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# Toxicological Review of Ethyl Tertiary Butyl Ether

(CASRN 637-92-3)

## Supplemental Information

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# ABBREVIATIONS

| | |
|---|---|
| ADAF | age-dependent adjustment factor |
| ADJ | adjusting experimental exposure concentrations to a value reflecting continuous exposure duration |
| ADME | absorption, distribution, metabolism, excretion |
| AIC | Akaike's information criterion |
| ALDH | aldehyde dehydrogenase |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase/transaminase |
| AST | aspartate aminotransferase/transaminase |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AUC | area-under-the-curve |
| BBN | *N*-butyl-*N*-(hydroxybutyl)nitrosamine |
| BMC | benchmark concentration |
| BMCL | benchmark concentration lower confidence limit |
| BMD | benchmark dose |
| BMDL | benchmark dose lower confidence limit |
| BMDS | Benchmark Dose Software |
| BMR | benchmark response |
| BUN | blood urea nitrogen |
| BW | body weight |
| CAAC | Chemical Assessment Advisory Committee |
| CAR | constitutive androstane receptor |
| CASRN | Chemical Abstracts Service registry number |
| $C_{max}$ | maximum concentration |
| CPHEA | Center for Public Health and Environmental Assessment |
| CPN | chronic progressive nephropathy |
| CSL | continuous simulation language |
| CYP450 | cytochrome P450 |
| DAF | dosimetric adjustment factor |
| DEN | diethylnitrosamine |
| df | degrees of freedom |
| DHPN | *N*-bis(2-hydroxypropyl)nitrosamine |
| DMH | 1,2-dimethylhydrazine dihydrochloride |
| DNA | deoxyribonucleic acid |
| EHEN | *N*-ethyl-*N*-hydroxyethylnitrosamine |
| EPA | Environmental Protection Agency |
| ETBE | ethyl tertiary butyl ether |
| GD | gestation day |
| GGT | γ-glutamyl transferase |
| GLP | good laboratory practice |
| HBA | hydroxyisobutyric acid |
| HEC | human equivalent concentration |
| HED | human equivalent dose |
| HERO | Health and Environment Research on Online |
| HGPRT | hypoxanthine-guanine phosphoribosyltransferase |
| HIBA | 2-hydroxyisobutyrate |
| HT | heterogeneous |
| IARC | International Agency for Research on Cancer |
| IRIS | Integrated Risk Information System |
| i.v. | intravenous |
| JPEC | Japan Petroleum Energy Center |
| Km | Michaelis-Menten constant |
| KO | knockout |
| LD | lactation day |
| LOAEL | lowest-observed-adverse-effect level |
| MN | micronucleus, micronucleated |
| MNPCE | micronucleated polychromatic erythrocytes |
| MNU | *N*-methyl-*N*-nitrosourea |
| MOA | mode of action |
| MPD | 2-methyl-1,2-propanediol |
| MTBE | methyl *tert*-butyl ether |
| MTD | maximum tolerated dose |
| N.D. | not detected |
| No. | number |
| NOAEL | no-observed-adverse-effect level |
| NR | not reported |
| NTP | National Toxicology Program |
| OECD | Organisation for Economic Co-operation and Development |
| ORD | Office of Research and Development |
| OSF | oral slope factor |
| PBPK | physiologically based pharmacokinetic |
| PCE | polychromatic erythrocyte |
| PND | postnatal day |
| PNW | postnatal week |
| POD | point of departure |
| PPARα | peroxisome proliferator-activated receptor α |
| PXR | pregnane X receptor |
| QA | quality assurance |
| QSAR | quantitative structure-activity relationship |
| RD | relative deviation |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| rho | Spearman's rank coefficient |
| RNA | ribonucleic acid |
| S-D | Sprague-Dawley |

| | |
|---|---|
| SAB | Science Advisory Board |
| SD | standard deviation |
| SE | standard error |
| SRBC | sheep red blood cell |
| SS IICA | Stoddard Solvent IICA |
| TBA | *tert*-butyl alcohol, *tert*-butanol |
| TSCATS | Toxic Substances Control Act Test Submissions |
| TWA | time-weighted average |
| UF | uncertainty factor |
| $UF_A$ | animal-to-human uncertainty factor |
| $UF_C$ | composite uncertainty factor |
| $UF_D$ | database deficiencies uncertainty factor |
| $UF_H$ | human variation uncertainty factor |
| $UF_L$ | LOAEL-to-NOAEL uncertainty factor |
| $UF_S$ | subchronic-to-chronic uncertainty factor |
| USGS | U.S. Geological Survey |
| $V_{max}$ | maximum substrate turnover velocity |
| VOC | volatile organic compounds |
| WT | wild type |
| wt | weight |

# APPENDIX A. OTHER AGENCY AND INTERNATIONAL ASSESSMENTS

**Table A-1. Health assessments and regulatory limits by other national and international health agencies**

| Organization | Toxicity value |
|---|---|
| National Institute for Public Health and the Environment (Bilthoven, The Netherlands) | Oral noncancer tolerable daily intake: 0.25 mg/kg-d<br>Inhalation noncancer tolerable concentration in air: 1.9 mg/$m^3$ |

# APPENDIX B. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-REPONSE ANALYSIS

## B.1. TOXICOKINETICS

### B.1.1. Absorption

***Absorption in Humans***

Most of the available human data on the uptake of ethyl tertiary butyl ether (ETBE) were obtained from volunteers. Nihlén et al. (1998) exposed eight healthy male volunteers (average age: 29 years) to 5, 25, or 50 ppm (20.9, 104, or 210 mg/m$^3$) ETBE by inhalation for 2 hours. Each volunteer was exposed at each concentration in sequence with 2-week intervals between exposures. The study was performed according to the Declaration of Helsinki after approval by the Regional Ethical Committee of the institution where the study was performed, and written informed consent was obtained from the volunteers. The volunteers performed light physical exercise (50 watts) on a bicycle ergometer during exposure. Exhaled air was collected before exposure, every 30 minutes during exposure, and 6 times after exposure. The concentrations of ETBE and one of its primary metabolites, *tert*-butanol (TBA), were determined in exhaled air samples. Blood was drawn before exposure, approximately every 10 minutes during exposure, approximately every 30 minutes from 1 to 4 hours after exposure, and an additional 4 times up to 48 hours after exposure. Urine was collected before exposure, at 0 and 2 hours, and at approximately 4, 7, 11, 20, 22, and 46 hours after exposure. ETBE, *tert*-butanol, and acetone (an ETBE metabolite) concentrations were determined in blood and urine. The blood profiles of the parent compound and metabolites were similar at all three exposure levels and reflected exposure concentrations, as judged by linear increases in blood area-under-the-curve (AUC) values for the concentration-time curve calculated (but only reported in graphical form by the authors).

Acetone levels were highly variable and seemed to reflect not only ETBE exposure, but also the physical activity of the volunteers. Nihlén et al. (1998) calculated the ETBE doses to the volunteers to be 0.58, 2.9, and 5.8 mmol for the 20.9-, 104-, and 210-mg/m$^3$ exposure levels, respectively. The concentrations of ETBE in blood rose sharply during the first 30 minutes of exposure and kept rising at a lower rate until the end of exposure, reaching peak concentrations of about 10, 5.4, and 1.1 µM at 210, 104, and 20.9 mg/m$^3$, respectively. By 6 hours, the concentrations of ETBE had fallen to very low levels (<1 µM), even after the 210-mg/m$^3$ exposure. Based on blood AUC values for ETBE, the authors calculated two types of respiratory uptake: (1) net respiratory

uptake = (concentration in inhaled air − concentration in exhaled air) multiplied by the pulmonary ventilation and (2) respiratory uptake = net respiratory uptake + amount exhaled during the exposure. During the 2 hours of exposure, the authors calculated that 32–34% of each dose was retained by the volunteers (respiratory uptake), and the net respiratory uptake was calculated to be 26% of the dose at all three exposure levels. Over 24 hours, the respiratory expiration was calculated as 45–50% of the respiratory uptake, and because the net respiratory uptake and expiration do not consider the amount of ETBE cleared during exposure, the net respiratory excretion was lower, at 30–31% of the net respiratory uptake. These authors determined that the ETBE blood:air partition coefficient in humans was 11.7.

Amberg et al. (2000) exposed six volunteers (three males and three females, average age 28 ± 2 years) to 4.5 ppm (18.8 mg/m$^3$) and 40.6 ppm (170 mg/m$^3$) ETBE. The exposures lasted 4 hours, and the two concentrations were administered to the same volunteers 4 weeks apart. These volunteers were healthy nonsmokers and were asked to refrain from alcohol and medication intake from 2 days before the experiment until its end. The study was performed according to the Declaration of Helsinki after approval by the Regional Ethical Committee of the institution where the study was performed, and written informed consent was obtained from the volunteers. Urine was collected at 6-hour intervals for 72 hours. Blood was drawn immediately after exposure and thereafter every 6 hours for 48 hours. Levels of ETBE and its primary metabolite, *tert*-butanol, were determined in blood. The same two substances, plus additional metabolites of *tert*-butanol, were assessed in urine. The authors estimated the retained doses to be 1,090 µmol following 170-mg/m$^3$ ETBE exposure and 121 µmol following 18.8-mg/m$^3$ exposure. These estimates were derived using a resting human respiratory rate of 9 L/minute (13 m$^3$/day) and a retention factor for ETBE of 0.3, which was based on data reported by Nihlén et al. (1998). These estimates of retained dose are lower than those reported during light exercise (Nihlén et al., 1998).

### *Absorption in Animals*

Amberg et al. (2000) exposed F344 NH rats (5/sex/dose group) concurrent with the human volunteers in the same exposure chamber. Blood was taken from the tail vein of each rat at the end of the exposure period, and urine was collected for 72 hours at 6-hour intervals following exposure. The authors reported that immediately after the 4-hour exposure period blood levels of ETBE were lower in the rats than in humans, although the exact values were not reported. The authors estimated that the rats received doses of 20.5 and 2.3 µmol at the 170- and 18.8-mg/m$^3$ exposures, respectively, using an alveolar ventilation rate of 0.169 L/minute and a retention factor of 0.3 for rats.

No published oral dosing studies of the absorption of ETBE in humans were identified. The Japan Petroleum Energy Center (JPEC), however, conducted an oral dosing study of the absorption of ETBE in rats after single and repeated dosing for 14 days (JPEC, 2008e, f). Seven-week-old Crl:CD(SD) male rats (4/dose group) were administered either a single oral dose of 5, 50, or 400 mg/kg [$^{14}$C]ETBE via gavage or 5 mg/kg-day [$^{14}$C]ETBE daily for 14 days. In the single-dose

study by JPEC (2008f), plasma levels were compared with those observed after a single intravenous dose of 5 mg/kg-day [$^{14}$C]ETBE. There is no indication that a similar comparison was conducted in the repeated-dose study (JPEC, 2008e). Plasma radioactivity was measured in rats at 1, 2, 4, 6, 8, 10, and 24 hours after the first exposure in the repeated-dose study; 8 and 24 hours after the second to 13th exposures; and at 1, 2, 4, 6, 8, 10, 12, 24, 32, 48, 72, 96, 120, 144, and 168 hours after the last exposure in the repeated-dose study and after the single-dose study.

Plasma radioactivity levels increased following a single dose of [$^{14}$C]ETBE; this increase was not proportional as the dose increased, especially at the high dose (i.e., the peak plasma radioactivity levels were 2,800, 22,100, and 89,900 ng equivalents of ETBE/mL [ng equivalent ETBE/mL] in the 5-, 50-, and 400-mg/kg dose groups, respectively). Maximum plasma [$^{14}$C]ETBE levels ($C_{max}$) were estimated to be reached at 9.0, 11.5, and 8.0 hours after administration in the 5-, 50-, and 400-mg/kg dose groups, respectively. The [$^{14}$C]ETBE levels in the plasma were higher following oral exposure than after intravenous exposure (see Table B-2). The estimated elimination plasma half-lives were 17.5, 19.8, and 9.9 hours for the 5-, 50-, and 400-mg/kg dose groups, respectively. With repeated dosing of 5 mg/kg-day [$^{14}$C]ETBE (JPEC, 2008e), the $C_{max}$ was achieved 6 hours after the first exposure and increased until it reached a steady state around the fifth day of exposure. After the last exposure on Day 14, the $C_{max}$ of 6,660 ± 407 ng equivalent ETBE/mL was achieved 10 hours after administration of [$^{14}$C]ETBE, and plasma radioactivity steadily decreased after this point. The elimination plasma half-life from $C_{max}$ to 24 hours was 17.9 hours after the first dose and 14.2 hours after the final dose. The elimination half-life from $C_{max}$ to 168 hours after the final dose following repeated dosing was 24.7 hours. Based on radioactivity levels measured in urine and exhalation, more than 90% of the administered dose was absorbed.

In two parallel studies, the pharmacokinetics of ETBE was studied in mice (Sun and Beskitt, 1995a) and male Fischer 344 (F344) rats (Sun and Beskitt, 1995b). The study authors investigated the pharmacokinetics of [$^{14}$C]ETBE in mice and rats (3/sex/dose) exposed by nose-only inhalation at target concentrations of 500, 750, 1,000, 1,750, 2,500, and 5,000 ppm (2,090, 3,130, 4,180, 7,310, 10,450, and 20,900 mg/m$^3$) for a single 6-hour period (the true doses differed by less than 10% from the targets). Specific activity of the administered [$^{14}$C]ETBE and localization of the label were not reported. Note, that in the absence of the specific activity and localization of the label, it is not clear how the "mg ETBE equivalents" were calculated in the Sun and Beskitt (1995a, 1995b) report or for the specific tissues. Of the three animals per sex exposed concurrently, two were used to determine blood and tissue concentrations of radiolabel, and the third was kept in a metabolism cage for up to 118 hours to quantify radiolabel excretion in urine, feces, exhaled carbon dioxide ($CO_2$), and as volatiles in expired air. Exhaled organic volatiles were trapped in charcoal filters. Exhaled $CO_2$ was trapped in aqueous 1 M KOH. Samples from the 20,900-mg/m$^3$ treated animals were collected at 3, 6, 12, 18, 24, 48, 72, 96, and 118 hours after termination of exposure. At the lower exposure concentrations listed above, the samples were collected at fewer time points,

generally at full-day intervals up to 96 hours. Animals were euthanized either immediately after exposure or after being removed from the metabolic cages, and blood and kidneys were collected. The cages were washed, and the wash fluid collected. Charcoal traps were eluted with methanol. Urine, cage wash, trapped $^{14}CO_2$, and charcoal filter eluates were measured directly by liquid scintillation spectrometry. Blood and kidney tissue from rats and blood and liver tissue from mice were combusted in a sample oxidizer and analyzed by liquid scintillation spectrometry.

Immediately upon cessation of exposure, radiolabel was quantified in the blood and kidneys of two rats and in the blood and liver of two mice. Results in Table B-1 demonstrate the absorption of radiolabel expressed as mg equivalents of ETBE into blood. Because the ETBE carbon(s) bearing the radiolabel was not identified, further speciation is not possible. The concentration of radiolabel in rat blood is proportionate with exposure concentration to the highest concentration (20,894 mg/m$^3$), although in mice, such proportionality is absent at concentrations of 10,447 mg/m$^3$ and above. These data indicate that ETBE is well absorbed following inhalation exposure, but that higher concentrations (e.g., 10,447 mg/m$^3$ and above) could result in reduced respiration rates or otherwise affect mechanisms of inhalation uptake. Additional support for reduction of absorption is presented in Table B-1, demonstrating the excretion of the radiolabel from rats and mice in these studies (Sun and Beskitt, 1995a, b).

**Table B-1. Radioactivity in blood and kidney of rats and blood and liver of mice, following 6 hours of [$^{14}$C]ETBE inhalation exposure**

| Exposure level (mg/m$^3$) | F344 rat[a] | | CD-1 mouse[a] | |
|---|---|---|---|---|
| | Blood[b] | Kidney[c] | Blood[b] | Liver[c] |
| 2,089 | 0.037 | 0.074 | 0.154 | 0.208 |
| 3,134 | 0.062 | 0.094 | 0.340 | 0.348 |
| 4,179 | 0.080 | 0.116 | 0.336 | 0.540 |
| 7,313 | 0.124 | 0.152 | 0.481 | 0.724 |
| 10,447 | 0.156 | 0.185 | 0.474 | 0.628 |
| 20,894 | 0.114 | 0.182 | 0.408 | 0.592 |

[a]Mean values of one male and one female per rat/mouse.
[b]In mg [$^{14}$C]ETBE equivalents per gram blood.
[c]In mg [$^{14}$C]ETBE equivalents.
Sources: Sun and Beskitt (1995a) and Sun and Beskitt (1995b).

In contrast, Borghoff and Asgharian (1996) evaluated the disposition of $^{14}$C radiolabel in F344 rats and CD-1 mice after whole-body and nose-only inhalation exposure to 500, 1,750, or 5,000 ppm [$^{14}$C]ETBE. Besides recovery of total radioactivity in urine, feces, and expired air, air and urine samples were analyzed for ETBE and *tert*-butanol. Urine samples were also analyzed for *tert*-butanol metabolites hydroxyisobutyric acid (HBA) and 2-methyl-1,2-propanediol (MPD), and

$^{14}CO_2$ was measured in exhaled air. Results obtained after both a single 6-hour exposure or after 13 days of preexposure to 0, 500, or 5,000 ppm ETBE indicate that total inhalation uptake increases linearly with exposure concentration over this range, although there are dose- and preexposure-related shifts in the form and route of excretion. Because the later study used four rats per sex and exposure level, rather than just two, it should be given higher weight.

No studies investigating dermal absorption of ETBE were identified, but because dermal absorption of homologous organic substances is thought to be a function of the octanol:water partition coefficient, ETBE might be assumed to penetrate rat skin relatively well. For humans, Potts and Guy (1992) proposed an equation to calculate the dermal permeability coefficient, $K_p$:

$$\log K_p \text{ (cm/sec)} = -6.3 + 0.71 \times \log K_{ow} - 0.0061 \times \text{(molecular weight)} \qquad \text{(B-1)}$$

Using the log $K_{ow}$ [identified as $K_{oct}$ in Potts and Guy (1992)] values for ETBE [0.95–2.2; Drogos and Diaz (2001)] and converting cm/second values to cm/hour, the estimated $K_p$ values are 0.0020–0.016 cm/hour for ETBE.

ETBE is moderately absorbed following inhalation exposure in rats and humans, and blood levels of ETBE approached—but did not reach—steady-state concentrations within 2 hours. Nihlén et al. (1998) calculated the net respiratory uptake of ETBE in humans to be 26%. The AUC for the concentration-time curve was linearly related to the ETBE exposure level, suggesting linear kinetics up to 209 mg/m$^3$. The JPEC studies (JPEC, 2008e, f) demonstrated that ETBE is readily absorbed following oral exposure in rats with >90% of a single dose (5–400 mg/kg-day) or repeated doses (5 mg/kg-day) estimated to be absorbed. In the repeated-dose study, peak plasma [$^{14}$C]ETBE levels were reached 6 hours after the first dose and 10 hours after the final (14th) dose, and the maximum plasma concentration reached a steady state on Day 5. No data are available on dermal absorption of ETBE.

**Table B-2. Plasma radioactivity after a single oral or intravenous dose of [$^{14}$C]ETBE to male Crl:CD(SD) rats**

| Time (h) | Radioactive concentration (ng eq of ETBE/mL) | | | |
|---|---|---|---|---|
| | Oral | | Intravenous | |
| Dose administered | 5 mg/kg | 50 mg/kg | 400 mg/kg | 5 mg/kg |
| 0.083 | – | – | – | 918 ± 188[a] |
| 0.25 | – | – | – | 822 ± 165 |
| 0.5 | – | – | – | 914 ± 156 |
| 1 | 2,150 ± 281 | 11,100 ± 1,007 | 47,000 ± 11,900 | 907 ± 143 |
| 2 | 2,400 ± 151 | 12,100 ± 883 | 58,200 ± 7,340 | 923 ± 158 |
| 4 | 2,620 ± 109 | 14,800 ± 659 | 73,300 ± 6,800 | 929 ± 193 |
| 6 | 2,750 ± 146 | 18,700 ± 1,550 | 82,900 ± 12,500 | 981 ± 216 |
| 8 | 2,760 ± 265 | 19,900 ± 2,430 | 89,900 ± 16,300 | 973 ± 196 |
| 10 | 2,710 ± 303 | 21,400 ± 2,830 | 87,300 ± 15,300 | 943 ± 203 |
| 12 | 2,660 ± 426 | 22,000 ± 3,060 | 78,500 ± 18,100 | 862 ± 205 |
| 24 | 1,330 ± 419 | 10,800 ± 2,820 | 17,200 ± 6,460 | 383 ± 184 |
| 32 | 1,170 ± 424 | 9,310 ± 2,510 | 13,100 ± 6,580 | 334 ± 190 |
| 48 | 443 ± 271 | 3,900 ± 1,480 | 3,180 ± 1,480 | 144 ± 93.8 |
| 72 | 204 ± 165 | 1,660 ± 845 | 2,000 ± 1,820 | 65.2 ± 34.0 |
| 96 | 81.3 ± 70.3 | 792 ± 338 | N.D. | 31.3 ± 11.4 |
| 120 | 35.9 ± 44.0 | 385 ± 110 | N.D. | 16.1 ± 3.8 |
| 144 | 19.6 ± 26.0 | 179 ± 129 | N.D. | 11.9 ± 13.8 |
| 168 | N.D. | 85.4 ± 103 | N.D. | N.D. |

– = not measured; N.D. = not detected.

[a]Mean ± standard deviation; *n* = 4.
Source: JPEC (2008e).

## B.1.2. Distribution

There are no in vivo data on the tissue distribution of ETBE in humans. Nihlén et al. (1995) measured the partitioning of ETBE and *tert*-butanol in air into human blood from 10 donors (5 males, 5 females), saline, or oil inside of sealed vials. Also, human tissue-to-blood partitioning coefficients were estimated in brain, fat, liver, kidney, lung, and muscle based on their relative water and fat contents. Kaneko et al. (2000) conducted a similar series of in vitro studies to measure the partitioning of ETBE and *tert*-butanol in air to various rat tissues (5 male Wistar rats),

including blood, brain, fat, liver, kidney, lung, muscle, and testis. The blood:air partition coefficients for ETBE were much lower than for *tert*-butanol. Both studies reported efficient uptake of these substances from air into blood, with blood:air partition coefficients of 11.7 and 11.6 for ETBE and 462 and 531 for *tert*-butanol in humans and rats, respectively. Nihlén et al. (1995) also estimated oil:water partition (log $K_{ow}$) coefficients and obtained values of 0.278 for *tert*-butanol and 22.7 for ETBE. These values have a similar ranking, but are not identical, to those listed in a report by Drogos and Diaz (2001) (namely, 0.35 for *tert*-butanol and 1.48–1.74 for ETBE). Nihlén et al. (1995) used the coefficients of tissue:air and blood:air partition coefficients to calculate human tissue:blood partition coefficients. These values are listed in Table B-3.

**Table B-3. Blood:tissue partition coefficients for ETBE and *tert*-butanol**

| Partition coefficient | *tert*-Butanol | ETBE |
|---|---|---|
| Blood:air | 462 | 11.7 |
| Brain:blood | 1.05 | 2.34 |
| Muscle:blood | 1.06 | 1.78 |
| Fat:blood | 0.646 | 11.6 |
| Lung:blood | 1.02 | 0.835 |
| Kidney:blood | 1.06 | 1.42 |
| Liver:blood | 1.05 | 1.44 |

Source: Nihlén et al. (1998).

