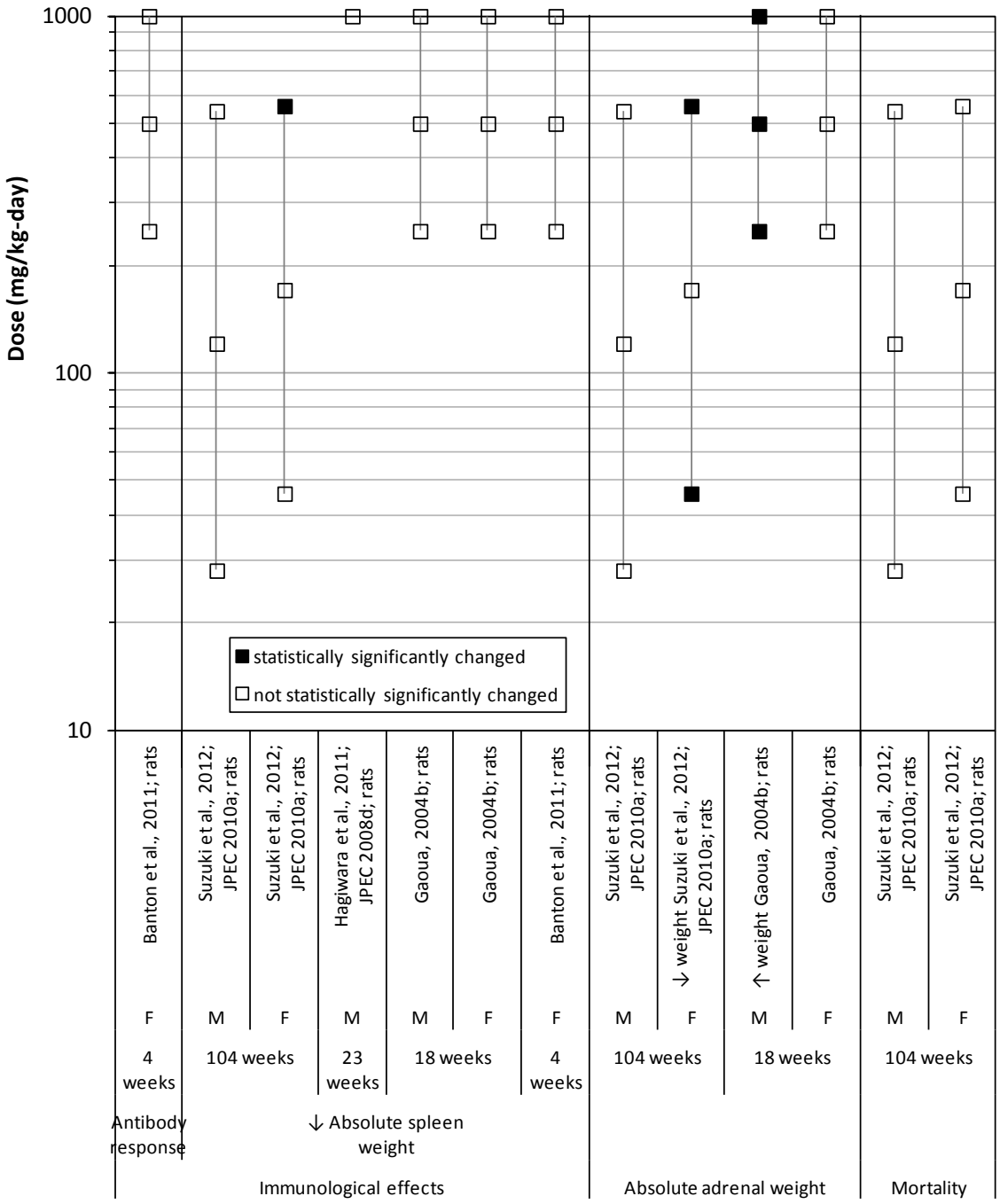


Figure 2-8. Exposure-response array of other systemic effects following oral exposure to ETBE.