The JPEC (2008e, 2008f) study examined the distribution of radioactivity in 7-week-old Crl:CD(SD) male rats (4/dose group) following either a single oral dose of 5 or 400 mg/kg [$^{14}$C]ETBE via gavage or a repeated dose of 5 mg/kg-day for 7 or 14 days. Tissue samples were collected at 8, 24, 72, and 168 hours after a single dose; 8 and 24 hours after 7 days of repeated dosing; and 8, 24, 72, and 168 hours after 14 days of repeated dosing. Although the highest radioactivity levels were generally detected in plasma, [$^{14}$C]ETBE was also detected in all tissues examined (brain, peripheral nerve, eyes, submaxillary gland, thyroid gland, thymus, lungs, kidneys, heart, liver, adrenal glands, spleen, pancreas, bone marrow, mesenteric lymph node, prostate, epididymis, testes, muscle, skin, adipose tissue, stomach, large intestines, and small intestines). Tissue concentrations after a single 400 mg/kg dose of [$^{14}$C]ETBE were higher than after a single 5 mg/kg dose; however, the percentage distribution of radioactivity in tissues was lower with the higher dose. Tissue radioactivity levels reached a maximum at 8 hours after a single dose of either 5 or 400 mg/kg [$^{14}$C]ETBE and rapidly decreased by 72 hours. In the repeated-dose study, the radioactivity was the same 8 hours after the seventh administration when compared to 8 hours after the 14th administration. The levels of [$^{14}$C]ETBE in the tissues declined steadily from 8 hours through 168 hours after the last exposure except in adipose tissue. In adipose tissue, there was a

rapid decline between 8 and 24 hours, but the levels remained consistent between the 24- and 168-hour time points. The percentage of radioactivity found in red blood cells was estimated to be 20–27% within 72 hours of administration, and little was found to be bound to plasma proteins.

Sun and Beskitt (1995a) and Sun and Beskitt (1995b) studied the distribution of radiolabel derived from [$^{14}$C]ETBE in rats and mice, respectively. The animals were subjected to a single nose-only inhalation exposure to [$^{14}$C]ETBE for 6 hours. Immediately upon cessation of exposure, radiolabel was quantified in the blood and kidneys of two rats and in the blood and liver of two mice. Results in Table B-1 (shown earlier) demonstrate the distribution of radiolabel expressed as mg equivalents of ETBE from blood to kidney (rats) and liver (mice) during exposure. The concentration of radiolabel in rat kidney and mouse liver parallels the concentration of radiolabel in blood of the respective species, leading to an expectation of the proportionate distribution of $^{14}$C from ETBE to rat kidney and mouse liver up to exposure concentrations of 7,313 mg/m$^3$ in rats and 10,447 mg/m$^3$ in mice. Because radiolabel levels do not distinguish between parent ETBE and its metabolites, these results need to be interpreted with some caution, as the distribution of individual chemical species may differ.

Leavens and Borghoff (2009) evaluated the distribution of the structurally similar compound, methyl *tert*-butyl ether (MTBE), and the common metabolite, *tert*-butanol, after inhalation exposure to those two compounds, specifically in the brain, kidney, and liver of male and female rats and testis of male rats. Concentrations of MTBE and *tert*-butanol were similar in the female rat brain, kidney, and liver, and concentrations in the male rat brain, liver, and testes, were similar for exposure level and across time points, indicating an even distribution of MTBE and *tert*-butanol in those tissues/sexes. While total concentrations of MTBE and *tert*-butanol were higher in male rat kidneys than other tissues, consistent with the mechanism of binding to alpha 2u-globulin for those two compounds (Leavens and Borghoff, 2009), the overall observations are consistent with the conclusion that unbound ETBE and *tert*-butanol distribute rapidly and evenly through the body, although additional accumulation of material bound to alpha 2u-globulin occurs for *tert*-butanol and may occur for ETBE in the male rat kidney.

### B.1.3. Metabolism

The metabolism of ETBE has been studied in rats and humans using both in vivo and in vitro methods. A schematic of the proposed metabolism of ETBE is presented in Figure B-1. Based on elucidated structures of urinary metabolites from rats that were exposed to ETBE by inhalation, ETBE is initially metabolized by cytochrome P450 (CYP450) enzymes via oxidative deethylation by the addition of a hydroxyl group to the α-carbon of the ethyl ether group (Bernauer et al., 1998). The resulting hemiacetal is unstable and decomposes spontaneously into *tert*-butanol and acetaldehyde. In human liver microsome preparations, this step is catalyzed mainly by CYP2A6, with some contribution from CYP3A4 and CYP2B6 and possible contribution from CYP2E1 (Le Gal et al., 2001; Hong et al., 1999a). Using data from rat hepatic microsome preparations, Turini et al.

(1998) suggested that CYP2B1 is the primary enzyme responsible for this step in rats but that CYP2A1 may also have an important role. Acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenase enzymes (some of which are polymorphically expressed) and eventually to $CO_2$. *tert*-Butanol can be sulfated, glucuronidated, and excreted into urine, or it can undergo further oxidation by the CYP enzymes (but not by alcohol dehydrogenases) to form MPD, and 2-hydroxyisobutyrate (HIBA), acetone, and formaldehyde (Bernauer et al., 1998). Note also that these metabolites have been identified in studies using liver preparations from human or rat studies using ETBE, MTBE, or *tert*-butanol (Bernauer et al., 1998; Cederbaum and Cohen, 1980); however, all the enzymes that perform these metabolic steps have not been fully described. Excretion studies indicate that final metabolism to $CO_2$ plays only a minor role (see Section **Error! Reference source not found.**).

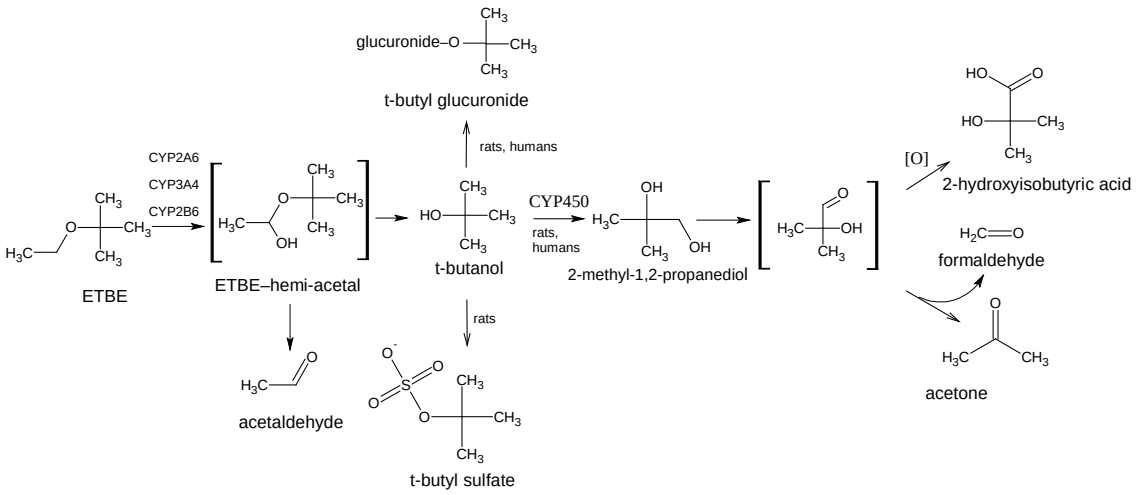



**Figure B-1. Proposed metabolism of ETBE.**

Source: Adapted from Dekant et al. (2001), NSF International (2003), ATSDR (1996), Bernauer et al. (1998), Amberg et al. (1999), and Cederbaum and Cohen (1980).

Zhang et al. (1997) used computer models to predict the metabolites of ETBE. The metabolism model correctly predicted cleavage into *tert*-butanol and acetaldehyde and that *tert*-butanol would undergo glucuronidation and sulfation. For the further metabolism of *tert*-butanol, however, the computer model predicted reductive steps leading to metabolites that have not been identified in vivo or in vitro. The software did not predict the formation of MPD or HIBA, which have been found in vivo.

### *Metabolism in Humans*

Metabolism of ethyl tertiary butyl ether (ETBE) in humans in vivo

Nihlén et al. (1998) exposed eight healthy male volunteers (average age: 29 years) to 0, 20.9, 104, or 209 mg/m$^3$ ETBE by inhalation for 2 hours. Profiles of ETBE, *tert*-butanol, and acetone

were established for blood throughout exposure and for up to 22 hours thereafter. The blood profiles of parent compounds and metabolites were similar at all three exposure levels and reflected exposure concentrations, as judged by linear increases in concentration-time AUC values calculated by the authors (only reported graphically). Acetone levels were highly variable before, during, and after the exposure period, and the variation could likely be due to variations in endogenous acetone production due to diet or physical activity.

The concentration of ETBE in blood rose sharply during the first 30 minutes of exposure and kept rising at a lower rate until the end of exposure to reach peak concentrations of about 10, 5, and 1 μM at 209, 104, and 20.9 mg/m$^3$, respectively. By 6 hours, ETBE concentrations had fallen to low levels even after exposure to 209 mg/m$^3$. The blood concentration of *tert*-butanol continued to rise for the full 2-hour exposure period, with peak values of about 12 and 7 μM at 209 and 104 mg/m$^3$, respectively. Blood concentrations leveled off for 3–4 hours and then began a slow decline to less than one-half maximum levels by 24 hours (*tert*-butanol levels could not be determined following 20.9 mg/m$^3$ exposure). Acetone blood levels began to increase after about 1 hour of exposure and continued to increase after the end of exposure (high dose) or leveled off for about 1.5 hours after exposure (lower doses and controls). Blood acetone levels fell rapidly during the next half hour but remained slightly above normal for the exposed volunteers until 4 hours after exposure when measurements were terminated.

Amberg et al. (2000) exposed six volunteers (three males and three females; average age: 28 ± 2 years) to 18.8 and 170 mg/m$^3$ of ETBE. The exposures lasted 4 hours, and the two concentrations were administered to the same volunteers 4 weeks apart. Urine was collected at 6-hour intervals for 72 hours. Blood was drawn immediately, at 4 or 6 hours after exposure, and thereafter every 6 hours for 48 hours. Levels of parent ETBE and its primary metabolite, *tert*-butanol, were determined in blood and urine. In urine, two further metabolites of *tert*-butanol, MPD and HIBA, were also assayed.

At 170 mg/m$^3$, the mean peak blood concentration of ETBE was 12.1 ± 4.0 μM, although for *tert*-butanol it was 13.9 ± 2.2 μM. The corresponding values at 18.8 mg/m$^3$ were 1.3 ± 0.7 and 1.8 ± 0.2 μM, respectively. The time courses of metabolite appearance in urine after 170 and 18.8 mg/m$^3$ were similar, but relative urinary levels of metabolites after 18.8 mg/m$^3$ differed from those after 170 mg/m$^3$. Using parent ETBE as the reference, molar ratios for total urinary excretion (ETBE:*tert*-butanol:MPD:HIBA) were 1:25:107:580 after 170 mg/m$^3$, and 1:17:45:435 after 18.8 mg/m$^3$. Individual variations were large, but the authors did not report any sex differences in the metabolism of ETBE based on data from only three subjects of each sex.

In vitro metabolism of ethyl tertiary butyl ether (ETBE) using human enzyme preparations

The metabolism of ETBE has been studied in vitro using microsomal protein derived from human liver and from genetically engineered cells expressing individual human CYP isozymes. Hong et al. (1997b) coexpressed human *CYP2A6* or *CYP2E1* with human CYP reductase in insect SF9 cells. In this heterologous expression system, in the presence of 1 mM ETBE, *tert*-butanol was

formed at rates of 13.6 nmol/min-nmol CYP2A6 and 0.8 nmol/min-nmol CYP2E1, indicating a greater capacity for ETBE metabolism by CYP2A6 than by CYP2E1 at high (e.g., 1 mM) concentrations of ETBE.

Hong et al. (1999a) obtained hepatic microsomal protein preparations from 15 human donor liver microsomal samples and used them to evaluate the contributions of several CYP enzymes to ETBE metabolism. The 15 samples displayed very large interindividual variations in metabolic activities towards ETBE ranging from 179 to 3,130 pmol/minute-mg protein. Michaelis-Menten constant (Km) values, estimated in three human liver microsomal samples using MTBE, ranged from 28 to 89 μM, with maximum substrate turnover velocity ($V_{max}$) values ranging from 215 to 783 pmol/minute-mg protein. The $V_{max}$:Km ratios, however, varied only between 7.7 and 8.8. After evaluating the activities of multiple different CYP forms in the 15 donor samples, the study authors demonstrated that the metabolism of ETBE was highly correlated with certain CYP forms. The highest degree of correlation was found for CYP2A6, which also displayed the highest metabolic capacity for ETBE.

As part of CYP inhibition studies in the same paper, human liver microsomes were coincubated with ETBE in the presence of chemical inhibitors or specific antibodies against either CYP2A6 or CYP2E1. For chemical inhibition, coumarin was added to the liver microsomes prior to initiation of the reaction. For antibody inhibition, monoclonal antibodies against human CPY2A6 or CYP2E1 were preincubated with liver microsomes prior to incubation with the rest of the reaction mixture. Methanol alone caused approximately 20% inhibition of the metabolism of ETBE, and coumarin, a CYP2A6 substrate, caused a significant dose-dependent inhibition of ETBE metabolism, which reached a maximal inhibition of 99% at 100-μM coumarin. The antibody against CYP2A6 inhibited metabolism by greater than 75% but did not do so against CYP2E1.

In the same paper, several specific human CYPs were expressed into human β-lymphoblastoid cells which were used to evaluate ETBE metabolism. Based on the ETBE metabolizing activities in the 15 human liver microsomes and the enzyme activity profiles towards known CYP specific substrates, correlation coefficients (ranging from 0.94 for CYP2A6 to 0 for CYP2D6) were calculated for each CYP enzyme. The correlation ranking for ETBE metabolism by nine human CYP isozymes was as follows:
2A6 > 3A4 ≈ 2B6 ≈ 3A4/5 >> 2C9 > 2E1 ≈ 2C19 >> 1A2 ≈ 2D6. The reported direct enzyme activities towards ETBE by the heterologous expression systems (in pmol *tert*-butanol formed per minute per pmol CYP enzyme) were 1.61 for CYP2A6; 0.34 for CYP2E1; 0.18 for CYP2B6; and 0.13 for CYP1A2. CYPs 1B1, 2C8, 2C9, 2C19, and 2D6 were not investigated. CYP3A4 and 1A1 did not metabolize ETBE. The authors concluded that CYP2A6 is the major enzyme responsible for the oxidative metabolism of ETBE in human livers. Furthermore, they concluded that the results of the correlation analysis and antibody inhibition study strongly suggest that CYP2E1 is not a major enzyme responsible for metabolism of ETBE. Le Gal et al. (2001) used similar human cytochrome preparations as Hong et al. (1999a) (i.e., from human donors) or used genetically modified human

β-lymphoblastoid cell lines transfected with CYP2A6, CYP2B6, CYP3A4, or CYP2E1 and human CYP reductase to elucidate the metabolism of ETBE, MTBE, and tertiary amyl methyl ether. They identified acetaldehyde and *tert*-butanol as primary metabolites from ETBE.

### *Metabolism in Animals*

Metabolism of ethyl tertiary butyl ether (ETBE) in animals in vivo

Bernauer et al. (1998) studied the metabolism and excretion of [$^{13}$C]ETBE and *tert*-butanol in rats. F344 rats, 2/sex, were exposed via inhalation to 2,000 ppm (8,400 mg/m$^3$) ETBE; three male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours. The excretion profile for ETBE metabolites was
MPD > HIBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with
HIBA > *tert*-butanol-sulfate > MPD >> *tert*-butanol-glucuronide ≈ *tert*-butanol. *tert*-Butanol could not be detected in urine following inhalation exposure to ETBE. Traces of acetone were also detected in urine. Amberg et al. (2000) exposed F344 NH rats, 5/sex/dose, to ETBE in the same exposure chamber described earlier for the human volunteers. Urine was collected for 72 hours following exposure. Blood samples were drawn from the tail vein every 6 hours up to 48 hours. Peak blood levels of ETBE and *tert*-butanol were 5.3 ± 1.2 and 21.7 ± 4.9 µM at 170 mg/m$^3$ and 1.0 ± 0.7 and 5.7 ± 0.8 µM at 18.8 mg/m$^3$, respectively. Peak levels of *tert*-butanol were higher in rats than in humans. Like humans, rats excreted mostly HIBA in urine, followed by MPD and *tert*-butanol. The molar ratios for total urinary excretion of *tert*-butanol:MPD:HIBA were 1:2.3:15 after exposure to 170 mg/m$^3$ and 1:1.5:11 after exposure to 18.8 mg/m$^3$. Parent ETBE was not identified in rat urine in this study.

In a review covering mostly their own work on fuel oxygenate metabolism, Dekant et al. (2001) focused on aspects of ETBE metabolism that were considered quantitatively similar in humans and rats, with no sex-dependent differences and no likely accumulation of metabolites or parent compound. They reported that at a high exposure level (8,400 mg/m$^3$ ETBE), rats predominantly excreted the glucuronide of *tert*-butanol in urine; however, at low exposure levels (16.7 or 167.1 mg/m$^3$ ETBE), the relative concentration of *tert*-butanol to the received dose was much smaller. This result seems to indicate that at high exposure levels, the normally rapid metabolism of *tert*-butanol to MPD and HIBA became saturated, forcing more of the *tert*-butanol through the glucuronidation pathway. The apparent final metabolite of ETBE was HIBA, which can undergo further metabolism to acetone. The latter process appeared to play a minor role in the overall metabolism of ETBE. Dekant et al. (2001) also noted that many metabolites of the fuel oxygenate ethers, such as formaldehyde, acetaldehyde, *tert*-butanol, HIBA, or acetone, occur naturally in normal mammalian physiology, providing a highly variable background that needs to be accounted for in metabolic experiments.

JPEC (2008e, 2008f) measured metabolite distribution in the plasma and urine of 7-week-old Crl:CD(SD) male rats (4/dose group) following either a single oral dose of 5 or 400 mg/kg [$^{14}$C]ETBE via gavage or a repeated dose of 5 mg/kg-day for 7 or 14 days. The metabolites were measured in the plasma 8 hours after single or repeated dosing and measured in urine collected on Days 1, 7, and 14 after repeated dosing or during a 24-hour period after administration of the single dose. The number of doses did not appear to affect the metabolic pattern. The study authors determined the identities of five metabolites, and the results in plasma and urine are summarized in Table B-4 and Table B-5, respectively. When combined with what is known of the metabolic pathway for ETBE, these data indicate that ETBE is efficiently metabolized to *tert*-butanol, which is then metabolized to *tert*-butanol glucuronide, 2-methyl-1,2-propanediol, and finally to 2-hydroxyisobutyrate.

Although Sun and Beskitt (1995a) did not identify the radiolabel excreted, their investigations do yield information pertinent to determining whether metabolic saturation might occur under bioassay conditions. In their single-exposure protocol, rats and mice were exposed via inhalation to ETBE. These investigators reported the fraction of absorbed dose that was excreted in urine and feces, as expired volatiles, and as expired $CO_2$ from one rat and one mouse. At inhaled concentrations between 4,180 and 7,310 mg/m$^3$ a shift in the primary route of excretion was observed, as demonstrated by a marked decrease in the fraction of radiolabel excreted in urine and a marked increase in the fraction of radiolabel eliminated as volatiles in expired air, and (in rats) a doubling of the fraction eliminated as exhaled $CO_2$. Given the different solubilities, molecular size, and other characteristics of ETBE and its multiple metabolites, it is thought that this shift in the excretion pattern of radiolabel is indicative of a shift in metabolism at these exposure levels.

Considering the potential shift in metabolic pattern relative to the pattern of toxicity can be informative, especially related to species and dose extrapolation. These data might still be considered preliminary because they are from one animal of each species, have not been replicated by other study authors, and the radiolabel has not been speciated as to chemical form. The unfortunate limitation of the application of the physiologically based pharmacokinetic (PBPK) model for human inhalation precludes its combination with rat PBPK models to complete species extrapolation. The inhalation toxicity study by Saito et al. (2013), however, demonstrated an increased incidence of urothelial hyperplasia at an exposure concentration of 6,270 mg/m$^3$ and higher, and an increased incidence of hepatocellular adenoma or carcinoma only at an exposure concentration of 20,900 mg/m$^3$. Additional data are required to determine whether increases in incidence could be related to pharmacokinetic effects (e.g., metabolic saturation).

**Table B-4. Unchanged ETBE and its metabolites in plasma 8 hours after a single oral dose or repeated (7 or 14) daily oral dosing of [$^{14}$C]ETBE to male Crl:CD(SD) rats**

| Compound | Metabolite | Percentage of dose | | | |
|---|---|---|---|---|---|
| | | 1 Dose | | 7 Doses | 14 Doses |
| | | 5 mg/kg-d | 400 mg/kg-d | 5 mg/kg-d | 5 mg/kg-d |
| Unchanged ETBE | ETBE | N.D. | N.D. | N.D. | N.D. |
| P-1 | 2-Hydroxyisobutyrate | 75.4 ± 8.1[a] | 35.7 ± 2.5 | 71.4 ± 4.7 | 69.8 ± 7.3 |
| P-2 | *tert*-Butanol glucuronide | N.D. | N.D. | N.D. | N.D. |
| P-3 | Not enough to determine | N.D. | N.D. | N.D. | N.D. |
| P-4 | 2-Methyl-1,2-propanediol | 9.7 ± 2.4 | 9.328 ± 0.9 | 9.1 ± 0.8 | 8.1 ± 1.4 |
| P-5 | *tert*-Butanol | 12.9 ± 3.1 | 55.0 ± 2.9 | 18.2 ± 3.8 | 22.2 ± 6.0 |

N.D. = not detected.

[a]Mean ± standard deviation; *n* = 4.

Source: JPEC (2008e, 2008f) unpublished reports.

**Table B-5. Unchanged ETBE and its metabolites in the urine (measured 0–24 hours) after a single oral dose or repeated (7 or 14) daily oral dosing of [$^{14}$C]ETBE to male Crl:CD(SD) rats**

| Compound | Metabolite | Percentage of dose | | | |
|---|---|---|---|---|---|
| | | 1 Dose | | 7 Doses | 14 Doses |
| | | 5 mg/kg-d | 400 mg/kg-d | 5 mg/kg-d | 5 mg/kg-d |
| Unchanged ETBE | ETBE | 0.7 ± 0.5[a] | N.D. | 0.9 ± 0.6 | 1.4 ± 0.4 |
| P-1 | 2-Hydroxyisobutyrate | 53.0 ± 3.4 | 55.4 ± 4.7 | 58.9 ± 4.2 | 56.0 ± 5.2 |
| P-2 | *tert*-Butanol glucuronide | 29.2 ± 3.0 | 25.9 ± 4.6 | 22.8 ± 3.2 | 25.2 ± 5.8 |
| P-3 | Not enough to determine | 2.5 ± 0.2 | 1.7 ± 0.4 | 2.2 ± 0.3 | 1.7 ± 0.4 |
| P-4 | 2-Methyl-1,2-propanediol | 13.1 ± 0.6 | 13.3 ± 2.5 | 13.4 ± 1.5 | 13.9 ± 2.3 |
| P-5 | *tert*-Butanol | 1.5 ± 0.5 | 3.7 ± 0.6 | 1.9 ± 0.2 | 1.8 ± 0.0 |

N.D. = not detected.

[a]Mean ± standard deviation; *n* = 4.

Source: JPEC (2008e, 2008f) unpublished reports.

Borghoff and Asgharian (1996) evaluated the disposition of a $^{14}$C radiolabel in F344 rats and CD-1 mice after whole-body and nose-only inhalation exposure to 500, 1,750, or 5,000 ppm [$^{14}$C]ETBE. Besides recovery of total radioactivity in urine, feces, and expired air, the air and urine samples were analyzed for ETBE and *tert*-butanol. Urine samples were also analyzed for *tert*-butanol metabolites, HBA and MPD. Results obtained after both a single 6-hour exposure or after 13 days of preexposure to 0, 500, or 5,000 ppm ETBE indicated dose- and preexposure-related shifts in the form and route, likely due to metabolic factors. Excretion shifted from being primarily in the urine after 500 ppm exposure to primarily by exhalation at 5,000 ppm in naïve rats, indicating a saturation of metabolism of ETBE to TBA. This shift was greater in female rats than in males. However, in rats preexposed to 5,000 ppm ETBE for 13 days, most of the excretion was in the urine even at 5,000 ppm. Rats preexposed to 500 ppm ETBE also showed a shift from exhalation to urinary excretion in comparison to naïve rats, but to a smaller degree than elicited by 5,000 ppm preexposure. The changes in excretion after preexposure indicated an induction of the metabolism of ETBE to *tert*-butanol. As with rats, the fraction of radiolabel in exhaled volatiles in mice increased with exposure level, while the fraction excreted in urine decreased. The exhalation pattern observed in rats showed levels of ETBE falling ~90% in the first 8 hours postexposure, whereas levels of TBA exhaled actually rose between 0 and 3 hours postexposure and then fell more slowly between 3 and 16 hours, particularly after 5,000 ppm ETBE exposure. The increase in TBA between 0 and 3 hours postexposure can be explained by the continued metabolism of ETBE

during that period. The slower decline after 3 hours can be explained as a result of the generally slower clearance of TBA, which is saturated by the higher ETBE exposure levels.

Metabolism of ETBE in animal tissues in vitro

Using microsomal protein isolated from the olfactory epithelium from male Sprague-Dawley (S-D) rats, Hong et al. (1997a) measured ETBE metabolism as the formation of TBA. They found that metabolism occurred only in microsomal protein (not in cytosol) and only in the presence of an nicotinamide adenine dinucleotide phosphate (NADP)-regenerating system. The metabolic activity was inhibited by 80% after treating the microsomal preparation with carbon monoxide and by 87% in the presence of coumarin (a CYP2A6 inhibitor), which indicates CYP involvement. Using an in vitro concentration of 1 mM ETBE, metabolic activity could not be detected in microsomal protein from the olfactory bulb, lungs, or kidneys. Activity toward ETBE was 8.78, 0.95, and 0.24 nmol/minute-mg microsomal protein in olfactory mucosa, respiratory mucosa, and liver, respectively. In olfactory mucosa, the study authors reported a Km value of 125 μM for ETBE.

Hong et al. (1999b) used hepatic microsomal protein derived from *Cyp2e1* knockout mice to investigate whether this enzyme plays a major role in ETBE metabolism. They compared the metabolizing activity of liver microsomes (incubated for 30 minutes at 37°C and with 0.1 mM ETBE) between the *Cyp2e1* knockout mice and their parental lineage strains using four or five female mice (7 weeks of age) per group. The ETBE-metabolizing activities were not significantly different between the *Cyp2e1* knockout strain (0.51 ± 0.24 nmol/minute-mg protein) compared with those observed in the *Cyp2e1* wild-type parental strains (0.70 ± 0.12 for C57BL/6N mice, and 0.66 ± 0.14 for 129/Sv mice). Therefore, microsomal protein from mice that did not express any CYP2E1 did not differ from microsomal protein derived from wild-type animals in their ability to metabolize ETBE in vitro, suggesting that CYP2E1 might contribute only little to ETBE metabolism in vivo. Furthermore, these authors evaluated potential sex- and age-dependent differences for the metabolism of 1 mM concentrations of ETBE by hepatic microsomal protein. Although activities in female knockout mice were approximately 60% of those in male knockout mice, the difference did not reach the level of statistical significance. Finally, observed rates of ETBE metabolism (approximately 0.5 to 0.9 nmol/min/mg microsomal protein) did not seem to differ when assayed at 0.1 or 1 mM, indicating that for mouse hepatic microsomal ETBE metabolism, saturation can occur at concentrations no higher than 0.1 mM in vitro, and that Km values would be expected to be lower than 0.1 mM in vitro.

Turini et al. (1998) investigated the effects of ETBE exposure on P450 content and activities, and characteristics of ETBE metabolism in hepatic microsomal protein from male Sprague-Dawley rats in an attempt to elucidate the role of CYP2E1 in ETBE metabolism. Administration of ETBE at 200 or 400 mg/kg for 4 days did not alter hepatic CYP profiles, but the administration of 2 mL ETBE/kg resulted in significant increases of metabolic activities toward substrates characteristic for CYP2B and CYP2E1 (p-NPH) forms, but not of activities catalyzed by

CYP3A or 1A forms. Studies of ETBE metabolism were based on high-performance liquid chromatography detection of the acetaldehyde ETBE metabolite. Induction of CYP2B forms in vivo via the administration of phenobarbital slightly reduced the Km value and produced a significant, approximate threefold increase in $V_{max}$; in these preparations; chemical inhibition of CYP2B forms resulted in significant inhibition of ETBE metabolism. Studies with CYP enzymes purified from rats confirmed metabolic competency of several CYP forms, with the activity of purified rat CYP forms 2B1 > 2E1 > 1A1 > 2C11. Chemical inhibition of CYP2E1 did not reduce ETBE metabolic activity; CYP2A forms were not evaluated. In microsomal preparations from rats treated with phenobarbital (a CYP2B inducer), incubation with chemical inhibitors of CYP2B forms produced a significant decrease in ETBE metabolism. Pretreatment of rats with chemicals known as inducers of CYP2E1, CYP3A, and CYP1A forms did not result in significant changes in Km or $V_{max}$ values for ETBE metabolism, as measured in vitro. The results of these investigations indicate that, in rats, CYP2E1 is apparently minimally involved in ETBE metabolism, and that under some conditions, CYP2B forms can contribute to ETBE metabolism. The role of CYP2A forms was not studied in this investigation. This study also investigated the kinetic constants for ETBE metabolism in control rat hepatic microsomal protein, indicating a Km value of 6.3 mM and a $V_{max}$ value of 0.93 nmol/min/mg microsomal protein. When compared with the kinetic constants indicated by the results of Hong et al. (1999b), the rate of ETBE metabolism at in vitro concentrations below 1 mM are expected to be higher in mouse than in rat microsomal preparations.

The enzymes that metabolize *tert*-butanol to MPD, HIBA, and even acetone, have not been fully characterized; however, *tert*-butanol is not subject to metabolism by alcohol dehydrogenases (Dekant et al., 2001).

### B.1.4. Excretion

#### *Excretion in Humans*

Nihlén et al. (1998) exposed eight healthy male volunteers (average age 29 years) to 20.9, 104, and 209 mg/m$^3$ ETBE by inhalation for 2 hours. ETBE, and two metabolites (*tert*-butanol and acetone) were measured in urine for up to 22 hours after exposure. The blood profiles of the parent compound and metabolites were similar at all three exposure levels and reflected exposure concentrations. The study authors estimated the inhaled amount of ETBE in the volunteers to be 0.58, 2.9, and 5.8 mmol for the 20.9-, 104-, and 209-mg/m$^3$ exposure levels, respectively. Based on blood AUC values for ETBE and metabolites, the authors calculated respiratory uptake to be 32–34% in humans and the net uptake (which excludes ETBE exhaled during exposure) to be 26% of the dose at all three exposure levels. During the 24 hours following the start of inhalation exposure, respiratory expiration was calculated at 45–50% of the inhaled ETBE (respiratory uptake), and net respiratory expiration was 31% (of the net respiratory uptake), of which *tert*-butanol accounted for only 1.4–3.8%. Urinary excretion of parent ETBE (as percentage of the respiratory uptake of ETBE) accounted for even less: 0.12, 0.061, and 0.056% after the exposures to

20.9, 104, and 209 mg/m$^3$, respectively. The authors identified four phases of excretion of ETBE from blood, with half-lives of about 2 and 20 minutes and 1.7 and 28 hours. Only one phase for excretion of *tert*-butanol from blood was identified with a half-life of 12 hours compared with 10 hours in another study with volunteers (Johanson et al., 1995). In urine, ETBE displayed two phases of excretion, with half-lives of about 8 minutes and 8.6 hours. The half-life of *tert*-butanol in urine was determined to be 8 hours (Johanson et al., 1995).

ETBE displayed a multiphasic excretion from blood. The first phase likely indicates uptake into highly perfused tissues. The other phases could indicate uptake into less perfused tissues and fat or result from metabolism events. The apparent total body clearance of ETBE (based on the net respiratory uptake) was 0.57 L/hour-kg (average of the three exposure levels). The metabolic clearance was calculated as 0.39 L/hour-kg and the exhalation clearance as 0.35 L/hour-kg. These authors reported that the kinetics of ETBE in humans was linear over the range of concentrations studied (Nihlén et al., 1998).

In the study by Amberg et al. (2000) that was described earlier, two excretion half-lives were found for ETBE (1.1 ± 0.1 and 6.2 ± 3.3 hours) at the high exposure concentration (170 mg/m$^3$), although *tert*-butanol displayed only one half-life (9.8 ± 1.4 hours). At the low exposure concentration (18.8 mg/m$^3$), only the short half-life for ETBE could be measured at 1.1 ± 0.2 hours, although that for *tert*-butanol was 8.2 ± 2.2 hours. The predominant urinary metabolite, identified was HIBA, was excreted in urine at 5–10 times the amount of MPD and 12–18 times the amount of *tert*-butanol (note: urine samples had been treated with acid before analysis to cleave conjugates). Excretion of unchanged ETBE in urine was minimal. The time courses of urinary excretion for 170 and 18.8 mg/m$^3$ were similar, but relative urinary levels of HIBA after 18.8 mg/m$^3$ were higher, although those for MPD were lower, as compared to 170 mg/m$^3$. HIBA in urine showed a broad maximum at 12–30 hours after exposure to both concentrations, with a slow decline thereafter. MPD in urine peaked at 12 and 18 hours after 170 and 18.8 mg/m$^3$, respectively, although *tert*-butanol peaked at 6 hours after both concentrations. The time to peak of the three metabolites reflected the sequence of their formation and interconversion as ETBE is metabolized. Interindividual variations were large, but the authors did not report differences in the toxicokinetics of ETBE by sex. Amberg et al. (2000) calculated that 43 ± 12% of the 170 mg/m$^3$ dose and 50 ± 20% of the 18.8 mg/m$^3$ dose had been excreted in urine by 72 hours. Respiratory elimination was not monitored.

### *Excretion in Animals*

Amberg et al. (2000) exposed F344 NH rats, 5/sex/dose, concurrent with the human volunteers in the same exposure chamber. Urine was collected for 72 hours following exposure. Like humans, rats excreted mostly HIBA in urine, followed by MPD and *tert*-butanol. Parent ETBE was not identified in rat urine. The half-life for *tert*-butanol in rat urine was 4.6 ± 1.4 hours at 170 mg/m$^3$ but could not be calculated at 18.8 mg/m$^3$. Corresponding half-lives were 2.6 ± 0.5 and 4.0 ± 0.9 hours for MPD, and 3.0 ± 1.0 and 4.7 ± 2.6 hours for HIBA. The authors concluded that rats

excreted ETBE considerably faster than humans. Urinary excretion accounted for 53 ± 15 and 50 ± 30% of the estimated dose at 170- and 18.8-mg/m$^3$ exposures, respectively, with the remainder of the dose being eliminated via exhalation, as suggested by the study authors.

Bernauer et al. (1998) studied the excretion of [$^{13}$C]ETBE and MTBE in rats. F344 rats, 2/sex, were exposed via inhalation to 8,400 mg/m$^3$ ETBE or 7,200 mg/m$^3$ MTBE for 6 hours, or 3 male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours, and the ETBE metabolite prevalence in urine was
MPD > HIBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with relative amounts of
HIBA > *tert*-butanol-sulfate > MPD >> *tert*-butanol-glucuronide ≈ *tert*-butanol.

Although there are several unpublished reports relevant to the elimination of ETBE following inhalation exposure, no additional peer-reviewed publications were identified. Unpublished reports have not gone through the public peer-review process and are of unknown quality. They are included here as additional information only.

During 96 hours in metabolic cages, rats excreted approximately 60% of the radioactivity in urine, approximately 38% was recovered as exhaled organic volatiles, and approximately 1% as exhaled $CO_2$. This pattern was maintained at an exposure concentration of 4,180 mg/m$^3$; above that, urinary excretion of radioactivity decreased to 34% of the recovered radioactivity, although exhalation of organic volatiles increased to 63%. A shift in the excretion profile of radiolabel was seen at concentrations of 7,310 mg/m$^3$ and above, which remained fairly constant to the highest exposure of 20,900 mg/m$^3$. In this range of concentrations, approximately 39% of the excreted radiolabel was found in urine, approximately 58% was exhaled as organic volatiles, and 2% was eliminated as exhaled $CO_2$.

A review of the data demonstrating the percentage of recovered radiolabel via various routes of elimination demonstrate, in the rat and mouse, a pattern indicative of metabolic saturation occurring at inhaled concentrations above 4,180 mg/m$^3$.

In rats, the time course of excretion indicated that exhalation of organic volatiles was essentially complete by 24 hours, although urinary excretion of ETBE-derived radioactivity displayed a broad peak at 12–48 hours. The bulk of each dose was excreted within 48 hours after the end of exposure. At 20,900 mg/m$^3$, $^{14}CO_2$ exhalation and fecal excretion of radioactivity remained rather constant from 12 to 118 hours. In comparing the total radiolabel excreted to the inhaled concentrations (see Table B-6), a proportionate relationship is observed in rats at all concentrations, but less than proportionate excretion of total radiolabel at the highest concentration in mice. The complete data set led the study authors to conclude that saturation of the inhalation absorptive processes might have occurred at concentrations of approximately 7,310 mg/m$^3$. The findings of Sun and Beskitt (1995a) in mice at 20,900 mg/m$^3$ were essentially confirmed by Borghoff (1996) (unpublished report, a pilot study) and Borghoff and Asgharian

(1996) (unpublished report, final study), which used the identical species, experimental protocol, materials, and methods but which were conducted later at a different laboratory (see Table B-6).

**Table B-6. Excretion of [$^{14}$C]ETBE-derived radioactivity from rats and mice within 96 hours following a single 6-hour inhalation exposure**

| Exposure level (mg/m$^3$) | Volatile organics[a] | Exhaled $CO_2$[a] | Urine[a] | Feces[a] | Total[b] |
|---|---|---|---|---|---|
| *F344 rat*[c] | | | | | |
| 2,090[d] | 37<br>[28, 32] | 1<br>[1.2, 1.3] | 60<br>[59, 59] | 2<br>[2.8, 1.0] | 9.9<br>[16.1, 13.6] |
| 3,130 | 36 | 1 | 62 | 2 | 17.5 |
| 4,180 | 42 | 1 | 56 | 2 | 22.1 |
| 7,310[d] | 58<br>[41, 52] | 2<br>[1.5, 1.7] | 38<br>[53, 41] | 3<br>[0.7, 0.5] | 56.9<br>[45, 31] |
| 10,400 | 52 | 2 | 45 | 2 | 56.2 |
| 20,900[d,e] | 63<br>(51)<br>[51, 64] | 2<br>(1)<br>[1.6, 2.0] | 34<br>(44)<br>[45, 30] | 1<br>(3)<br>[0.2, 0.2] | 97.5<br>(116)<br>[143, 94] |
| *CD-1 mouse*[f] | | | | | |
| 2,090[d] | 10<br>[12.7, 26.8] | 1<br>[1.2, 1.2] | 74<br>[81.3, 67.2] | 16<br>[3.2, 2.3] | 6.38<br>[10.4, 6.8] |
| 3,130 | 28 | 2 | 60 | 10 | 7.9 |
| 4,180 | 29 | 2 | 64 | 6 | 12.8 |
| 7,310[d] | 42<br>[23, 36] | 2<br>[2.2, 1.9] | 46<br>[71, 61] | 10<br>[1.1, 0.6] | 13.7<br>[22.4, 17.3] |
| 10,400 | 42 | 2 | 47 | 10 | 22.7 |
| 20,900[d,e] | 44<br>(37)<br>[40, 47] | 5<br>(2)<br>[2.9, 3.3] | 39<br>(57)<br>[53, 47] | 12<br>(2)<br>[0.6, 0.8] | 18.9<br>(28)<br>[37.1, 25.2] |

[a]Percentage of total excreted radioactivity; mean of one male and one female.
[b]In mg [$^{14}$C]ETBE equivalents.
[c]Sun and Beskitt (1995b).
[d]Values in brackets: [males, females], nose-only exposures, excretion up to 48 h Borghoff and Asgharian (1996).
[e]Values in parentheses: Borghoff (1996).
[f]Sun and Beskitt (1995b).

Similarities between rats (Sun and Beskitt, 1995b) and mice (Sun and Beskitt, 1995a) are evident. Both species demonstrate similar excretion pathways and present evidence of saturation of metabolic pathways at concentrations lower than those that demonstrate saturation of absorptive pathways. Metabolic saturation (evidenced as a shift from urine as the predominant

excretion pathway and an increase in the fraction of dose eliminated via exhalation) occurred in both species at concentrations approximating 7,310 mg/m$^3$. Noteworthy differences between the two species were that, in general, mice excreted a smaller percentage of the dose in the form of volatile organics and a higher amount in urine, at least up to 4,180 mg/m$^3$ (see Table B-6), compared with rats and that mice excreted about five times as much [$^{14}$C]ETBE-derived radioactivity via feces than did rats. The total amounts of excreted radioactivity (mg equivalents) were considerably higher in rats than in mice; however, the values in the respective columns of Table B-6 are not corrected for body weight (BW). When normalized to body weight, it is apparent that mice absorbed a higher dose than rats; however, the total excreted radioactivity at 20,900 mg/m$^3$ showed no further increase over the values at 10,450 mg/m$^3$, indicating that the absorptive capacities of mice had become saturated; however, this analysis conducted in rats does not indicate a saturation of absorptive capacities over the range of concentrations studied.

Borghoff (1996), in an unpublished report, conducted studies to establish experimental conditions for future bioassays of ETBE, based on the two studies previously conducted by Sun and Beskitt (1995a, 1995b). The experimental protocol and materials were identical to the ones used by Sun and Beskitt (1995a, 1995b); however, in this pilot study, only three male F344 rats and three male CD-1 mice were used per experiment, with only one exposure level at 20,900 mg/m$^3$. Also, only blood was collected from the animals, while the whole carcasses were liquefied and assayed for retained radioactivity immediately after exposure and after the end of the animals' stay in metabolic cages. The carbon at "the central position of the *tert*-butyl group" was radiolabeled. Radioactive ETBE was obtained by mixing [$^{14}$C]ETBE with unlabeled material in the gas phase for a specific activity of 2.74 µCi/mmol. The rats, when assayed immediately after exposure, were found to have absorbed 2.57 ± 0.14 µCi radioactivity, although the balance of radioactivity after 96 hours in metabolic cages from other animals accounted for 3.17 ± 0.08 µCi (mean ± standard deviation [SD], $n$ = 3). The authors could not make any suggestion as to the origin of this discrepancy. Absorbed doses in mice were 0.85 ± 0.08 µCi immediately after exposure and 0.77 ± 0.16 µCi for other mice placed in metabolism cages. Excretion values detected in these rats and mice are shown in parentheses in Table B-6; the percentage values shown in this table were based on the total body burden of the individual animals from which the excretion data were obtained, not on group means.

Mice had excreted most of the dose within 12 hours after exposure, rats within 24 hours. Organic volatiles collected on charcoal filters were analyzed for ETBE and *tert*-butanol contents. Rats exhaled 22% of the absorbed ETBE within 1 hour after exposure, 12% during the following 2 hours, and only another 3% during the next 3 hours. *tert*-Butanol exhalation accounted for 1% of the total during the first hour, 3% during the following 2 hours, and 4% during the last 3 hours of the experimental period. Mice, on the other hand, exhaled 16% of the unmetabolized ETBE within 1 hour after exposure and 1% during the following 2 hours, with immeasurable amounts thereafter. *tert*-Butanol exhalation made up 6% of total during the first hour, 8% in the next 2 hours, and 4% during the final 3 hours. Excretion of ETBE, *tert*-butanol, HIBA, and MPD in urine were assayed.

During 24 hours of collection, the rats excreted about 7 times as much *tert*-butanol as ETBE in urine; in mice, the ratio was >60. HIBA was detected in urine of both species but could not be quantified. MPD was not detected. These results could be interpreted as suggesting that mice metabolize, and hence excrete, ETBE faster than rats.

A subsequent larger study by Borghoff and Asgharian (1996) (see previous details) essentially confirmed the results of the pilot study (Borghoff, 1996). F344 rats and CD-1 mice were exposed by inhalation to 500, 1,750, or 5,000 ppm [$^{14}$C]ETBE. Concentrations of ETBE and *tert*-butanol were measured in exhaled breath up to 16 hours postexposure. The exhalation pattern observed in rats showed levels of ETBE falling ~90% in the first 8 hours postexposure, while levels of TBA exhaled actually rose between 0 and 3 hours postexposure and then fell more slowly between 3 and 16 hours, particularly after 5,000 ppm ETBE exposure. The increase in TBA between 0 and 3 hours postexposure can be explained by the continued metabolism of ETBE during that period. The slower decline after 3 hours can be explained by the generally slower clearance of TBA, which is saturated by the higher ETBE exposure levels. Exhaled breath levels declined much more rapidly in mice than in rats.

Unpublished reports by JPEC (2008e) determined that following oral exposure of 7-week-old Crl:CD(SD) male rats to [$^{14}$C]ETBE, the largest amount of radioactivity was recovered in expired air, followed by urinary excretion, with very little excretion occurring via the feces. With increasing dose, increasing proportions of radioactivity were found in expired air. The total radioactivity recovered by 168 hours after a single dose of 5 mg/kg [$^{14}$C]ETBE was 39.16% in the urine, 0.58% in the feces, and 58.32% in expired air, and, after a single dose of 400 mg/kg, 18.7% in the urine, 0.15% in the feces, and 78.2% in expired air. With repeated dosing, the recovery of radioactivity through excretion increased through Day 6 when a steady state was achieved; however, the radioactivity level in the feces increased throughout the 14 days, but the level was too low to affect the total recovery. After 14 days, 36.3% of the administered dose was recovered in the urine, 2.33% was recovered in the feces, and 56.7% was recovered in expired air.

### B.1.5. Physiologically Based Pharmacokinetic Models

Two PBPK models have been developed specifically for describing the absorption, distribution, metabolism, and excretion of ETBE in rats (Borghoff et al., 2016; Salazar et al., 2015). A detailed summary of these and other toxicokinetic models is provided in U.S. EPA (2017). The PBPK model described in Borghoff et al. (2016) and in U.S. EPA (2017) was considered to conduct a route-to-route extrapolation based on an equivalent internal dose (the rate of ETBE metabolism in the liver), but was not ultimately used for this purpose because of feedback from external peer review by EPA's Science Advisory Board (SAB). The SAB recommended that in the absence of a consistent dose-response relationship for ETBE when combining oral and inhalation studies to assess liver tumors, extrapolation between inhalation and oral routes of exposure not be performed. Regarding the extrapolation from animals to humans, the existing human PBPK model

was not considered adequate (see below); therefore, default methodologies were applied to extrapolate toxicologically equivalent exposures from adult laboratory animals to adult humans.

The PBPK model described in Borghoff et al. (2016) and in U.S. EPA (2017) includes a possible adjustment for induction of *tert*-butanol metabolism; however, this induction has only been observed in mice exposed directly to *tert*-butanol (McComb and Goldstein, 1979). Furthermore, implementing metabolic induction does not allow for dependence on exposure or dose, nor for any de-induction that might occur during periods without exposure, such as weekends during 5 days/week exposures. Finally, because induction is expected to have an equal impact on oral and inhalation exposures—and only if *tert*-butanol levels or metabolism is used as a dose metric—induction's potential impact on risk evaluation for ETBE is considered minimal. Therefore, this adjustment was not turned off in the model; instead, the maximum induction level was set to zero.

While model simulations accounted for variations during the day and week (e.g., 6 hours/day, 5 days/week inhalation exposure), simulations reached a condition of "periodicity" by the second week, such that the time course of internal doses were identical between the second week and subsequent weeks of exposure with metabolic induction turned off. However, to ensure applicability should metabolic induction is considered (predicted to take 2–3 weeks), simulations were generally run for 7 weeks, with results for the last 1–2 weeks used to estimate average tissue or blood concentrations or metabolic rates.