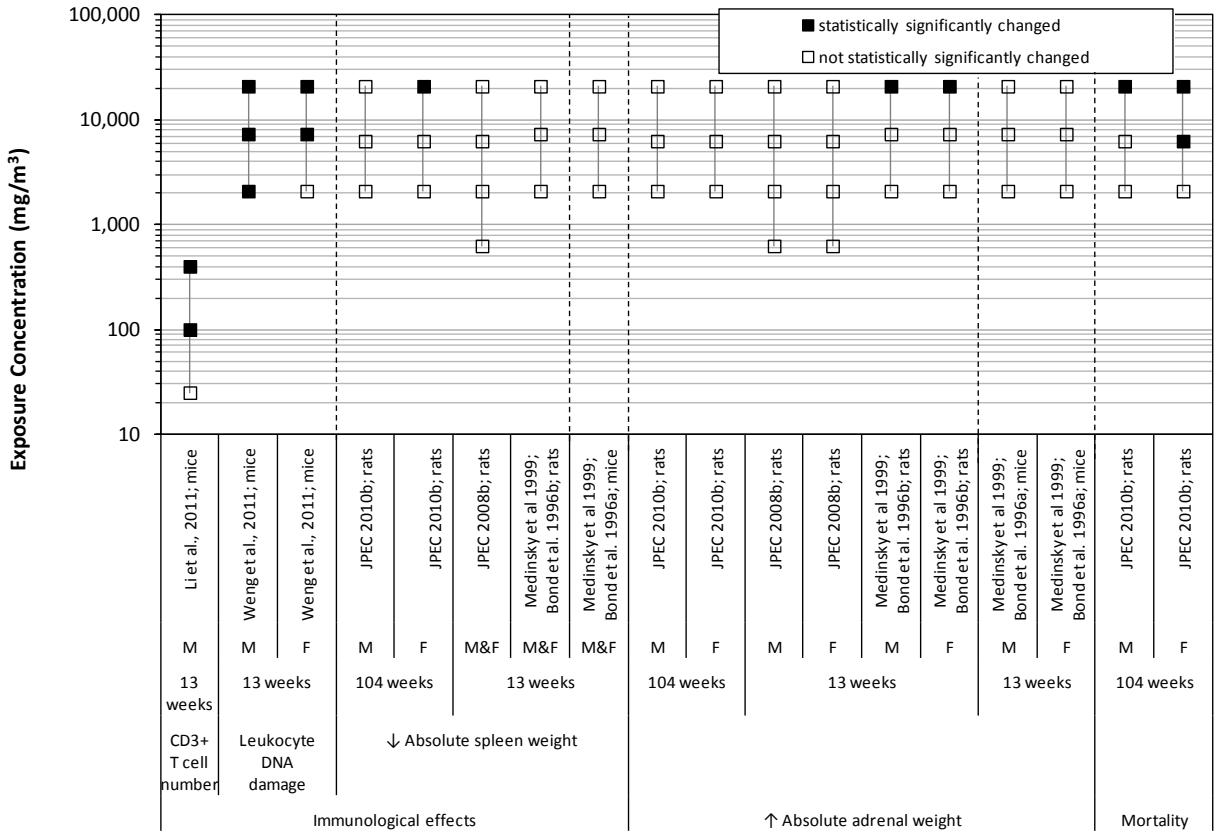


Figure 2-9. Exposure-response array of other systemic effects following inhalation exposure to ETBE.

2.7. Carcinogenic Effects

Table 2-10. Evidence pertaining to carcinogenic effects in animals exposed to ETBE

Reference and study design	Results				
Neoplastic lesions					
Suzuki et al. (2012) Rats, F344, male and female, 50/sex/group 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d in males; 0, 46, 171, 560 mg/kg-d in females)[a] Drinking water 104 weeks **Related reference**: JPEC (2010a) (unpublished study)	Neoplasms				
	No treatment related effects				
Maltoni et al. (1999) Rats, Sprague Dawley, Male and female, 60/sex/group 0, 250, 1,000 mg/kg-d Gavage 104 weeks	Incidence of uterine malignancies				
	F	0	250	1,000	
		2/60	10/60*	2/60	
	Incidence of mouth epithelium tumors				
	M	0	250	1,000	
		6/60	14/60	15/60*	
Hagiwara et al. (2011) Rats, F344, male 30/sex/group 0, 300, 1,000 mg/kg-d Gavage 23 weeks following a 4 week tumor initiation with DMBDD	Incidence of neoplastic lesions				
	M	0	300	1,000	
	Thyroid	8/30	17/30*	20/30*	
	Forestomach	0/30	4/30	3/30	
	Colon	25/30	21/30	28/30*	
	Liver	1/30	1/30	6/30*	
	Kidney	11/30	6/30	13/30	
JPEC (2010b) Rats, F344, male and female, 50/sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Incidence of hepatocellular adenomas and carcinomas				
	M	0	2,090	6,270	20,900
		0/50	2/50	1/49	10/50*
	F	0	2,090	6,270	20,900
		1/50	0/50	1/50	1/50
Preneoplastic lesions					
JPEC (2010b) Rats, F344, male and female, 50/sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] Whole body inhalation 6 hours/day, 5 days/week 104 weeks	Incidence of acidophilic and basophilic foci in liver				
	M	0	2,090	6,270	20,900
	Acidophilic	31/50	28/50	36/49	39/50*
	Basophilic	18/50	10/50	13/49	33/50*
	F	0	2,090	6,270	20,900
	Acidophilic	2/50	1/50	4/50	2/50
	Basophilic	36/50	31/50	32/50	28/50