For simulating exposure to drinking water, the water consumption was modeled as episodic, based on the pattern of drinking observed in rats (Spiteri, 1982). In particular, rats were assumed to ingest water in pulses or "bouts," which were treated as periods of continuous ingestion, interspersed with periods of no ingestion. During the active dark period (12 hours/day), 80% of total daily ingestion is assumed to occur in 45-minute bouts alternating with 45 minutes of other activity. During the relatively inactive light period (12 hours/day), the remaining 20% of daily ingestion is assumed to occur; the bouts are only assumed to last 30 minutes, with 2.5 hours between. This pattern is thought to be more realistic than assuming continuous 24 hours/day ingestion. The resulting ingestion rate for one exposure is shown in Figure B-2.

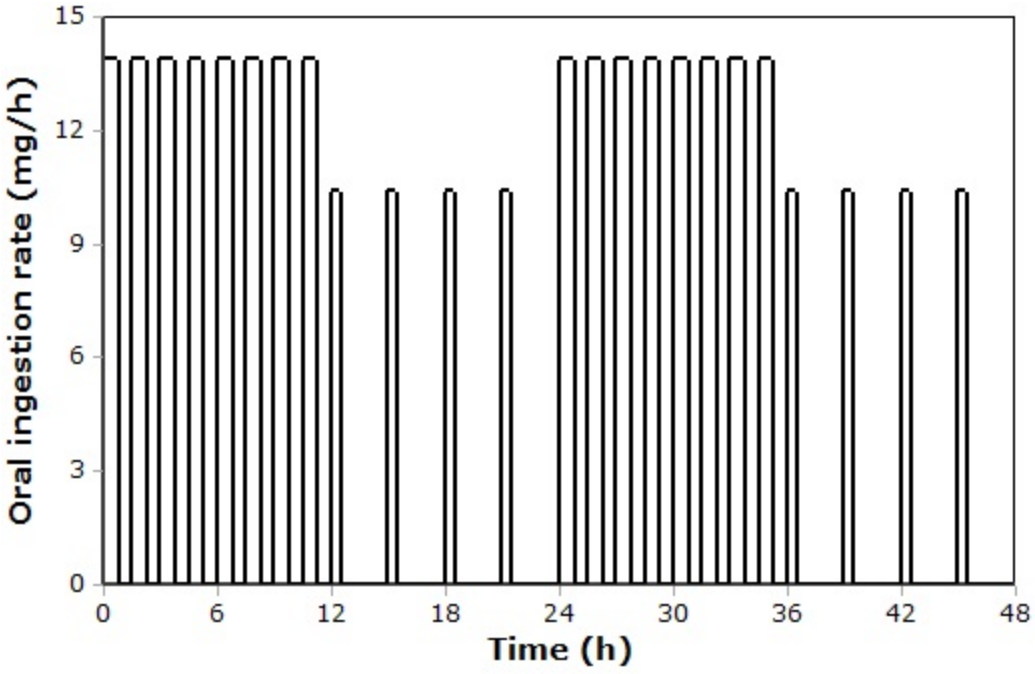
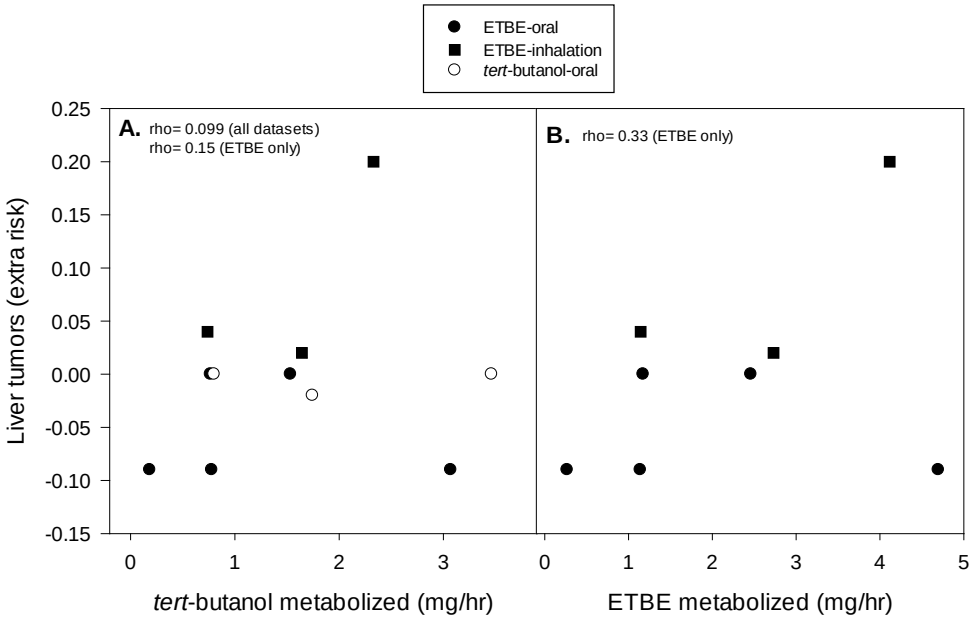
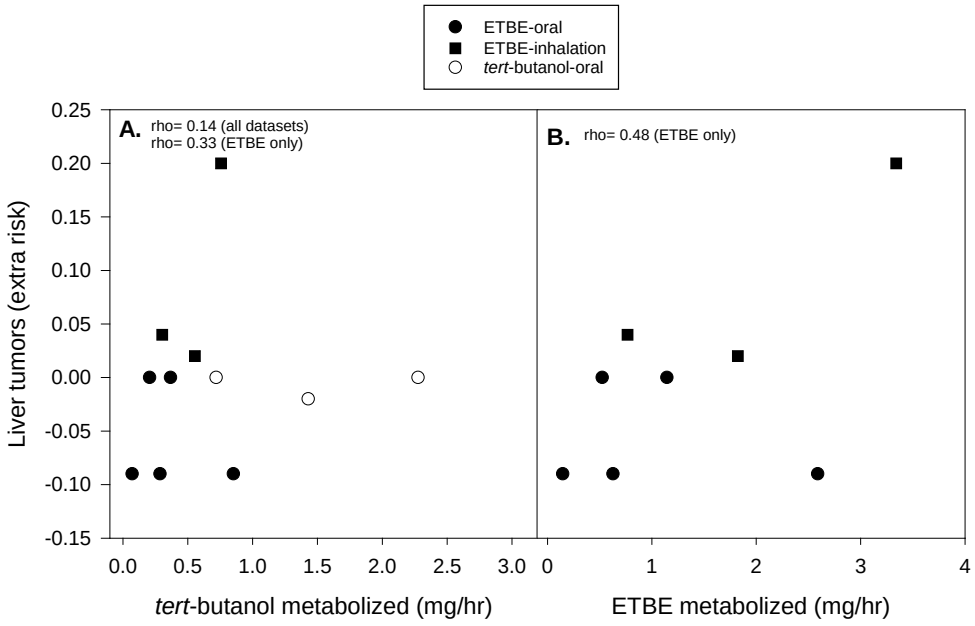



**Figure B-2. Example oral ingestion pattern for rats exposed via drinking water.**

PBPK modeling was also used to evaluate a variety of internal dose metrics (daily average TBA blood concentration, daily amount of TBA metabolized in liver, daily average of ETBE blood concentration, and daily amount of ETBE metabolized in liver) to assess the correlation with different endpoints following exposure to ETBE or TBA (Salazar et al., 2015). Administering ETBE either orally or via inhalation achieved similar or higher levels of TBA blood concentrations or TBA metabolic rates as those induced by direct TBA administration. Altogether, the PBPK model-based analysis by Salazar et al. (2015) [which applied a model structurally similar to Borghoff et al. (2016)] indicated a consistent dose-response relationship between kidney weight, urothelial hyperplasia, and chronic progressive nephropathy (CPN) and TBA blood concentration (as the dose metric for both ETBE and TBA). Kidney and liver tumors, however, were not consistently correlated with any dose metric. These data are consistent with TBA mediating the noncancer kidney effects following ETBE administration, but additional factors besides internal dose are necessary to explain the induction of liver and kidney tumors.




Salazar et al. (2015)




Borghoff et al. (2016)

**Figure B-3. Comparisons of liver tumors in male rats following 2-year oral or inhalation exposure to ETBE or *tert*-butanol with internal dose metrics calculated from the PBPK model.** Results applying the model of **Salazar et al. (2015)** (top) and **Borghoff et al. (2016)** (bottom).

Dose metrics expressed are metabolism rate of *tert*-butanol (A) and metabolism rate of ETBE (B). Liver tumor incidences following ETBE oral or inhalation exposure did not present a consistent dose-response relationship using either the ETBE or *tert*-butanol metabolism rate dose

metric, and the correlation coefficients was not statistically significant. These data indicate that internal dose is inadequate to explain differences in tumor response across these studies.

### B.1.6. Physiologically Based Pharmacokinetic (PBPK) Model Code

The PBPK acslX model code is available electronically through EPA's Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2016).

### B.1.7. Physiologically Based Pharmacokinetic (PBPK) Model Evaluation

All available PBPK models of ETBE and its principal metabolite *tert*-butanol were evaluated for potential use in the assessment.

#### *Overview of Available Models*

A PBPK model of ETBE and its principal metabolite *tert*-butanol has been developed for humans exposed while performing physical work (Nihlén and Johanson, 1999). The Nihlén and Johanson model is based on measuring blood concentrations of 8 individuals exposed to 5, 25, and 50 ppm ETBE for 2 hours while physically active. This model differs from conventional PBPK models in that the tissue volumes and blood flows are calculated from individual data on body weight and height. Additionally, to account for physical activity, blood flows to tissues are expressed as a function of the workload. These differences from typical PBPK models preclude allometric scaling of this model to other species for cross-species extrapolation. As there are no oral exposure toxicokinetic data in humans, this model does not have a mechanism for simulating oral exposures, which prevents its use in animal-to-human extrapolation for that route.

A number of PBPK models were developed previously for the related compound, MTBE and the metabolite *tert*-butanol that is common to both MTBE and ETBE (Borghoff et al., 2010; Leavens and Borghoff, 2009; Blancato et al., 2007; Kim et al., 2007; Rao and Ginsberg, 1997; Borghoff et al., 1996). A PBPK model for ETBE and *tert*-butanol in rats was then developed by the EPA (Salazar et al., 2015) by integrating information from across these earlier models. Another model for ETBE and *tert*-butanol was published by Borghoff et al. (2016), adapted with modest structural differences from the Leavens and Borghoff (2009) MTBE/*tert*-butanol model. Brief descriptions below highlight the similarities and differences between the MTBE/*tert*-butanol models of Blancato et al. (2007) and Leavens and Borghoff (2009), and the ETBE/*tert*-butanol models of Salazar et al. (2015), and Borghoff et al. (2016).

#### *The Models of Blancato et al. (2007) and Leavens and Borghoff (2009)*

The Blancato et al. (2007) model is an update of the earlier Rao and Ginsberg (1997) model, and the Leavens and Borghoff (2009) model is an update of the Borghoff et al. (1996) model. Both the Blancato et al. (2007) and Leavens and Borghoff (2009) models are flow-limited models that predict amounts and concentrations of MTBE and its metabolite *tert*-butanol in blood and six tissue

compartments: liver, kidney, fat, brain, and rapidly and slowly perfused tissues. These tissue compartments are linked through blood flow, following an anatomically accurate, typical, physiologically based description (Andersen, 1991). The parent (MTBE) and metabolite (*tert*-butanol) models are linked by the metabolism of MTBE to *tert*-butanol in the liver. Oral and inhalation routes of exposure are included in the models for MTBE; Leavens and Borghoff (2009) also included oral and inhalation exposure to *tert*-butanol. Oral doses are assumed to be 100% bioavailable and 100% absorbed from the gastrointestinal tract represented with a first-order rate constant. Following inhalation of MTBE or *tert*-butanol, the chemical is assumed to enter the systemic blood supply directly, and the respiratory tract is assumed to be at pseudo-steady state. Metabolism of MTBE by CYP450s to formaldehyde and *tert*-butanol in the liver is described with two Michaelis-Menten equations representing high- and low-affinity enzymes. *tert*-Butanol is either conjugated with glucuronide or sulfate or further metabolized to acetone through MPD and HBA; the total metabolic clearance of *tert*-butanol by both processes is described by a single Michaelis-Menten equation in the models. All model assumptions are considered valid for MTBE and *tert*-butanol.

In addition to differences in fixed parameter values between the two models and the addition of exposure routes for *tert*-butanol, the Leavens and Borghoff (2009) model has three features not included in the Blancato et al. (2007) model: (1) the alveolar ventilation was reduced during exposure, (2) the rate of *tert*-butanol metabolism was increased over time to account for induction of CYP enzymes, and (3) binding of MTBE and *tert*-butanol to alpha 2u-globulin was simulated in the kidney of male rats. The Blancato et al. (2007) model was configured through EPA's PBPK modeling framework, Exposure-Related Dose Estimating Model, which includes explicit pulmonary compartments. The modeling assumptions related to alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol are discussed in the model evaluation section below.

MTBE and *tert*-butanol binding to alpha 2u-globulin in the kidneys of male rats were incorporated in the PBPK model of MTBE by Leavens and Borghoff (2009). Binding to alpha 2u-globulin is one hypothesized mode of action for the observed kidney effects in MTBE-exposed animals. For a detailed description of the role of alpha 2u-globulin and other modes of action in kidney effects, see the kidney mode-of-action section of the Toxicological Review. In the Leavens and Borghoff (2009) model, binding of MTBE to alpha 2u-globulin was applied to describe sex differences in kidney concentrations of MTBE and *tert*-butanol, but acceptable estimates of MTBE and *tert*-butanol pharmacokinetics in the blood are predicted in other models that did not consider alpha 2u-globulin binding. Moreover, as discussed below, the EPA's implementation of the Leavens and Borghoff (2009) model did not adequately fit the available *tert*-butanol i.v. dosing data, adding uncertainty to the parameters they estimated.

The Blancato et al. (2007) and Leavens and Borghoff (2009) PBPK models for MTBE were specifically evaluated by comparing predictions from the *tert*-butanol portions of the models with

the *tert*-butanol i.v. data of Poet et al. (1997) (see Figure B-4). Neither model adequately represented the *tert*-butanol blood concentrations. Modifications of model assumptions for alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol did not significantly improve model fits to the data.

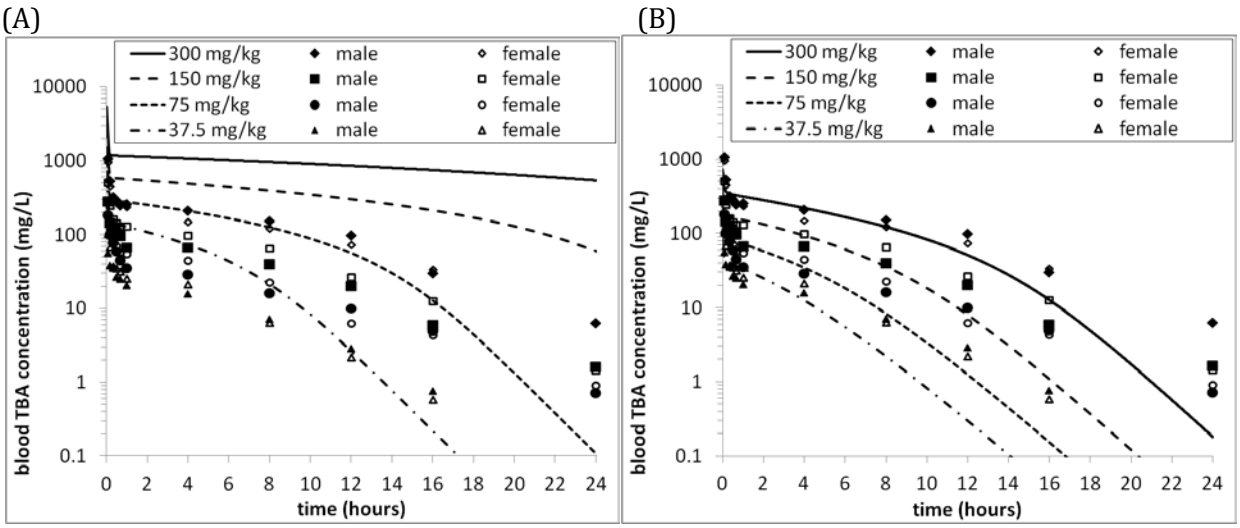



**Figure B-4. Comparison of the *tert*-butanol portions of existing methyl tertiary butyl ether models with *tert*-butanol blood concentrations from i.v. exposure by Poet et al. (1997).** Neither the (A) **Blancato et al. (2007)** nor the (B) **Leavens and Borghoff (2009)** model adequately represents the measured *tert*-butanol blood concentrations.

### *The Model of Salazar et al. (2015)*

To better account for the *tert*-butanol blood concentrations after intravenous *tert*-butanol exposure, the model by Leavens and Borghoff (2009) was modified by adding a pathway for reversible sequestration of *tert*-butanol in the blood (Salazar et al., 2015). Sequestration of *tert*-butanol was modeled using an additional blood compartment, into which *tert*-butanol can enter reversibly, represented by a differential mass balance (see Figure B-5). Other differences in model structure are that the brain was included in the other richly perfused tissues compartment and that binding to alpha 2u-globulin was not included. Binding to alpha 2u-globulin was neglected because it was assumed to not significantly affect the blood concentration or metabolic rate of ETBE to TBA, the two dose metrics being used for route-to-route extrapolation. This model improved the fit to *tert*-butanol blood concentrations after *tert*-butanol i.v. exposures [see Salazar et al. (2015)]. Additionally, the model adequately estimated the *tert*-butanol blood concentrations after inhalation and gavage exposures. The ETBE submodel was based on the MTBE component of the Leavens and Borghoff (2009) model. The model assumed two-pathways for metabolism of

ETBE to TBA, and the metabolic parameters were optimized to fit toxicokinetic data. Partition coefficients of ETBE were based on data of Nihlén and Johanson (1999).

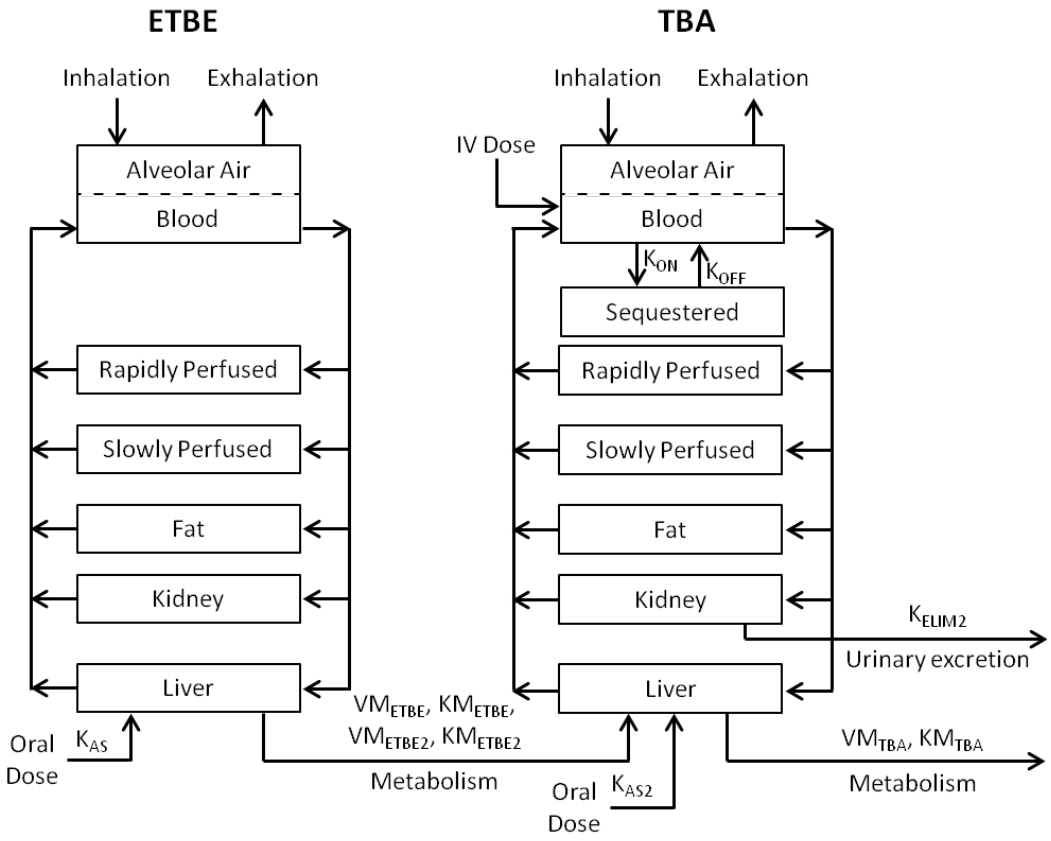



**Figure B-5. Schematic of the Salazar et al. (2015) PBPK model for ETBE and its major metabolite *tert*-butanol in rats.** Exposure can be via multiple routes including inhalation, oral, or i.v. dosing. Metabolism of ETBE and *tert*-butanol occur in the liver and are described by Michaelis-Menten equations with two pathways for ETBE and one for *tert*-butanol. ETBE and *tert*-butanol are cleared via exhalation, and *tert*-butanol is additionally cleared via urinary excretion.

### *The Model of Borghoff et al. (2016)*

The Borghoff et al. (2016) models for ETBE and *tert*-butanol were based on Leavens and Borghoff (2009), including binding of ETBE and TBA to alpha 2u-globulin and induction of *tert*-butanol metabolism, with some structural changes. The revised model lumped gastrointestinal tract tissue and brain tissue into the richly perfused compartment [Leavens and Borghoff (2009) modeled these compartments separately]. Borghoff et al. (2016) assumed that urinary clearance was a function of central venous blood concentration and effectively occurs from that compartment, as opposed to clearance from the kidney venous blood assumed by Leavens and Borghoff (2009). Using the new structure, urinary clearance was reparameterized to fit the intravenous data by Poet

et al. (1997). The model assumed a single oxidative metabolic pathway for metabolism of ETBE to *tert*-butanol using parameters from Rao and Ginsberg (1997), instead of the two-pathway models assumed by Leavens and Borghoff (2009) (for MTBE) and Salazar et al. (2015). The model did not incorporate the *tert*-butanol blood sequestration kinetics included in the *tert*-butanol model. It did, however, incorporate the oral absorption rate of *tert*-butanol estimated by Salazar et al. (2015). Partition coefficients for ETBE were obtained from Kaneko et al. (2000) and from metabolic parameters. Rate constants for binding of ETBE to alpha 2u-globulin and its dissociation were assumed to be the same as estimated for MTBE by Leavens and Borghoff (2009). Finally, unlike Leavens and Borghoff (2009), Borghoff et al. (2016) assumed a lower-bound alveolar ventilation for all times and exposures, not just during periods of inhalation exposure.

To simulate induction of *tert*-butanol metabolism, the default metabolic rate of *tert*-butanol clearance is multiplied by an exponential function of the form $[1 + A(1 - e^{-kt})]$, where $A$ is the maximum fold increase above baseline metabolism, $k$ is the rate constant for the ascent to maximum induction, and $t$ is time. Because metabolic induction does not occur instantaneously, but involves a delay for induction of ribonucleic acid transcription and translation, Borghoff et al. (2016) assumed that induction did not begin until 24 hours after the beginning of exposure. But the computational implementation then treated the effect as if the enzyme activity suddenly jumped each 24 hours to the level indicated by the time-dependent equation shown in the paper. This stepwise increase in activity was not considered realistic. Therefore, in evaluating the impact of induction, the EPA treated the induction as occurring continuously with time but beginning at 12 hours after the start of exposure. This change would not affect long-term steady-state or periodic simulations, in particular those used to characterize bioassay conditions, but has a modest effect on simulations between 12 and 24 hours, which are compared to experimental data below for the purpose of model validation. However, with further review of the existing data on liver histology (which would also reflect metabolic induction if it occurs, as detailed below), the EPA determined that it is likely to only occur at the very highest exposure levels and hence not at levels where the model is applied for route-to-route extrapolation. Therefore, the maximal induction was set to zero unless otherwise noted.

The form of the equations for hepatic metabolism in the Borghoff et al. (2016) model was revised to be a function of the free liver concentration, specifically the concentration in the venous blood leaving the liver, rather than the concentration in the liver tissue. To maintain the integrity of all prior model simulations and parameter estimations, EPA updated the Michaelis-Menten constants (Km's) for ETBE and TBA by scaling them by the liver:blood partition coefficients. As a result, the model produces identical results as before without reestimating a fitted parameter.

Finally, a discrepancy between the pulmonary ventilation value as described by Borghoff et al. (2016), in particular as the lower limit of values reported by Brown et al. (1997), should be noted. Borghoff et al. (2016) claimed that an allometric coefficient of 18.9 L/hour/kg$^{0.75}$ (allometric coefficient provided here reflects actual use in model code) is the lower limit. For a 0.25 kg rat, this

value yields an absolute ventilation rate of 6.6822 L/hour or 111.37 mL/min. In Table 31 of Brown et al. (1997), the mean and range of values given for the rat are 52.9 and 31.5–137.6 mL/min/(100 g BW). From the text immediately following this table, it is clear that these mean and range values are not scaled to $BW^{0.75}$, but exactly as indicated. Hence for a 250 g rat they correspond to 132.25 and 78.75–344 mL/min. Use of 18.9 L/hour/$kg^{0.75}$ corresponds to a ventilation rate 61% of the way between the lower limit and the mean for a 0.25 kg rat. Note that 31.5 mL/min/100 g BW, the actual lower limit, equals 18.9 L/hour/$kg^{1.0}$ (i.e., the respiration per kg BW, not per kg $BW^{0.75}$). Thus, the discrepancy appears due to a mistaken translation in allometric scaling.

The fact that Borghoff et al. (2016) and Leavens and Borghoff (2009) used a ventilation rate closer to the mean than the lower limit may explain why it was also necessary to incorporate a fraction of TBA available for alveolar absorption of 0.6. From considering the plots of model simulations versus data below, it appears that model fits to the data would be improved by further decreasing ventilation, which could now be justified. But EPA has chosen to keep the value of alveolar ventilation (QPC) and absorption fraction as published by Borghoff et al. (2016).

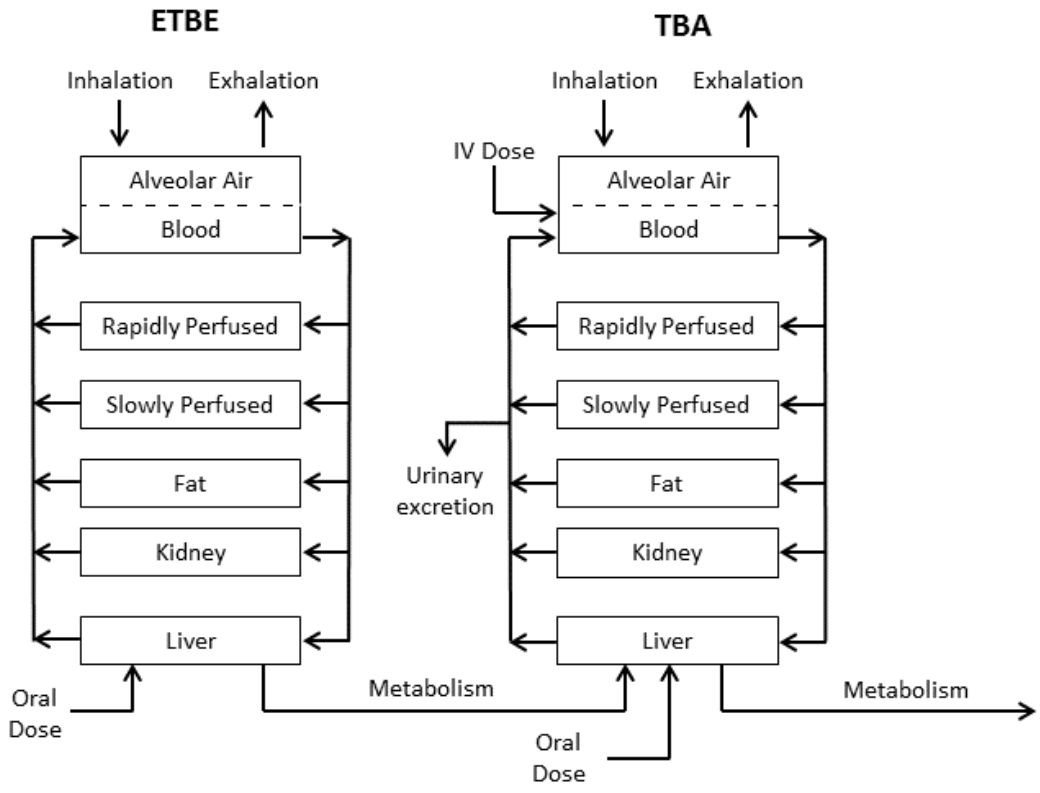



**Figure B-6. Schematic of the Borghoff et al. (2016) PBPK model for ETBE and its major metabolite *tert*-butanol in rats.**

**Table B-7. PBPK model physiological parameters and partition coefficients[a]**

| Parameter | Value | Source or reference |
|---|---|---|
| *Body weight and organ volumes as fraction of body weight* | | |
| Body weight (kg) | 0.25 | Brown et al. (1997) |
| Liver | 0.037 | Brown et al. (1997) |
| Kidney | 0.0073 | Brown et al. (1997) |
| Fat | 0.35 × BW + 0.00205 | Brown et al. (1997) |
| Richly perfused (total) | 0.136 | Brown et al. (1997) |
| Richly perfused | 0.0177 | [b] |
| Poorly perfused (total) | 0.757 | Brown et al. (1997) |
| Poorly perfused | 0.75495 − 0.35 × BW | |
| Blood | 0.074 | Brown et al. (1997) |
| Rest of body (not perfused) | 0.107 | Brown et al. (1997) |
| *Cardiac output and organ blood flows as fraction of cardiac output* | | |
| Cardiac output (L/h-kg) | 18.9 | Brown et al. (1997)[c] |
| Alveolar ventilation (L/h-kg) | 18.9 | Brown et al. (1997)[c] |
| Liver | 0.174 | Brown et al. (1997)[d] |
| Kidney | 0.141 | Brown et al. (1997) |
| Fat | 0.07 | Brown et al. (1997) |
| Richly perfused (total) | 0.47 | [e] |
| Richly perfused | 0.155 | [f] |
| Poorly perfused (total) | 0.53 | Brown et al. (1997) |
| Poorly perfused | 0.46 | [g] |
| *Partition coefficients for ETBE* | | |
| Blood:air | 11.6 | Kaneko et al. (2000) |
| Liver:blood | 2.9 | Kaneko et al. (2000) |
| Fat:blood | 11.7 | Kaneko et al. (2000) |
| Richly perfused:blood | 2.9 | Kaneko et al. (2000) |
| Poorly perfused:blood | 1.9 | [h] |
| Kidney:blood | 2.9 | [i] |
| *Partition coefficients for tert-butanol* | | |
| Blood:air | 481 | Borghoff et al. (1996) |
| Liver:blood | 0.83 | Borghoff et al. (1996) |

**Table B-7. PBPK model physiological parameters and partition coefficients[a] (continued)**

| Parameter | Value | Source or reference |
|---|---|---|
| Fat:blood | 0.4 | Borghoff et al. (1996) |
| Richly perfused:blood | 0.83 | Borghoff et al. (1996) |
| Poorly perfused:blood | 1.0 | Borghoff et al. (1996) |
| Kidney:blood | 0.83 | Borghoff et al. (2001) |

[a]Values have been updated to incorporate corrections from a quality assurance review and to include values to the number of digits used in the model code.
[b]0.165 − Σ(kidney, liver, blood).
[c]Lower limit of alveolar ventilation for rat reported in Brown et al. (1997); alveolar ventilation is set equal to cardiac output.
[d]Sum of liver and gastrointestinal blood flows.
[e]Brown et al. (1997) only accounts for 94% of the blood flow. This assumes unaccounted 6% is richly perfused.
[f]0.47 − Σ(kidney, liver).
[g]0.53 − fat.
[h]Set equal to muscle tissue (Borghoff et al., 2016).
[i]Set equal to richly perfused tissue (Borghoff et al., 2016).

**Table B-8. PBPK model rate constants**

| Parameter | Value | Source or reference |
|---|---|---|
| *tert-Butanol rate constants* | | |
| TBA first-order absorption constant (1/h) | 5.0 | Salazar et al. (2015) |
| Fraction of TBA absorbed in alveolar region | 0.6 | Medinsky et al. (1993) |
| Urinary clearance of TBA (L/h/kg$^{0.75}$) | 0.015 | Borghoff et al. (2016) |
| Scaled maximum metabolic rate of TBA (µmol/h/kg) | 54 | Borghoff et al. (1996), Rao and Ginsberg (1997) |
| Michaelis-Menten constant (µmol/L) | 457[a] | Borghoff et al. (1996), Rao and Ginsberg (1997) |
| Maximum percentage increase in metabolic rate | 0.0 | 124.9 used by Leavens and Borghoff (2009) |
| Rate constant for ascent to maximum (1/d)[b] | 0.3977 | Leavens and Borghoff (2009) |
| *ETBE rate constants* | | |
| ETBE first-order absorption constant (1/h) | 1.6 | Leavens and Borghoff (2009) |
| Scaled maximum metabolic rate of ETBE (µmol/h/kg$^{0.75}$) | 499 | Rao and Ginsberg (1997) |
| Michaelis-Menten constant for ETBE (µmol/L) | 430[a] | Rao and Ginsberg (1997) |
| *Alpha 2u-globulin binding parameters* | | |
| Steady-state free kidney alpha 2u-globulin (µmol/L) | 550[c] | Leavens and Borghoff (2009) |
| First-order constant for hydrolysis of free alpha 2u-globulin (1/h) | 0.31 | Leavens and Borghoff (2009) |
| First-order constant for hydrolysis of bound alpha 2u-globulin (1/h) | 0.11 | Leavens and Borghoff (2009) |
| Second-order binding constant for TBA to alpha 2u-globulin (L/µmol/h) | 1.3 | Leavens and Borghoff (2009) |
| Alpha 2u-globulin dissociation constant for TBA (µmol/L) | 120 | Leavens and Borghoff (2009) |
| First-order constant for unbinding of TBA from alpha 2u-globulin (1/h) | Calculated[d] | |
| Second-order binding constant for ETBE to alpha 2u-globulin (L/µmol/h) | 0.15 | Leavens and Borghoff (2009) |
| Alpha 2u-globulin dissociation constant for ETBE (µmol/L) | 1 | Leavens and Borghoff (2009) |

**Table B-8. PBPK model rate constants (continued)**

| | | |
|---|---|---|
| First-order constant for unbinding of ETBE from alpha 2u-globulin (1/h) | Calculated[e] | |

[a]Based on dividing the original values in Borghoff et al. (1996) and Rao and Ginsberg (1997) [used by Borghoff et al. (2016)] by the corresponding liver partition coefficients: 379/0.83 = 457 for *tert*-butanol kinetics, and 1,248/2.9 = 430 for ETBE kinetic pathway 1.
[b]Note: model revised from a daily stepwise induction change to a continuous change (with a 12-h time lag), while still maintaining the default parameters.
[c]Based on values ranging from ~160 to 1,000 µmol/L (Carruthers et al., 1987; Charbonneau et al., 1987; Olson et al., 1987; Stonard et al., 1986).
[d]Product of alpha 2u-globulin dissociation constant for *tert*-butanol and second-order binding constant for *tert*-butanol to alpha 2u-globulin.
[e]Product of alpha 2u-globulin dissociation constant for ETBE and second-order binding constant for ETBE to alpha 2u-globulin.

## B.1.8. Toxicokinetic Data Extraction and Selected Model Outputs

### *Data Extraction and Adjustments*

A study by ARCO (1983) reported *tert*-butanol blood levels after gavage exposure, primarily as *tert*-butanol equivalents based on total $^{14}C$ activity, which does not distinguish between *tert*-butanol and its metabolites. However, for oral doses of 1 and 500 mg/kg, the fraction of activity identifiable as *tert*-butanol was also reported, although not at identical time points. To estimate total equivalents at other times, the study authors used empirical bi-exponential curves (see Figure B-7) to interpolate between the time points at which total *tert*-butanol equivalents were measured. The total equivalents calculated this way were then multiplied by the fraction of *tert*-butanol reported at 0.5, 3, 6, and 12 hours for 1 mg/kg [ARCO (1983), Table 24] and 500 mg/kg [ARCO (1983), Table 25] to obtain the data used for PBPK modeling (see Table B-10).

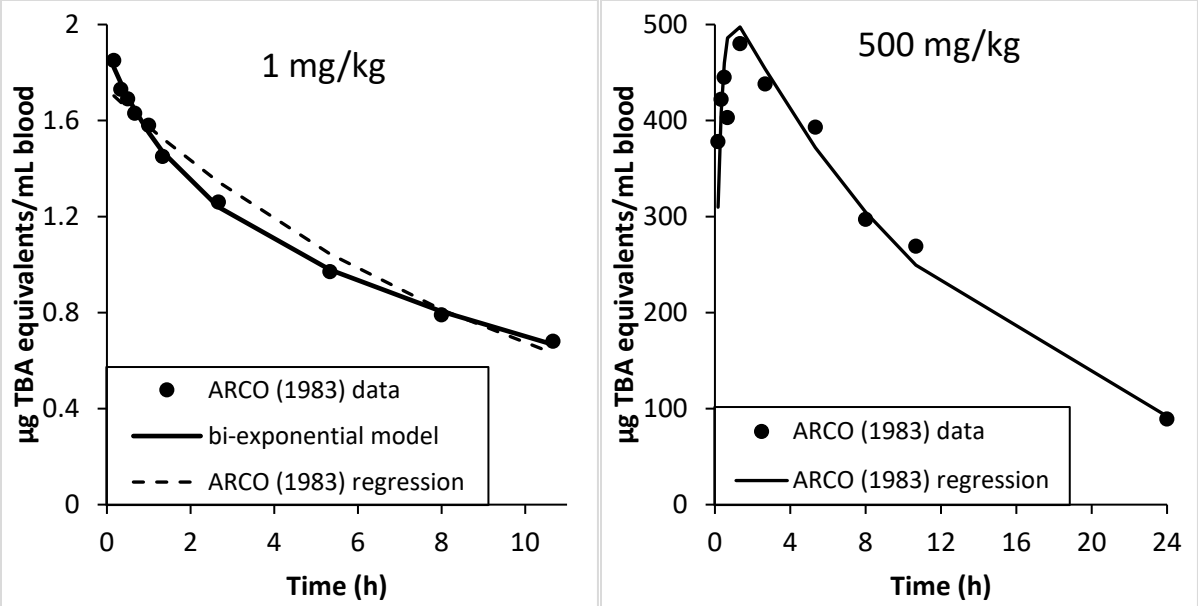



**Figure B-7. *tert*-Butanol PK data for 1 and 500 mg/kg oral exposures from ARCO (1983).**

Time-course data and empirical regressions for *tert*-butanol equivalents in rats following oral exposure to 1 or 500 mg/kg [$^{14}$C]TBA (ARCO, 1983). For 1 mg/kg, the single exponential regression reported by ARCO (1983) was 1.73 × exp(−0.0946*t*) (dashed line), but it did not appear to adequately fit the data. A bi-exponential regression (solid line) was found by minimizing the sum of square errors between the regression and data in Excel: 0.4874 × exp(−0.7055*t*) + 1.404 × exp(−0.06983*t*). For 500 mg/kg, the bi-exponential regression reported by ARCO (1983) appeared sufficient: 554 × exp(−0.0748*t*) − 426 × exp(−3.51*t*).

The single-dose data from JPEC (2008f) were taken from Appendix Table 12 of that report. The values for the P-5 component were converted from ETBE equivalents to mg/L *tert*-butanol. For example, at 5 mg/kg-day, 416 ng ETBE eq/mL is reported for P-5 in animal # 17. The corresponding concentration in mg/L for *tert*-butanol are then calculated as (416 ng ETBE eq/mL) × (1,000 mL/L) × ($10^{-6}$ mg/ng) × (74.12 [MW *tert*-butanol])/(102.17 [MW ETBE]) = 0.302 mg *tert*-butanol eq/L, where MW represents molecular weight. Likewise the data for the repeated-dose study (JPEC, 2008e), Days 7 and 14, were converted from the P-5 values in Appendix Table 7, p. 53 of that report (The data from the single-dose study were combined with the Day 7 and 14 data from the multiple dose study for comparison with model simulations of 14-day dosing.).

The JPEC (2008a) and JPEC (2008b) studies measured *tert*-butanol in plasma only, unlike the Poet et al. (1997) and Leavens and Borghoff (2009) studies, which measured *tert*-butanol in whole blood. Based on the measurements of plasma and whole blood by JPEC (2008a, 2008b), the concentration of *tert*-butanol in plasma is approximately 130% of the concentration in whole blood (see Table B-11). The *tert*-butanol plasma concentrations measured by JPEC were therefore divided by 1.3 to obtain the expected concentration in whole blood for comparison with the PBPK model.

**Table B-9. Summary of pharmacokinetic data used for model calibration and evaluation**

| Exposure | | Measured | | Data source | Figure no. in Salazar et al. (2015) | Conversion | Notes |
|---|---|---|---|---|---|---|---|
| Chemical | Route | Chemical | Medium | | | | |
| TBA | i.v. | TBA | Blood | Poet et al. (1997) Figure 1 and 2 | 3A | µM to mg/L | Digitized from the figure |
| | Inhalation | TBA | Blood | Leavens and Borghoff (2009) Figure 8A–B | 3B | µM to mg/L | Digitized from the figure, showing only 1 d of exposure |
| | Gavage | TBA | Blood | ARCO (1983), % total TBA, Tables 24–25; TBA equivalents, Figure 6 | 3C | TBA equivalents to TBA concentration | |
| ETBE | Gavage | TBA | Blood | JPEC (2008f) Appendix 12 | 4A | ETBE equivalents to mg/L TBA | "P5" is TBA |
| | | TBA | Urine | JPEC (2008f) Appendix 13 | 4B | ETBE equivalents to mg/L TBA | "P5" is TBA |
| ETBE | Inhalation | ETBE | Blood | Amberg et al. (2000) Table 5 | 4C | µM to mg/L | |
| | | TBA | Blood | Amberg et al. (2000)Table 5 | 4D | µM to mg/L | |
| | | TBA | Urine | Amberg et al. (2000) Table 6 and Figure 4 | 4E | µM to mg/L | |
| | | ETBE | Exhaled air | Borghoff (1996) | 4F | µmoles to mg | Cumulative mass |
| | | TBA | Exhaled air | Borghoff (1996) | 4G | µmoles to mg | Cumulative mass |
| TBA | Inhalation | TBA | Blood | Leavens and Borghoff (2009) Figure 8B | 5A–B | µM to mg/L | Digitized from the figure |
| | | TBA | Blood | Leavens and Borghoff (2009) Figure 8A | 5C–D | µM to mg/L | Digitized from the figure |
| ETBE | Gavage | TBA | Blood | JPEC (2008f) Appendix 12 | 5E | ETBE equivalents to mg/L TBA | "P5" is TBA |

**Table B-10. Conversion of ARCO (1983) total *tert*-butanol (TBA) equivalents and serum fraction data to TBA concentrations**

| Time (h) | % TBA[a] | Total TBA equivalents interpolated (µg/mL)[b] | TBA concentration using interpolated equivalents (µg/mL = mg/L)[c] | Total TBA equivalents measured at nearest time point (time measured)[d] | TBA concentration using nearest time point (mg/L)[e] |
|---|---|---|---|---|---|
| *1 mg/kg data* | | | | | |
| 0.5 | 57.3 | 1.6982 | 0.9731 | 1.69 (0.5 h) | 0.9684 |
| 3 | 25 | 1.1972 | 0.2993 | 1.26 (2.67 h) | 0.3150 |
| 6 | 18.1 | 0.9304 | 0.1684 | 0.97 (5.33 h) | 0.1756 |
| 12 | 1 | 0.6074 | 0.006074 | 0.68 (10.67 h) | 0.006800 |
| *500 mg/kg data* | | | | | |
| 0.5 | 22.9 | 460.0 | 105.34 | 445 (0.5 h) | 101.91 |
| 3 | 20.4 | 442.6 | 90.30 | 438 (2.67 h) | 89.35 |
| 6 | 18.7 | 353.7 | 66.14 | 393 (5.33 h) | 73.49 |
| 12 | 18.5 | 225.8 | 41.77 | 269 (10.67 h) | 49.77 |

[a]From Table 24, p. 48 of ARCO (1983) (1 mg/kg) and Table 25, p. 49 of ARCO (1983) (500 mg/kg).
[b]Using bi-exponential functions.
[c]Values used in PBPK modeling; %TBA × total TBA equivalents interpolated.
[d]From Table 14, p. 32 of ARCO (1983) (1 mg/kg) and Table 11, p. 27 of ARCO (1983) (500 mg/kg).
[e]%TBA × total TBA equivalents at nearest time point.

**Table B-11. Ratio of $^{14}C$ activity in blood vs. plasma after [$^{14}C$]ETBE exposures in rats JPEC (2008a) JPEC (2008b)**

| Time (h) | Animal no. | Plasma (ng $^{14}C$ eq/mL) | Blood (ng $^{14}C$ eq/mL) | Plasma/blood (%) |
|---|---|---|---|---|
| *Single dose, JPEC (2008f) Appendix Table 5, p. 94* | | | | |
| **8** | 97 | 78,133 | 40,667 | 192.1 |
| | 98 | 95,533 | 80,000 | 119.4 |
| | 99 | 89,367 | 64,667 | 138.2 |
| | 100 | 72,400 | 62,333 | 116.2 |
| **24** | 37 | 10,900 | 8,800 | 123.9 |
| | 38 | 19,133 | 14,433 | 132.6 |
| | 39 | 19,433 | 15,400 | 126.2 |
| | 40 | 30,767 | 22,967 | 134.0 |
| **72** | 41 | 2,133 | 1,600 | 133.3 |
| | 42 | 2,833 | 3,033 | 93.4 |
| | 43 | 4,033 | 3,200 | 126.0 |
| | 44 | 3,167 | 2,333 | 135.7 |
| | | | **Mean ± SD** | **130.9 ± 22.8** |
| *Single dose, JPEC (2008f) Appendix Table 3, p. 91* | | | | |
| **8** | 17 | 2,853 | 1,784 | 159.9 |
| | 18 | 2,850 | 1,802 | 158.2 |
| | 19 | 2,629 | 1,568 | 167.7 |
| | 20 | 3,918 | 2,718 | 144.2 |
| **24** | 21 | 1,692 | 1,255 | 134.8 |
| | 22 | 846.7 | 642.9 | 131.7 |
| | 23 | 1,048 | 785 | 133.5 |
| | 24 | 761.7 | 591.3 | 128.8 |
| **72** | 25 | 49.6 | 40 | 124.0 |
| | 26 | 34.2 | 29.2 | 117.1 |
| | 27 | 79.2 | 60.8 | 130.3 |
| | 28 | 107.9 | 84.6 | 127.5 |

**Table B-11. Ratio of $^{14}C$ activity in blood vs. plasma after [$^{14}C$]ETBE exposures in rats IPEC (2008a) IPEC (2008b) (continued)**

| Time (h) | Animal no. | Plasma (ng $^{14}C$ eq/mL) | Blood (ng $^{14}C$ eq/mL) | Plasma/blood (%) |
|---|---|---|---|---|
| **168** | 29 | 12.9 | 13.3 | 97.0 |
| | 30 | 17.5 | 13.8 | 126.8 |
| | 31 | 26.7 | 24.2 | 110.3 |
| | 32 | 40 | 35.8 | 111.7 |
| | | | **Mean ± SD** | **131.5 ± 18.9** |
| *Repeated dose, IPEC (2008e), Appendix Table 3, p. 49* | | | | |
| **8 (7 d dosing)** | | 3,789 | 3,029 | 125.1 |
| | | 5,041 | 3,988 | 126.4 |
| | | 4,914 | 3,938 | 124.8 |
| | | 5,608 | 4,638 | 120.9 |
| **24 (7 d dosing)** | | 2,740 | 1,908 | 143.6 |
| | | 3,433 | 2,575 | 133.3 |
| | | 2,488 | 1,888 | 131.8 |
| | | 963.3 | 812.5 | 118.6 |
| **8 (14 d dosing)** | | 5,665 | 4,546 | 124.6 |
| | | 5,175 | 4,075 | 127.0 |
| | | 3,889 | 3,058 | 127.2 |
| | | 5,090 | 3,858 | 131.9 |
| **24 (14 d dosing)** | | 2,003 | 1,508 | 132.8 |
| | | 2,121 | 1,692 | 125.4 |
| | | 1,948 | 1,354 | 143.9 |
| | | 1,037 | 804.2 | 128.9 |
| **72 (14 d dosing)** | | 1,378 | 1,138 | 121.1 |
| | | 301.3 | 245.8 | 122.6 |
| | | 110 | N.D. | |
| | | 421.3 | 337.5 | 124.8 |
| | | | **Mean ± SD** | **128.1 ± 6.85** |

N.D. = not detected.