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
*Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.

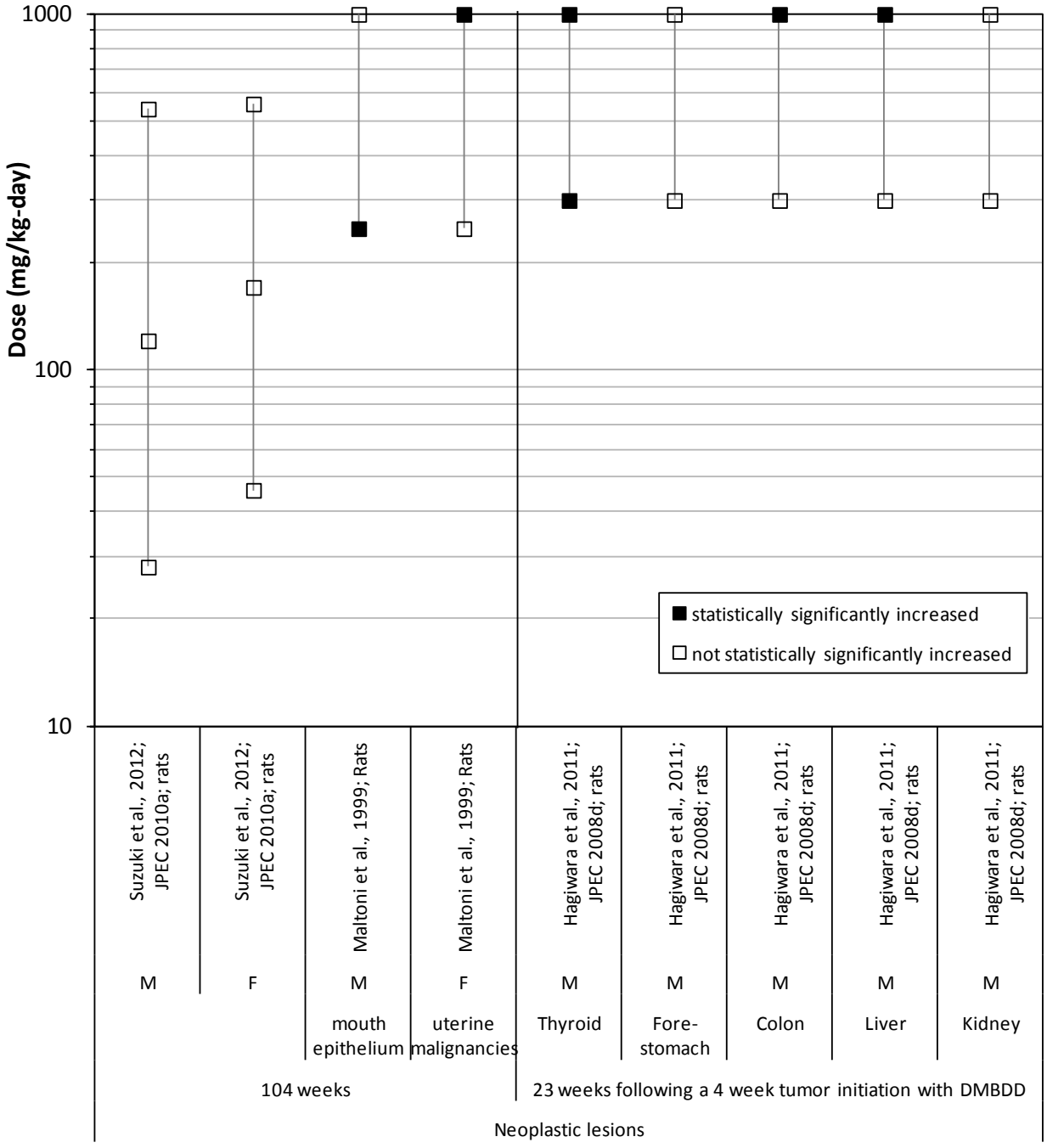


Figure 2-10. Exposure-response array of