***Selected Model Comparisons Applying the Borghoff et al. (2016) Model***

The modeling code was obtained by the authors of Borghoff et al. (2016). The modeling language and platforms is acslX (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, AL).

The following modifications were made:

1) Periodic drinking water pathway was incorporated into the continuous simulation language (CSL) file, and the continuous oral dose rate function was modified slightly to improve flexibility of the model.

2) For simulations showing the effect of including enzyme induction, the code was modified slightly in the CSL file to improve continuity. Daily step functions in metabolic chances were replaced with a continuous function but delayed by 12 hours.

3) Otherwise, enzyme induction was not used (set to zero).

4) In the PBPK model code, the changes to the Michaelis-Menten constants described as footnotes in Table B-8 above were not made in the PBPK parameter script (MTBEparam.m). Instead, parameters were redefined in the core model *CSL file as scaling calculations in the parameters section of the INITIAL bloc:

   a. Km1vetbe = Km1etbe/Pletbe

   b. Km2vetbe = Km2etbe/Pletbe

   c. Kmvtba = Kmtba/Pltba

5) Tissue volumes and the rate of hydrolysis of free alpha 2u-globulin were corrected (slightly) to values shown in Table B-7.

6) All model scripts previously used to evaluate model fits of the Salazar et al. (2015) model were adapted to run the Borghoff et al. (2016) model. Model parameters were set to uniform values for all simulations highlighted in this section, unless otherwise noted.

7) Digitized data from Amberg et al. (2000) were updated after a quality assurance (QA) review.

8) Tabulated data from Borghoff and Asgharian (1996) were updated subsequent to a QA review.

The PBPK acslX model code is available electronically through EPA's HERO database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2016). The model contains workspaces for EPA's implementation of the Salazar et al. (2015) model, the unchanged version of the of Borghoff et al. (2016) model, and the EPA implementation of the Borghoff et al. (2016) model.

Selected model outputs compared with experimental data sets are provided below.

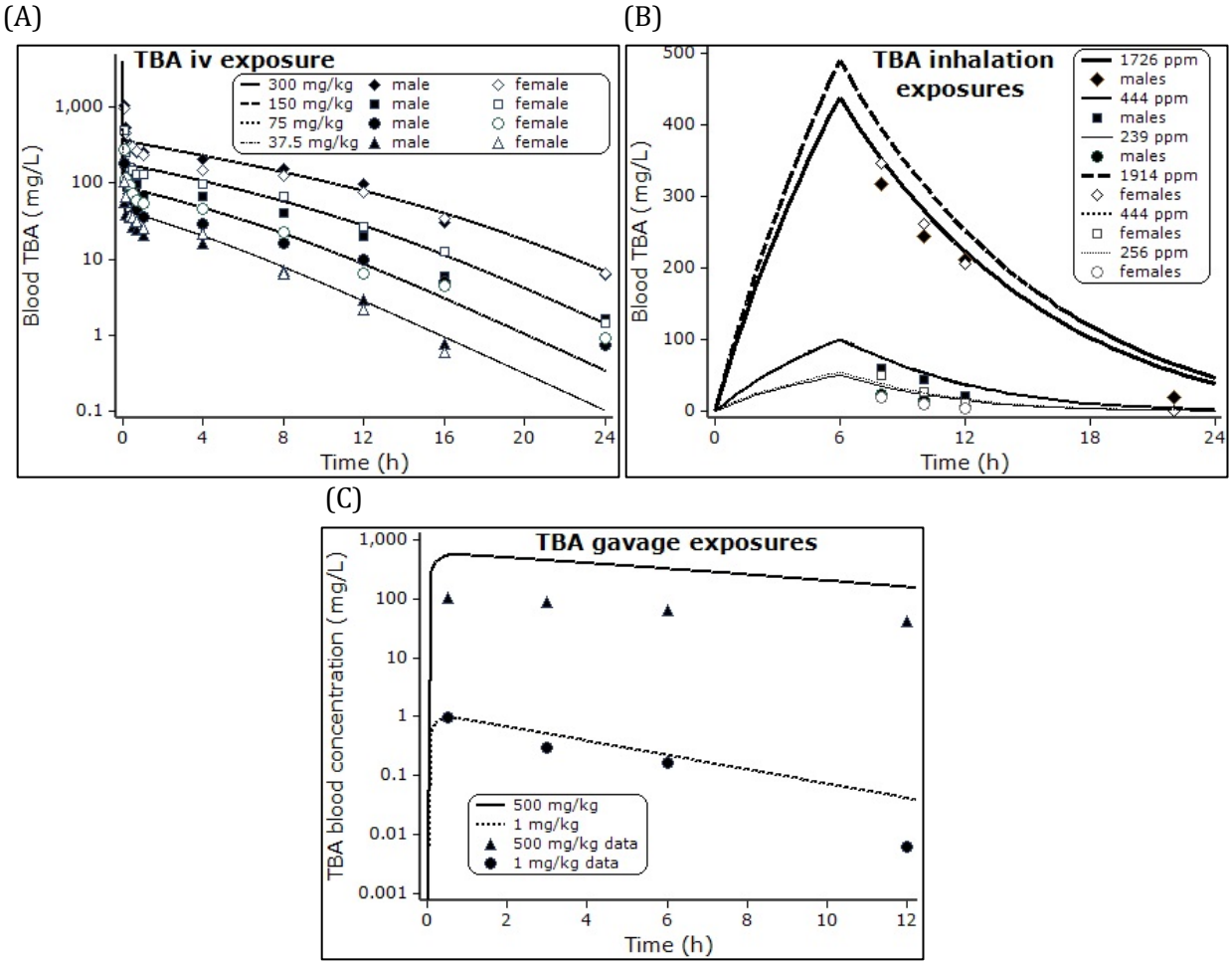



**Figure B-8. Comparison of the Borghoff et al. (2016) model predictions with measured *tert*-butanol blood concentrations for i.v., inhalation, and gavage exposure to *tert*-butanol.**

Source: (A) i.v. data from Poet et al. (1997); (B) inhalation data from Leavens and Borghoff (2009); and (C) gavage data from ARCO (1983).

The model results for the i.v. data are significantly improved from the Blancato et al. (2007) and Leavens and Borghoff (2009) model results presented previously. As evident here and in the Borghoff et al. (2016) study, the Borghoff et al. (2016) model generally overpredicts TBA blood and urine concentrations. Some attempts were made to improve model fit in the EPA model implementation (such as adjusting inhalation, urinary, and induction parameter values); however, the default values were maintained in the final model.

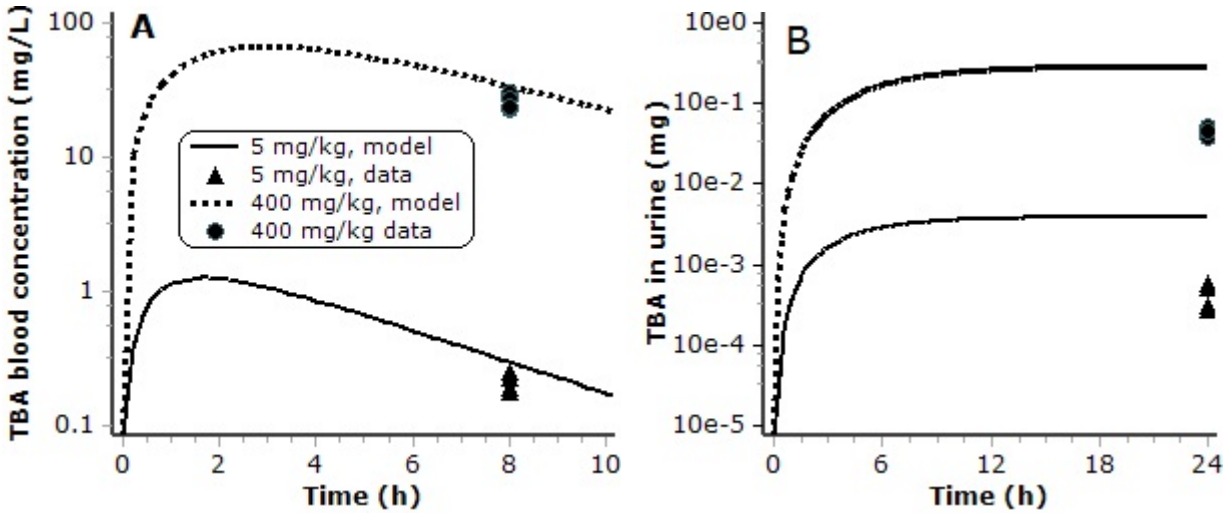



**Figure B-9. Comparison of Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol after gavage of ETBE.**

The data points show the measurements from the four individual rats in the JPEC (2008f) study. The concentrations of *tert*-butanol in blood are shown in (A). The amount of *tert*-butanol in urine is shown in (B). Note that the overprediction of *tert*-butanol in urine (B) is by a factor of 3- to 10-fold.

The predictions of the model are compared with amounts measured by Amberg et al. (2000) after ETBE inhalation in Figure B-10A. The predicted *tert*-butanol blood concentrations are slightly higher than the measured ones. The *tert*-butanol blood concentration would be reduced if the exposed animals were reducing their breathing rate or other breathing parameters, but the exposure concentration of ETBE are unlikely to be high enough to cause a change in breathing parameters, because at the much higher ETBE concentration in the ARCO (1983) study (5,000 ppm), changes in breathing were not noted. The model already uses a lower bound estimate of respiration rate and cardiac output for all simulations, and the model predictions fit most measured concentrations well. However, the urinary excretion of *tert*-butanol is significantly overestimated (~3- to 10-fold) by the *tert*-butanol submodel (see Figure B-10B).

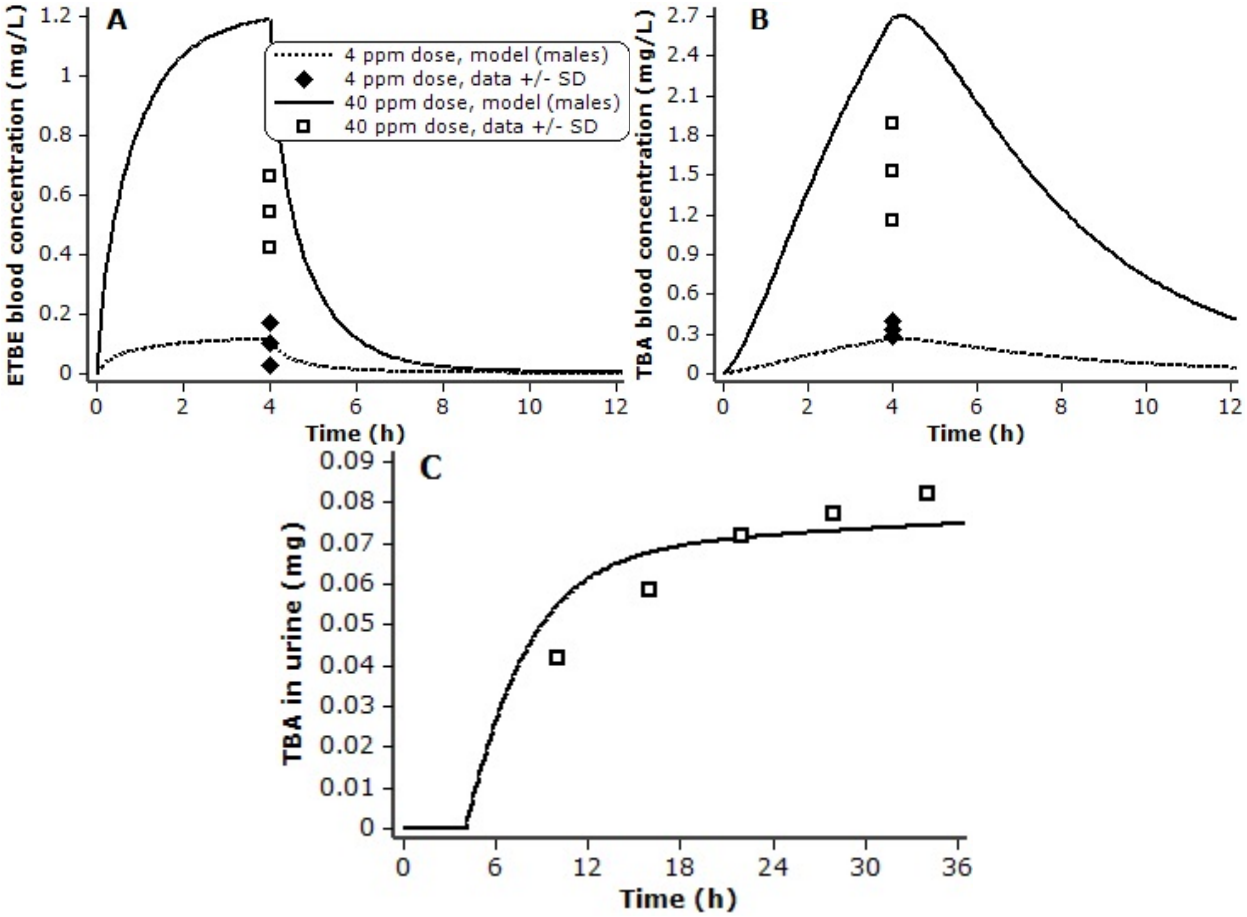



**Figure B-10. Comparison of Borghoff et al. (2016) model predictions with measured amounts after a 4-hour inhalation exposure to 4 and 40 ppm ETBE.**

Concentrations in blood are shown in (A) for ETBE and (B) for *tert*-butanol. The amount of *tert*-butanol in urine is shown in (C) for the 40-ppm exposure. The data are from Amberg et al. (2000).

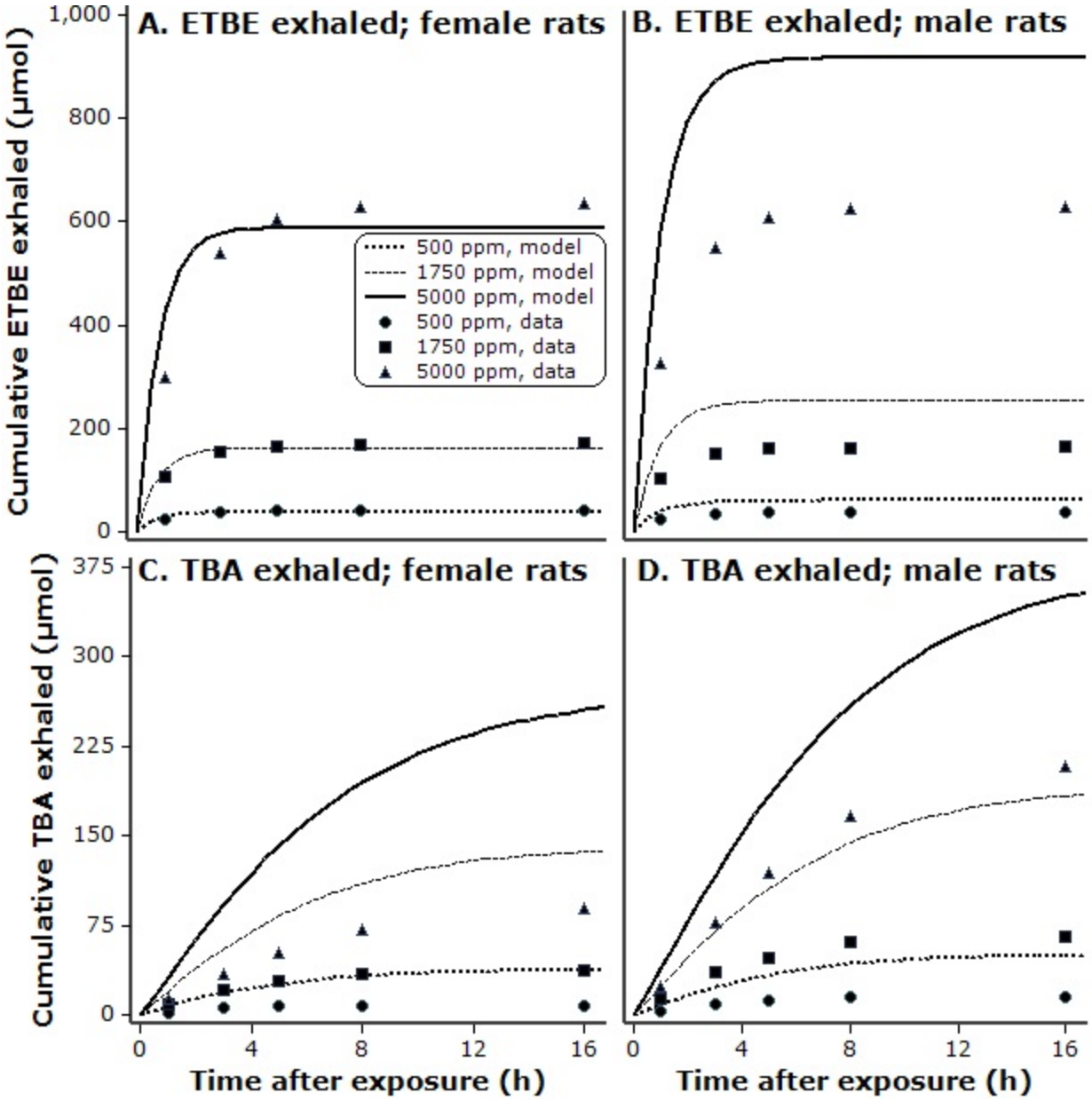



**Figure B-11. Comparison of Borghoff et al. (2016) model predictions with measured amounts of ETBE and *tert*-butanol in exhaled breath after a 6-hour inhalation exposure to 500, 1,750, and 5,000 ppm ETBE.**

The data points are from the Borghoff and Asgharian (1996) study. The model significantly overpredicted the concentrations of both ETBE and *tert*-butanol in the exhaled breath of male rats and of *tert*-butanol in female rats following ETBE inhalation exposure. The model currently assumes that 100% of inhaled ETBE, though only 60% of inhaled *tert*-butanol, is available for alveolar absorption. The inhalation availability may have a significant impact on estimated exhaled breath amounts but was not adjusted to fit this data set.

The increased *tert*-butanol metabolism better estimates the measured *tert*-butanol blood concentrations as shown in a comparison of the model predictions and experimental measurements in Figure B-12. The male rats have lower *tert*-butanol blood concentrations after repeated exposures than female rats, and this difference could indicate greater induction of *tert*-butanol metabolism in males or other physiologic changes such as ventilation or urinary excretion.

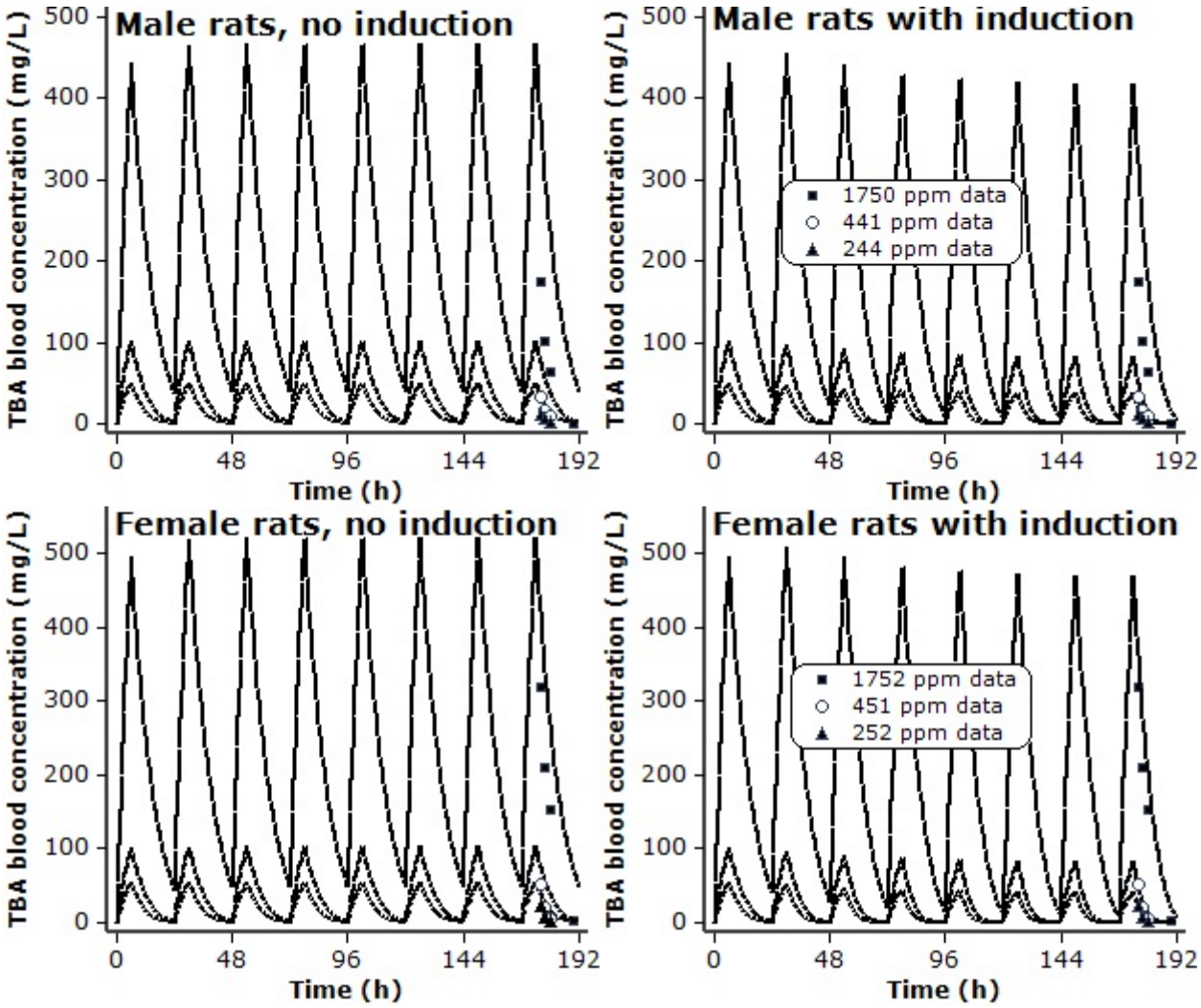



**Figure B-12. Comparison of the Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol in blood after repeated inhalation exposure to *tert*-butanol.**

Male rats were exposed to 239, 444, or 1,726 ppm and female rats were exposed to 256, 444, or 1,914 ppm *tert*-butanol for up to 8 consecutive days (Borghoff et al., 2001). *tert*-Butanol blood concentrations are better predicted by the model after 8 days of exposure with enzyme induction (right panels) than without enzyme induction (left panels).