carcinogenic effects following oral exposure to ETBE.

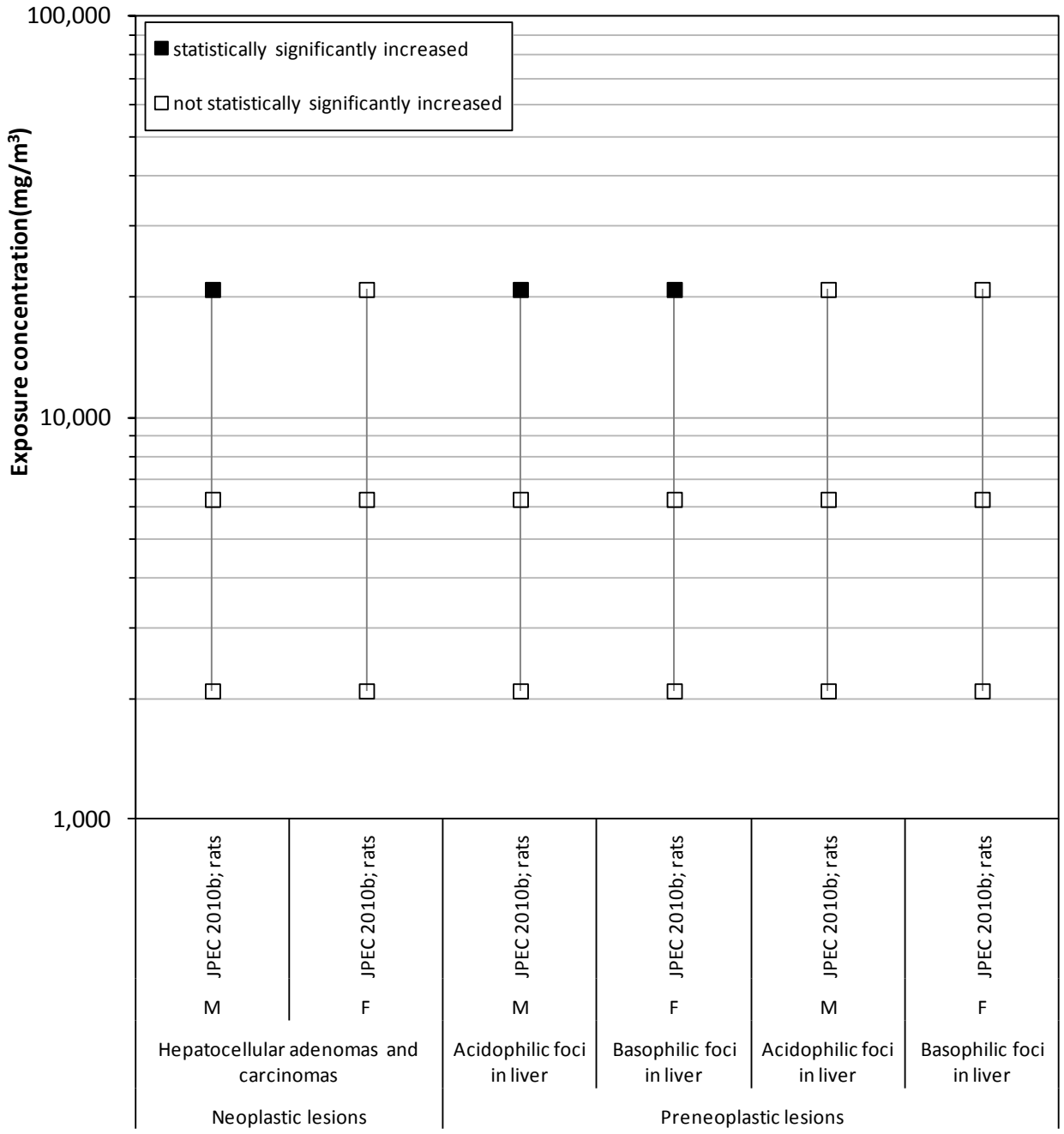


Figure 2-11. Exposure-response array of carcinogenic effects following inhalation exposure to ETBE.