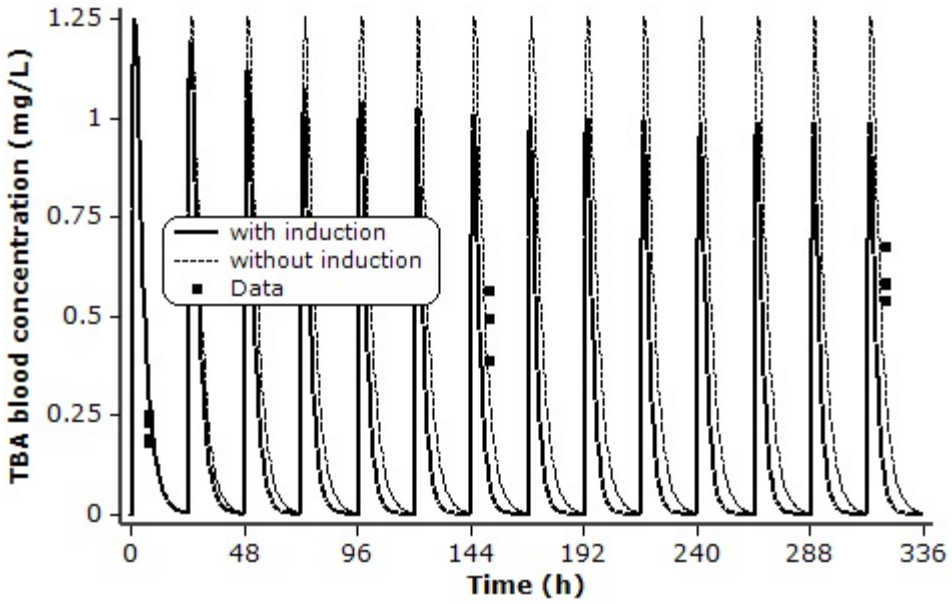



**Figure B-13. Comparison of EPA model predictions with measured amounts of *tert*-butanol in blood after 5 mg/kg-day ethyl tertiary butyl ether (ETBE) gavage for up to 14 days in male rats.**

The data show the individual measurements of the four rats in the JPEC (2008e, 2008f) study. Adding enzyme induction to the model has a small effect on the predicted *tert*-butanol blood concentrations and the model predictions are closer to measured data when induction is not included.

## B.2. OTHER PERTINENT TOXICITY INFORMATION

### B.2.1. Other Toxicological Effects

***Synthesis of Other Effects***

The database for effects other than kidney, liver, reproduction, and cancer contain only 11 rodent studies. These effects included decreased body weight, increased adrenal weights, altered spleen weights, and increased mortality. All selected studies used inhalation, gavage, or drinking water exposures for ≥90 days. Shorter duration, multiple-exposure studies that examined immunological endpoints were also included. No studies were removed for methodological concerns.

Kidney effects

Absolute kidney-weight data are presented in Table B-12.

**Table B-12. Evidence pertaining to absolute kidney-weight effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Fujii et al. (2010); JPEC (2008d)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>P0, male (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 16 wk beginning 10 wk prior to mating<br>P0, female (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 17 wk beginning 10 wk prior to mating to LD 21 | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 100 | 5 | 100 | −2 |
| | 300 | 8 | 300 | 0 |
| | 1,000 | 18[a] | 1,000 | 7[a] |
| Gaoua (2004b)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>P0, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups<br>P0, female (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until PND 21<br>F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 11[a] | 250 | −1 |
| | 500 | 15[a] | 500 | 2 |
| | 1,000 | 21[a] | 1,000 | 5 |
| | **F1, Male** | | **F1, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 10 | 250 | 4 |
| | 500 | 22[a] | 500 | 3 |
| | 1,000 | 58[a] | 1,000 | 11[a] |
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0 or 1,000 mg/kg-d<br>Daily for 23 wk | **Male** | | | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | | |
| | 0 | – | | |
| | 1,000 | 19[a] | | |

**Table B-12. Evidence pertaining to absolute kidney-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Miyata et al. (2013); JPEC (2008b)<br>Rat, Crl:CD(SD)<br>Oral—gavage<br>Male (15/group): 0, 5, 25, 100, or 400 mg/kg-d; female (15/group): 0, 5, 25, 100, or 400 mg/kg-d<br>Daily for 26 wk | Male | | Female | |
| | Dose (mg/kg-d) | Absolute weight (%) | Dose (mg/kg-d) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 5 | 1 | 5 | 1 |
| | 25 | 6 | 25 | 0 |
| | 100 | 5 | 100 | 7 |
| | 400 | 25[a] | 400 | 10[a] |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (34–37/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg d); Female (36–38/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)<br>Daily for 104 wk<br><br>(Organ weights measured for animals surviving to study termination) | Male | | Female | |
| | Dose (mg/kg-d) | Absolute weight (%) | Dose (mg/kg-d) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 28 | –4 | 46 | 3 |
| | 121 | 5 | 171 | 10[b] |
| | 542 | 18[b] | 560 | 14[b] |
| Saito et al. (2013); JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (30–44/group)<br>Female (29–39/group)<br>0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m$^3$)[b]<br>Dynamic whole-body inhalation; 6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration reported<br><br>(Organ weights measured for animals surviving to study termination) | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 5 | 2,090 | 5 |
| | 6,270 | 8 | 6,270 | 6[a] |
| | 20,900 | 18[a] | 20,900 | 18[b] |

**Table B-12. Evidence pertaining to absolute kidney-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$); female (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$);<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | **Dose (mg/m$^3$)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 627 | 10 | 627 | 1 |
| | 2,090 | 11 | 2,090 | −1 |
| | 6,270 | 18[a] | 6,270 | 4 |
| | 20,900 | 16[a] | 20,900 | 7 |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$);[c] female (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk followed by a 28 d recovery period; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | **Dose (mg/m$^3$)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 20,900 | 19 | 20,900 | 8 |
| Medinsky et al. (1999); US EPA (1997)<br>Rat, F344<br>Inhalation—vapor<br>Male (11/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (10-11/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | **Dose (mg/m$^3$)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 2,090 | 7 | 2,090 | 4 |
| | 7,320 | 10[a] | 7,320 | 12[a] |
| | 20,900 | 19[a] | 20,900 | 21[a] |

**Table B-12. Evidence pertaining to absolute kidney-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Dose ($mg/m^3$) | Absolute weight (%) | Dose ($mg/m^3$) | Absolute weight (%) |
| Medinsky et al. (1999); Bond et al. (1996)<br>Mice, CD-1<br>Inhalation—vapor<br>Male (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 $mg/m^3$);[b] female (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 $mg/m^3$)[b]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | 0 | – | 0 | – |
| | 2,090 | 9 | 2,090 | 0 |
| | 7,320 | 10 | 7,320 | 6 |
| | 20,900 | 5 | 20,900 | 4 |

– = for controls, no response relevant; for other doses, no quantitative response reported; LD = lactation day; *n* = number evaluated from group; NR = not reported; PND = postnatal day.

[a]Result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
[b]4.18 $mg/m^3$ = 1 ppm.

Body weight

As presented in Table B-12, body weights were significantly reduced compared with vehicle controls following 2-year oral and inhalation exposures to ETBE (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Reductions were also reported in studies of exposure durations shorter than 2 years (Banton et al., 2011; Hagiwara et al., 2011; Fujii et al., 2010; JPEC, 2008a, b; Gaoua, 2004b; Medinsky et al., 1999); however, these effects were frequently not statistically significant. Food consumption did not correlate well with body weight (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Water consumption was reduced in the 2-year oral exposure study (JPEC, 2010a). Reduced water consumption due to ETBE exposure and the chemical's unpalatability might contribute to the reduced body weight, particularly for dietary or drinking water exposures. Hypersalivation, which is frequently observed with unpalatable chemicals following gavage, was observed in rats gavaged for 18 weeks (Gaoua, 2004b). Body-weight changes are poor indicators of systemic toxicity but are important when evaluating relative organ-weight changes.

Adrenal weight

Adrenal weights were increased in the 13- and 23-week studies (see Table B-13). For instance, a 13-week inhalation study found that absolute adrenal weights were increased in male and female rats (Medinsky et al., 1999). In another study, absolute and relative adrenal weights were increased in male rats (Hagiwara et al., 2011). None of the observed organ-weight changes

corresponded with functional or histopathological changes; thus, adrenal effect data are inadequate to draw conclusions as a human hazard of ETBE exposure.

Immune system

In general, functional immune assays represent clear evidence of immunotoxicity and outweigh immune organ weight and cell population effects when establishing hazard [WHO (2012); see Table B-15]. The single published functional assay available for ETBE reported that the number of $IgM^+$ sheep red blood cell (SRBC)-specific antibody forming cells was not significantly affected after a 28-day oral exposure to ETBE (Banton et al., 2011). Relative spleen weights were inconsistently affected in male and female rats following oral and inhalation >13-week exposures to ETBE (see Table B-15). The only dose-responsive changes in spleen weights were increased relative weights in male rats and decreased absolute weights in female rats following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b) and increased relative weights in female rats following 2-year oral exposure (Suzuki et al., 2012; JPEC, 2010a). Spleen weights are heavily influenced by the proportion of red blood cells, which do not impact immune function of the organ (Elmore, 2006). Thus, spleen-weight changes must be correlated with histopathological and functional changes for evidence of immunotoxicity (Elmore, 2006), none of which are observed for ETBE. $CD3^+$, $CD4^+$, and $CD8^+$ T cells were modestly reduced in male mice after 6 or 13 weeks of ETBE exposure via inhalation but are not correlated with any change in T cell function as indicated by the SRBC assay (Li et al., 2011). No other indicators of histopathological or functional changes were reported with a single chemical exposure. The ETBE database contains no evidence of altered immune function that correlate with modest T cell population reductions and altered splenic organ weights; thus, the immune effect data are inadequate to draw conclusions as a human hazard of ETBE exposure.

**Table B-13. Evidence pertaining to body-weight effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Banton et al. (2011)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>Female (10/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for 28 consecutive d | **Female** | | | |
| | **Dose (mg/kg-d)** | **Body weight (%)** | | |
| | 0 | – | | |
| | 250 | 3 | | |
| | 500 | 5 | | |
| | 1,000 | −1 | | |
| Fujii et al. (2010); JPEC (2008d)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>P0, male (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 16 wk beginning 10 wk prior to mating; P0, female (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 17 wk beginning 10 wk before mating to LD 21 | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Body weight (%)** | **Dose (mg/kg-d)** | **Body weight (%)** |
| | 0 | – | 0 | – |
| | 100 | −4 | 100 | 1 |
| | 300 | −4 | 300 | 1 |
| | 1,000 | −7 | 1,000 | 5 |
| Gaoua (2004b)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>P0, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups<br>P0, female (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until PND 21<br>F1, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups<br>F1, female (24–25/group): 0, 250, 500, or 1,000 mg/kg-d<br>P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Final body weight (%)** | **Dose (mg/kg-d)** | **Final body weight (%)** |
| | 0 | – | 0 | – |
| | 250 | −1 | 250 | −7 |
| | 500 | −3 | 500 | −2 |
| | 1,000 | −5[a] | 1,000 | 0 |
| | **F1, Male** | | **F1, Female** | |
| | **Dose (mg/kg-d)** | **Final body weight (%)** | **Dose (mg/kg-d)** | **Final body weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 0 | 250 | −2 |
| | 500 | 3 | 500 | −3 |
| | 1,000 | 1 | 1,000 | 2 |

**Table B-13. Evidence pertaining to body-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0 or 1,000 mg/kg-d<br>Daily for 23 wk | **Male** | | | |
| | **Dose (mg/kg-d)** | **Final body weight (%)** | | |
| | 0 | – | | |
| | 1,000 | −5[a] | | |
| Miyata et al. (2013);JPEC (2008b)<br>Rat, Crl:CD(SD)<br>Oral—gavage<br>Male (15/group): 0, 5, 25, 100, or 400 mg/kg-d; female (15/group): 0, 5, 25, 100, or 400 mg/kg-d<br>Daily for 26 wk | **Male** | | **Female** | |
| | **Dose (mg/kg-d)** | **Body weight (%)** | **Dose (mg/kg-d)** | **Body weight (%)** |
| | 0 | – | 0 | – |
| | 5 | −6 | 5 | −5 |
| | 25 | 0 | 25 | −2 |
| | 100 | −5 | 100 | −2 |
| | 400 | 2 | 400 | −3 |
| Maltoni et al. (1999)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>Male (60/group): 0, 250, or 1,000 mg/kg-d; female (60/group): 0, 250, or 1,000 mg/kg-d; 4 d/wk for 104 wk; observed until natural death | **Male**<br>No significant difference at any dose<br>**Female**<br>No significant difference at any dose | | | |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (50/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg-d);[b] female (50/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)[b]<br>Daily for 104 wk | **Male** | | **Female** | |
| | **Dose (mg/kg-d)** | **Terminal body weight (%)** | **Dose (mg/kg-d)** | **Terminal body weight (%)** |
| | 0 | – | 0 | – |
| | 28 | −4 | 46 | −10[a] |
| | 121 | −7[a] | 171 | −11[a] |
| | 542 | −9[a] | 560 | −17[a] |

**Table B-13. Evidence pertaining to body-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$)<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Body weight (%) | Dose (mg/m$^3$) | Body weight (%) |
| | 0 | – | 0 | – |
| | 627 | 0 | 627 | −6 |
| | 2,090 | 1 | 2,090 | −7 |
| | 6,270 | −1 | 6,270 | −7 |
| | 20,900 | −7 | 20,900 | −11 |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$);[c] female (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk followed by a 28 d recovery period; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Body weight (%) | Dose (mg/m$^3$) | Body weight (%) |
| | 0 | – | 0 | – |
| | 20,900 | 3 | 20,900 | 4 |
| Medinsky et al. (1999); US EPA (1997)<br>Rat, F344<br>Inhalation—vapor<br>Male (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Body weight (%) | Dose (mg/m$^3$) | Body weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 2 | 2,090 | −3 |
| | 7,320 | 4 | 7,320 | 3 |
| | 20,900 | 2 | 20,900 | 6[a] |
| Medinsky et al. (1999); US EPA (1997)<br>Mice, CD-1<br>Inhalation—vapor<br>Male (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Body weight (%) | Dose (mg/m$^3$) | Body weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 0 | 2,090 | −2 |
| | 7,320 | −1 | 7,320 | −1 |
| | 20,900 | −3 | 20,900 | 2 |

**Table B-13. Evidence pertaining to body-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Dose ($mg/m^3$) | Body weight (%) | Dose ($mg/m^3$) | Body weight (%) |
| Saito et al. (2013);JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 $mg/m^3$);[c] female (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 $mg/m^3$)[c]<br>Dynamic whole-body inhalation; 6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method were reported | 0 | – | 0 | – |
| | 2,090 | −7[a] | 2,090 | −6[a] |
| | 6,270 | −7[a] | 6,270 | −10[a] |
| | 20,900 | −26[a] | 20,900 | −23[a] |

– = for controls, no response relevant; for other doses, no quantitative response reported; NR = not reported; PND = postnatal day.

[a]Result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
[b]Conversion performed by study authors.
[c]4.18 $mg/m^3$ = 1 ppm.
Percentage change compared to controls calculated as 100 × [(treated value − control value) ÷ control value].

**Table B-14. Evidence pertaining to adrenal effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| *Adrenal weight* | | | | |
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0, 1,000 mg/kg-d<br>Daily for 23 wk | **Male** | | | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Relative weight (%)** | |
| | 0 | – | – | |
| | 1,000 | 16[a] | 19[a] | |
| Medinsky et al. (1999); US EPA (1997)<br>Rat, F344<br>Inhalation—vapor<br>Male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m$^3$);[b] female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m$^3$)[b]<br>dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | **Dose (mg/m$^3$)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 2,090 | 11 | 2,090 | 7 |
| | 7,320 | 9 | 7,320 | 7 |
| | 20,900 | 34[a] | 20,900 | 18[a] |
| Medinsky et al. (1999); Bond et al. (1996)<br>Mice, CD-1<br>Inhalation—vapor<br>Male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m$^3$);[b] female (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m$^3$)[b]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | **Dose (mg/m$^3$)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 2,090 | 0 | 2,090 | −8 |
| | 7,320 | 50 | 7,320 | 8 |
| | 20,900 | 0 | 20,900 | −8 |

– = for controls, no response relevant; for other doses, no quantitative response reported; *n* = number evaluated from group.

[a]Result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
[b]4.18 mg/m$^3$ = 1 ppm.

**Table B-15. Evidence pertaining to immune effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| *Functional immune effects* | | | | |
| Banton et al. (2011)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>Female (10/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for 28 consecutive d<br>Immunized i.v. 4 d prior to sacrifice with sheep red blood cells | **Female** | | | |
| | **Dose (mg/kg-d)** | **IgM antibody forming cells/$10^6$ spleen cells (%)** | **IgM antibody forming cells/spleen (%)** | |
| | 0 | – | – | |
| | 250 | −21 | −20 | |
| | 500 | 42 | 36 | |
| | 1,000 | 8 | 8 | |
| *Immune cell populations* | | | | |
| Li et al. (2011)<br>Mice, 129/SV<br>Inhalation—vapor<br>Male (6/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[a]<br>Whole body, 6 h/d for 5 d/wk over 6 wk; generation method not reported; analytical concentration and method were reported | **Male** | | | |
| | **Dose (mg/m$^3$)** | **Number of CD3$^+$ T cells (%)** | **Number of CD4$^+$ T cells (%)** | **Number of CD8$^+$ T cells (%)** |
| | 0 | – | – | – |
| | 2,090 | −18[b] | −16 | −13 |
| | 7,320 | −16 | −11 | −14 |
| | 20,900 | −21[b] | −17[b] | −25 |
| Li et al. (2011)<br>Mice, C57BL/6<br>Inhalation—vapor<br>Male (6/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[a]<br>Whole body, 6 h/d for 5 d/wk over 6 wk; generation method not reported; analytical concentration and method were reported | **Male** | | | |
| | **Dose (mg/m$^3$)** | **Number of CD3$^+$ T cells (%)** | **Number of CD4$^+$ T cells (%)** | **Number of CD8$^+$ T cells (%)** |
| | 0 | – | – | – |
| | 2,090 | −14 | −15 | −12 |
| | 7,320 | −13 | −11 | −13[b] |
| | 20,900 | −24[b] | −23[b] | −23[b] |
| Li et al. (2011)<br>Mice, C57BL/6<br>Inhalation—vapor<br>Male (5/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[a]<br>Whole body, 6 h/d for 5 d/wk over 13 wk; generation method not reported; analytical concentration and method were reported | **Male** | | | |
| | **Dose (mg/m$^3$)** | **Number of CD3$^+$ T cells (%)** | **Number of CD4$^+$ T cells (%)** | **Number of CD8$^+$ T cells (%)** |
| | 0 | – | – | – |
| | 2,090 | −9 | −11 | −8 |
| | 7,320 | −17[b] | −28[b] | −12 |
| | 20,900 | −24[b] | −37[b] | −20 |

**Table B-15. Evidence pertaining to immune effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | | | |
|---|---|---|---|---|---|---|
| *Spleen weight* | | | | | | |
| Banton et al. (2011)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>Female (10/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for 28 consecutive d | | | | Female | | |
| | | | | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) |
| | | | | 0 | – | – |
| | | | | 250 | −3 | 0 |
| | | | | 500 | −15 | −18 |
| | | | | 1,000 | −9 | 0 |
| Fujii et al. (2010); JPEC (2008d)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>P0, male (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 16 wk beginning 10 wk prior to mating<br>P0, female (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 17 wk beginning 10 wk prior to mating to LD 21 | P0, Male | | | P0, Female | | |
| | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) |
| | 0 | – | – | 0 | – | – |
| | 100 | −4 | −1 | 100 | 0 | −2 |
| | 300 | −2 | 2 | 300 | −2 | −3 |
| | 1,000 | 0 | 8 | 1,000 | −1 | −5 |
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0 or 1,000 mg/kg-d<br>Daily for 23 wk | Male | | | | | |
| | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) | | | |
| | 0 | – | – | | | |
| | 1,000 | −5 | 0 | | | |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (50/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg-d);[a] female (50/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)[a]<br>Daily for 104 wk | Male | | | Female | | |
| | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) | Dose (mg/kg-d) | Absolute weight (%) | Relative weight (%) |
| | 0 | – | – | 0 | – | – |
| | 628 | −3 | −35 | 46 | −35 | 2 |
| | 121 | 19 | 3[b] | 171 | −1 | 28 |
| | 542 | 39 | −45 | 560 | −50[b] | 55[b] |

**Table B-15. Evidence pertaining to immune effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | | | |
|---|---|---|---|---|---|---|
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$)<br>Dynamic whole-body chamber;<br>6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | Male | | | Female | | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) |
| | 0 | – | – | 0 | – | – |
| | 627 | 0 | 0 | 627 | −9 | −3 |
| | 2,090 | 7 | 5 | 2,090 | −2 | 5 |
| | 6,270 | −1 | 1 | 6,270 | −5 | 1 |
| | 20,900 | −9 | −2 | 20,900 | 1 | 12 |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$);[c] female (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber;<br>6 h/d, 5 d/wk for 13 wk followed by a 28-d recovery period; generation method, analytical concentration and method were reported | Male | | | Female | | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) |
| | 0 | – | – | 0 | – | – |
| | 20,900 | 10 | 6 | 20,900 | 6 | 0 |
| Saito et al. (2013); JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body inhalation;<br>6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported | Male | | | Female | | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) | Dose (mg/m$^3$) | Absolute weight (%) | Relative weight (%) |
| | 0 | – | – | 0 | – | – |
| | 2,090 | 4 | 15 | 2,090 | 5 | 30 |
| | 6,270 | 32 | 43[b] | 6,270 | −39 | −31 |
| | 20,900 | 17 | 66[b] | 20,900 | −43[b] | −25 |

**Table B-15. Evidence pertaining to immune effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | | | |
|---|---|---|---|---|---|---|
| Medinsky et al. (1999); US EPA (1997)<br>Rat, F344<br>Inhalation—vapor<br>Male (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | | **Female** | | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | | **Dose (mg/m$^3$)** | **Absolute weight (%)** | |
| | 0 | – | | 0 | – | |
| | 2,090 | 6 | | 2,090 | −3 | |
| | 7,320 | 3 | | 7,320 | 3 | |
| | 20,900 | 5 | | 20,900 | 0 | |
| Medinsky et al. (1999); Bond et al. (1996)<br>Mice, CD-1<br>Inhalation—vapor<br>Male (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | **Male** | | | **Female** | | |
| | **Dose (mg/m$^3$)** | **Absolute weight (%)** | | **Dose (mg/m$^3$)** | **Absolute weight (%)** | |
| | 0 | – | | 0 | – | |
| | 2,090 | −5 | | 2,090 | −11 | |
| | 7,320 | 0 | | 7,320 | −2 | |
| | 20,900 | −15 | | 20,900 | −11 | |

− = for controls, no response relevant; for other doses, no quantitative response reported; LD = lactation day; *n* = number evaluated from group; NR = not reported.