2.8. Genotoxic Effects

Table 2-11. Evidence pertaining to genotoxic effects in animals exposed to ETBE

Reference and study design	Results				
Micronucleus assays					
JPEC (2007c) Rats, F344, Male and female, 10/sex/group 0, 1,600, 4,000, 10,000 ppm (0, 101, 259, 626 mg/kg-d in males; 0, 120, 267, 629 mg/kg-d in females)[a] Drinking water 13 weeks	Frequency of micronucleated polychromatic erythrocytes				
	M	0 0.2	101 0.2	259 0.1	626 0.2
	F	0 0.1	120 0.2	267 0.1	629 0.1
	Ratio of polychromatic erythrocytes / erythrocytes				
	M	0 24	101 26	259 25	626 24
	F	0 24	120 25	267 25	629 24
JPEC (2007d) Rats, F344 Male and female, 10/sex/group 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] Whole body inhalation 6 hours/day, 5 days/week 13 weeks	Frequency of micronucleated polychromatic erythrocytes				
	M	0 0.1	2,090 0.2	6,270 0.2	20,900 0.2
	F	0 0.2	2,090 0.2	6,270 0.2	20,900 0.2
	Ratio of polychromatic erythrocytes / erythrocytes				
	M	0 23	2,090 24	6,270 24	20,900 24
	F	0 24	2,090 23	6,270 25	20,900 25
JPEC (2007a) Rats, F344 Male and female, 5/sex/group 0, 500, 1,000, 2,000 mg/kg-d Gavage 2 doses, 24 hr apart 2 days	Frequency of micronucleated polychromatic erythrocytes				
	M	0 0.1	500 0.1	1,000 0.2	2,000 0.1
	F	0 0.1	500 0.1	1,000 0.1	2,000 0.1
	Ratio of polychromatic erythrocytes / erythrocytes				
	M	0 22	500 22	1,000 23	2,000 23
	F	0 21	500 21	1,000 20	2,000 26
JPEC (2007b) Rats, F344 Male and female, 5/sex/group 0, 250, 500, 1,000 mg/kg-d Intraperitoneal injection 2 doses, 24 hr apart 2 days	Frequency of micronucleated polychromatic erythrocytes				
	M	0 0.1	250 0.1	500 0.1	1,000 0.2
	F	0 0.1	250 0.2	500 0.1	1,000 0.1
	Ratio of polychromatic erythrocytes / erythrocytes				
	M	0 26	250 27	500 28	1,000 25
	F	0 23	250 26	500 27	1,000 30

Table 2-11. Evidence pertaining to genotoxic effects in animals exposed to ETBE (continued)

Reference and study design	Results				
Micronucleus assays					
Vergnes and Kubena (1995b)	Frequency of micronucleated polychromatic erythrocytes				
Mice, CD-1	M	0	1,670	8,360	20,900
Male and female, 5/sex/group		0.2	0.2	0.2	0.2
0, 400, 2,000, 5,000 ppm	F	0	1,670	8,360	20,900
(0, 1,670, 8,360, 20,900 mg/m^3)[b]		0.2	0.1	0.1	0.2
Whole body inhalation	Mean polychromatic erythrocytes / 1,000 erythrocytes (% of control)				
6 hours/day for 5 days	M	0	1,670	8,360	20,900
		-	99%	95%	92%
	F	0	1,670	8,360	20,900
		-	100%	96%	97%

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
[*]Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.

Table 2-12. Summary of in vitro studies of ETBE genotoxicity

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* (TA97, TA98, TA100, TA1535)	10,000 µg/plate	–	–		Zeiger et al. (1992)
SOS repair induction	ND					
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal malsegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	Chinese hamster ovary (HGPRT)	5 mg/mL	–	–		Vergnes and Kubena (1995a)
Chromosomal aberrations	Chinese hamster ovary	5 mg/mL	–	–		Vergnes (1995)
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive, ± = equivocal or weakly positive, − = negative, T = cytotoxicity, NA = not applicable, ND = no data.