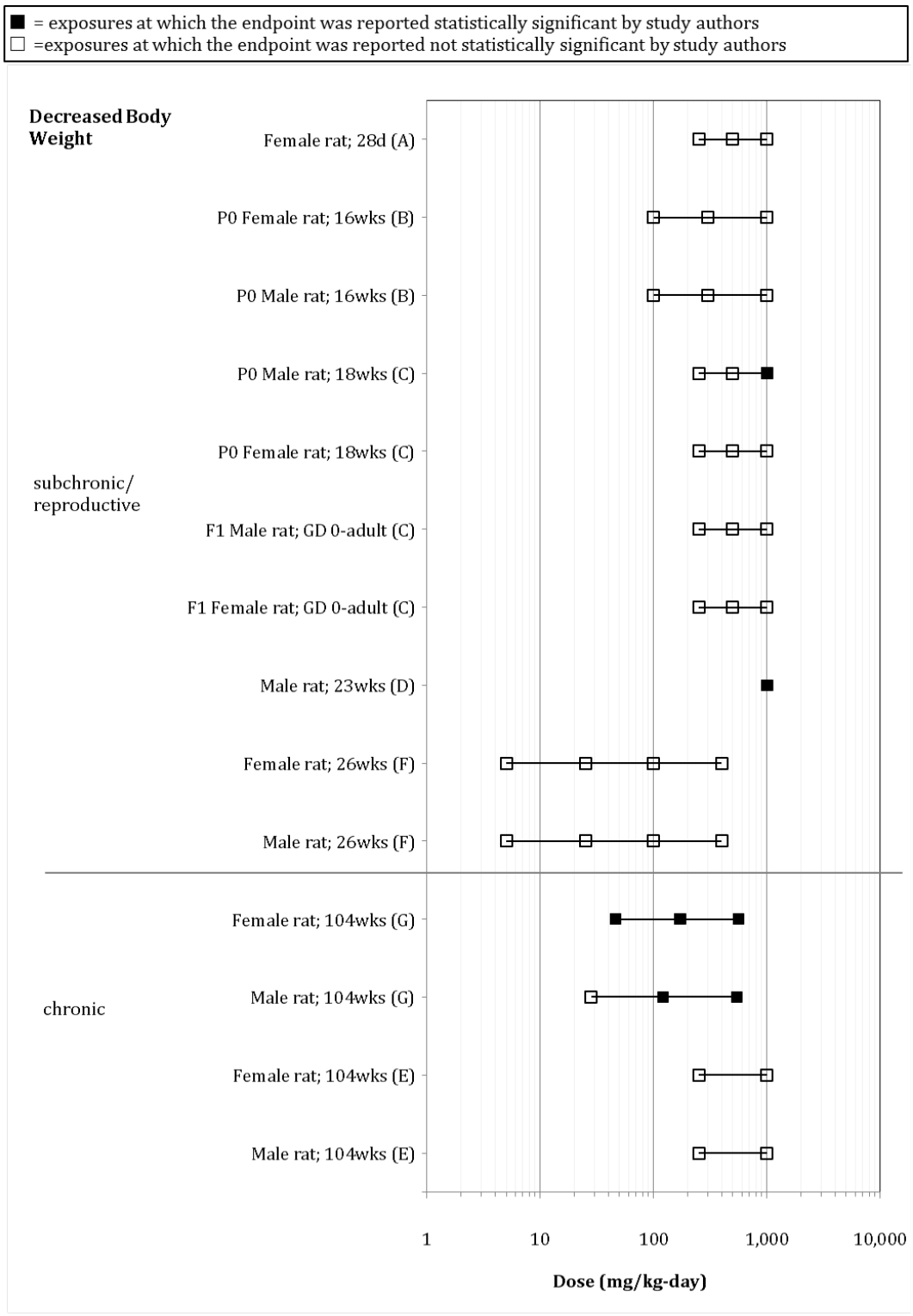
[a]Conversion performed by the study authors.
[b]Result is statistically significant ($p < 0.05$) based on analysis of data by the study authors.
[c]4.18 mg/m$^3$ = 1 ppm.

**Table B-16. Evidence pertaining to mortality in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Maltoni et al. (1999)<br>Rat, Sprague-Dawley<br>Oral—gavage<br>Male (60/group): 0, 250, or 1,000 mg/kg-d; female (60/group): 0, 250, or 1,000 mg/kg-d<br>4 d/wk for 104 wk; observed until natural death | **Male** | | **Female** | |
| | **Dose (mg/m³)** | **Survival at 104 wk (%)** | **Dose (mg/m³)** | **Survival at 104 wk (%)** |
| | 0 | – | 0 | – |
| | 250 | −8 | 250 | −8 |
| | 1,000 | −54 | 1,000 | 18 |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (50/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg-d);[a] female (50/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)[a]<br>Daily for 104 wk | **Male** | | **Female** | |
| | **Dose (mg/kg-d)** | **Percentage survival (%)** | **Dose (mg/kg-d)** | **Percentage survival (%)** |
| | 0 | – | 0 | – |
| | 628 | −3 | 46 | 3 |
| | 121 | −11 | 171 | 6 |
| | 542 | −11 | 560 | 6 |
| Saito et al. (2013);JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m³);[b] female (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m³)[b]<br>Dynamic whole-body inhalation; 6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported | **Male** | | **Female** | |
| | **Dose (mg/m³)** | **Survival at 104 wk (%)** | **Dose (mg/m³)** | **Survival at 104 wk (%)** |
| | 0 | – | 0 | – |
| | 2,090 | −14 | 2,090 | 3 |
| | 6,270 | −9 | 6,270 | −21[c] |
| | 20,900 | −32[c] | 20,900 | −21[c] |

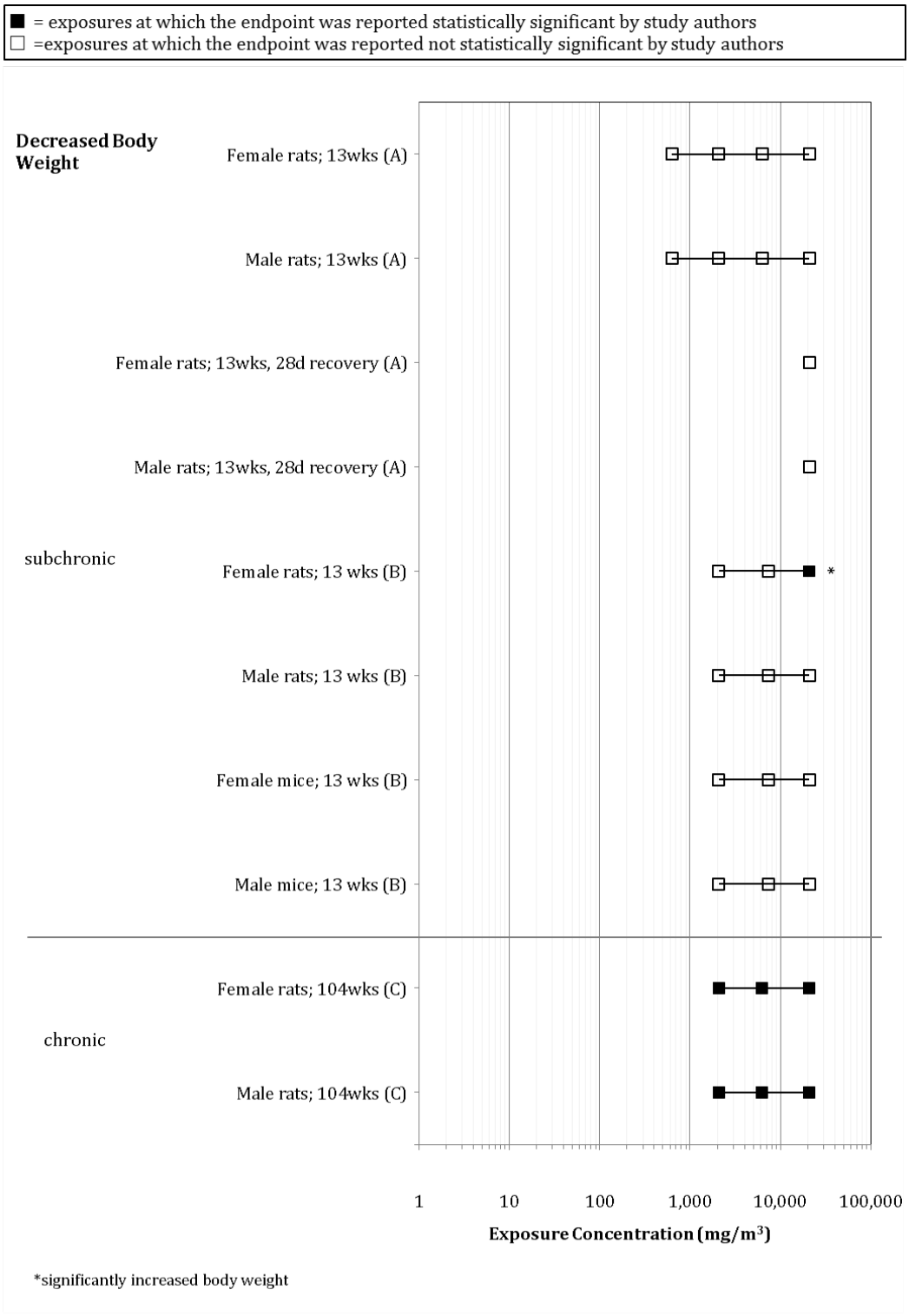
− = for controls, no response relevant; for other doses, no quantitative response reported; *n* = number evaluated from group.

[a]Conversion performed by the study authors.
[b]4.18 mg/m³ = 1 ppm.
[c]Result is statistically significant ($p < 0.05$) based on analysis of data by the study authors.




Sources: (A) Banton et al., 2011 (B) Fujii et al., 2010; JPEC, 2008e (C) Gaoua, 2004b (D) Hagiwara et al., 2011 (E) Maltoni et al., 1999 (F) Miyata et al., 2013; JPEC, 2008c (G) Suzuki et al., 2012; JPEC, 2010a

**Figure B-14. Exposure-response array of body-weight effects following oral exposure to ETBE.**




Sources: (A) JPEC, 2008b (B) Medinsky et al., 1999; Bond et al., 1996 (C) Saito et al., 2013; JPEC, 2010b

**Figure B-15. Exposure-response array of body-weight effects following inhalation exposure to ETBE.**

Mortality

Mortality was significantly increased in male and female rats following a 2-year ETBE inhalation exposure (Saito et al., 2013; JPEC, 2010b) but not significantly affected following a 2-year drinking water exposure (Suzuki et al., 2012; JPEC, 2010a). Increased mortality in male rats correlated with increased CPN severity in the kidney. The study authors attributed increased mortality in females to pituitary tumors; however, pituitary tumors were not dose responsively increased by ETBE exposure. Survival was also reduced in a lifetime gavage study at the highest exposure in males and females after 72 weeks (data not shown), and after 104 weeks, survival was reduced 54% in males at the highest dose (Maltoni et al., 1999). After 104 weeks, however, survival in the controls was approximately 25% in males and 28% in females, percentages that are much lower than expected for a 2-year study (Maltoni et al., 1999). The survival data in this study was likely confounded by chronic respiratory infections, which could have contributed to the reduced survival (Malarkey and Bucher, 2011). These data do not suggest that mortality was increased in these studies due to excessively high exposure concentrations of ETBE; thus, the mortality data are inadequate to draw conclusions as a human hazard of ETBE exposure.

***Mechanistic Evidence***

No relevant mechanistic data are available for these endpoints.

***Summary of Other Toxicity Data***

EPA concluded that the evidence is insufficient to support body-weight changes, adrenal and immunological effects, and mortality as potential human hazards of ETBE exposure based on confounding factors, lack of progression, and study quality concerns.

### B.2.2. Genotoxicity Studies

***Bacterial Systems***

The mutagenic potential of ETBE has been tested by Zeiger et al. (1992) using different *Salmonella typhimurium* strains for 311 chemicals, including ETBE, both in the absence and presence of metabolic activation (S9). Preincubation protocol was followed and precaution was exercised to account for the volatility of the compound. Five doses ranging from 100 to 10,000 µg/plate were tested using different *Salmonella* strains, including TA97, TA98, TA100, and TA1535. The results showed that the ETBE did not cause mutations in any of the *Salmonella* strains tested. It should be noted that TA102, a sensitive strain for oxidative metabolite, was not used in this study. The available genotoxicity data *for* ETBE are discussed below, and the summary of the data is provided in Table B-17.

**Table B-17. Summary of genotoxicity (both in vitro and in vivo) studies of ETBE**

| Species | Test system | Dose/ concentration | Results[a] | | Comments | Reference |
|---|---|---|---|---|---|---|
| | | | –S9 | +S9 | | |
| *Bacterial systems* | | | | | | |
| *Salmonella typhimurium* (TA97, TA98, TA100, TA1535) | Mutation assay | 100, 333, 1,000, 3,333, 10,000 µg/plate | – | – | Preincubation procedure was followed. Experiment was conducted in capped tubes to control for volatility | Zeiger et al. (1992) |
| *In vitro systems* | | | | | | |
| Chinese hamster ovary cells (*hgprt* locus) | Gene mutation assay | 100, 300, 1,000, 3,000, 5,000 µg/mL | – | – | Experiments conducted both with and without metabolic activation | Vergnes and Kubena (1995b) (unpublished report) |
| Chinese hamster ovary cells | Chromosomal aberration assay | 100, 300, 1,000, 3,000, 5,000 µg/mL | – | – | Experiments conducted both with and without metabolic activation | Vergnes (1995) (unpublished report) |
| *In vivo animal studies* | | | | | | |
| CD-1 mice (male and female) | Bone marrow micronucleus test | 0, 400, 2,000, 5,000 ppm (0, 1,670, 8,360, 20,900 mg/m$^3$)[b] | – | | Whole-body inhalation, 6 h/d, 5 d, 5/sex/group | Vergnes and Kubena (1995a) (unpublished report) |
| B6C3F$_1$ mice (male) | Bone marrow micronucleus test | 0, 1,300, 1,700, 2,100, 2,500 mg/kg | – | | Intraperitoneal injection 3×, 72 h; 5/group, 3 animals in dose 1,700 mg/kg dose. Surviving animals were not scored at doses of 2,100 and 2,500 mg/kg | NTP (1996a) |
| F344 rats (male) | Bone marrow micronucleus test | 0, 625, 1,250, 2,500 mg/kg | – | | Intraperitoneal injection 3×, 72 h; 5/group, 3 animals in 2,500 mg/kg dose group | NTP (1996b) |
| F344 rats (male and female) | Bone marrow micronucleus test | 0, 500, 1,000, 2,000 mg/kg-d | – | | Gavage, 24 h apart, 2 d, 5/sex/group | JPEC (2007b) (unpublished report) |

**Table B-17. Summary of genotoxicity (both in vitro and in vivo) studies of ETBE (continued)**

| Species | Test system | Dose/ concentration | Results[a] | | Comments | Reference |
|---|---|---|---|---|---|---|
| | | | −S9 | +S9 | | |
| F344 rats (male and female) | Bone marrow micronucleus test | 0, 250, 500, 1,000, 2,000 mg/kg-d | − | | Intraperitoneal injection, 24 h apart, 2 d, 5/sex/group | Noguchi et al. (2013); JPEC (2007b), unpublished report |
| F344 rats (male and female) | Bone marrow micronucleus test | 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)[c] | − | | Drinking water, 13 wk, 10/sex/group | Noguchi et al. (2013); JPEC (2007d), unpublished report |
| F344 rats (male and female) | Bone marrow micronucleus test | 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m$^3$)[b] | − | | Whole-body inhalation, 6 h/d, 5 d/wk, 13 wk. 10/sex/group | Noguchi et al. (2013); JPEC (2007d), unpublished report |
| C57BL/6 WT and Aldh2 KO mice | DNA strand breaks (alkaline comet assay); leukocytes | 0, 500, 1,750, 5,000 ppm | Male WT/KO | +[d]/+ | Whole-body inhalation, 6 h/d, 5 d/wk, 13 wk | Weng et al. (2011) |
| | | | Female WT/KO | −/+[d] | | |
| C57BL/6 WT and Aldh2 KO mice | DNA strand breaks (alkaline comet assay) | 0, 500, 1,750, 5,000 ppm | Male WT/KO | +[d]/+ | Whole-body inhalation, 6 h/d, 5 d/wk, 13 wk | Weng et al. (2012) |
| | | | Female WT/KO | −/+[d] | | |
| C57BL/6 WT and Aldh2 KO mice | Micronucleus assay; erythrocytes | 0, 500, 1,750, 5,000 ppm | Male[e] WT/KO | +[d]/+ | Whole-body inhalation, 6 h/d, 5 d/wk, 13 wk | Weng et al. (2013) |
| | | | Female[e] WT/KO | −/+ | | |
| C57BL/6 WT and Aldh2 KO mice | DNA strand breaks (alkaline comet assay); sperm | 0, 50, 200, 500 ppm | WT/HT/KO | −/+/+ | Whole-body inhalation, 6 h/d, 5 d/wk, 9 wk | Weng et al. (2014) |

**Table B-17. Summary of genotoxicity (both in vitro and in vivo) studies of ETBE (continued)**

| Species | Test system | Dose/ concentration | Results[a] | | Comments | Reference |
|---|---|---|---|---|---|---|
| | | | –S9 | +S9 | | |
| C57BL/6 WT and Aldh2 KO mice | DNA strand breaks (alkaline comet assay); sperm | 0, 500, 1,750, 5,000 ppm | WT/KO | +/+ | Whole-body inhalation, 6 h/d, 5 d/wk, 13 wk | Weng et al. (2014) |

KO = knockout; WT = wild type.

[a]+ = positive; – = negative; (+) = equivocal.
[b]4.18 mg/m$^3$ = 1 ppm.
[c]Conversions performed by study authors.
[d]Positive in highest dose tested.
[e]When the data of ETBE-induced MNRETs were normalized with corresponding control, the effect disappeared.

### *In Vitro Mammalian Studies*

The two available studies in in vitro mammalian systems were unpublished reports. Vergnes and Kubena (1995b) evaluated the mutagenicity of ETBE using the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) forward mutation assay in Chinese hamster ovary K1-BH4 cells. Duplicate cultures were treated with five concentrations of ETBE (>98% purity; containing 13 ppm AO22, an antioxidant stabilizer) ranging from 100 to 5,000 µg/mL, both in the presence and absence of S9 activation. No statistically significant or concentration-related increase in the HGPRT mutation frequencies were observed at any of the ETBE concentrations tested, either in the absence or in the presence of metabolic (S9) activation.

The same authors [Vergnes and Kubena (1995b) unpublished report] studied the clastogenic potential of ETBE in vitro using a chromosome aberration assay in Chinese hamster ovary cells. The cells were exposed from 100 to 5,000 µg/mL of ETBE in culture medium, both in the presence and absence of S9 metabolic activation system. No statistically significant or concentration-related increase in the frequency of chromosomal aberrations, in the presence or absence of the S9 metabolic activation system, was observed. Neither the effect of the antioxidant stabilizer used in ETBE nor control for volatility of the compound was described for both studies although capped glass bottles were used in the experiments.

### *In Vivo Animal Studies*

In vivo studies were conducted by the same authors that tested ETBE for in vitro genotoxicity. Vergnes and Kubena (1995a), in an unpublished report, performed an in vivo bone marrow micronucleus (MN) test in mice in response to ETBE exposure. Male and female CD-1 mice

(5/sex/group) were exposed to ETBE by inhalation at target concentrations of 0, 400, 2,000, or 5,000 ppm (0, 1,671, 8,357, or 20,894 mg/m$^3$) for 6 hours/day, for 5 days. Following treatment, polychromatic erythrocytes (PCE) from bone marrow were analyzed for micronucleus formation. The results showed that no statistically significant increases in the mean percentages of micronucleated polychromatic erythrocytes (MNPCE) were observed in mice (male or female) when exposed to ETBE.

In addition to Vergnes and Kubena (1995a), four animal studies were conducted by JPEC in rats using different routes of exposure (oral, inhalation, intraperitoneal, or drinking water) to detect micronucleus as a result of exposure to ETBE [JPEC (2007a, 2007b, 2007c, 2007d) published as Noguchi et al. (2013)].

The first two studies (oral and intraperitoneal injection) were part of an acute (2-day) exposure. In the first study, both male and female F344 rats (5/sex/dose group) were administered ETBE (99.3% pure) via gavage in olive oil at doses of 0, 500, 1,000, or 2,000 mg/kg-day every 24 hours [JPEC (2007a), unpublished report]. The animals were sacrificed, and bone marrow smears were collected and stained 24 hours after the final administration. Following treatment, polychromatic erythrocytes from bone marrow were analyzed for MN formation. The results were expressed as the ratio of PCE to total erythrocytes. No treatment-related effects on the number of MNPCE or the ratio of PCE to total erythrocytes were found. ETBE was determined to be negative for micronuclei induction in rat bone marrow cells after acute oral exposure.

In the second study (intraperitoneal injection), male and female F344 rats (5/sex/dose group) were administered two ETBE intraperitoneal injections separated by 24 hours at doses of 0, 250, 500, 1,000, or 2,000 mg/kg-day in olive oil (Noguchi et al., 2013; JPEC, 2007b). The animals were sacrificed, and bone marrow smears were collected and stained 24 hours after the final injection. All animals in the 2,000 mg/kg-day group died on the first day of treatment. There were no treatment-related effects on either the number of MNPCEs or the ratio of polychromatic erythrocytes to total erythrocytes. In addition, no dose-dependent tendencies for the increase in the MNPCE to PCE ratio or alterations in the ratios of PCE to total erythrocytes were noted in either sex of the treated groups. ETBE was determined to be negative for micronuclei induction in rats after acute intraperitoneal exposure.

The next two studies (drinking water and inhalation) were part of 13-week toxicity studies in rats in which the effects of ETBE on the micronuclei in PCE were examined at the end of the study. In the first 13-week study, male and female F344 rats (10/sex/dose group) were given drinking water containing 0, 1,600, 4,000, or 10,000 ppm ETBE for 13 weeks (Noguchi et al., 2013; JPEC, 2007c). The concentrations were stated to be equivalent to 0, 101, 259, and 626 mg/kg-day in males and 0, 120, 267, and 629 mg/kg-day in females. Following treatment, polychromatic erythrocytes from bone marrow were analyzed for MN formation. The results were expressed as

the ratio of PCE to total erythrocytes. There were no treatment-related effects on the number of MNPCEs or the ratio of PCE to total erythrocytes.

In the second 13-week study (inhalation), male and female F344 rats (10/sex/dose group) were exposed to ETBE (99.2–99.3% pure) through whole-body inhalation exposure at 0, 500, 1,500, or 5,000 ppm (0, 2,089, 6,268, or 20,894 mg/m$^3$) for 6 hours/day, 5 days/week (Noguchi et al., 2013; JPEC, 2007b). Normochromatic and polychromatic erythrocytes and micronuclei were counted as in the previous study. There were no treatment-related effects on the number of MNPCE or the ratio of PCE to total erythrocytes. ETBE was determined to be negative for micronuclei induction in rat bone marrow cells after a 13-week inhalation exposure.

Furthermore, NTP (1996a, 1996b) performed an in vivo bone marrow micronucleus test in both B6C3F$_1$ mice and Fischer rats. The animals were exposed through intraperitoneal injection 3 times in a period of 72 hours ($n$ = 5). Doses for the mice were 0, 1,300, 1,700, 2,100, or 2,500 mg/kg, and the doses for rats were 0, 625, 1,250, or 2,500 mg/kg. No increase in micronucleated PCEs were observed in either mice or rats. Two of five mice died in the 1,700 mg/kg dose group, while 3 of 5 and 4 of 5 animals died in the 2,100 and 2,500 mg/kg dose groups, respectively, and the surviving animals in the two highest dose groups were not scored. In the rat study, 2 of 5 animals died in the highest dose group.

Weng et al. (2011) conducted several studies evaluating the differences in genotoxicity of ETBE in various tissues or systems (i.e., erythrocytes, leukocytes, liver, and sperm) in C57BL/6 wild type and Aldh2 knockout mice after subchronic inhalation exposure. All studies used the same exposures (i.e., 0, 500, 1,750, or 5,000 ppm ETBE for 6 hours/day, 5 days/week for 13 weeks). Deoxyribonucleic acid (DNA) strand breaks were observed in leukocytes of male (all concentrations) and female (high dose only) Aldh2 knockout mice and with the high dose in wild-type male mice (Weng et al., 2011).

Weng et al. (2012) studied the differential genotoxic effects of subchronic exposure to ETBE in the liver of C57BL/6 wild-type and Aldh2 knockout mice. DNA strand breaks in the hepatocytes of male and female with different *Aldh2* genotypes were determined using the alkaline comet assay. In addition, 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified oxidative base modification, and 8-hydroxydeoxyguanosine were determined as endpoints for genetic damage. There was significant increase in damage in all three exposure groups in the knockout male mice, although the increase was only found in the 5,000-ppm exposure group for the knockout female mice. In the wild type, significant DNA damage was seen only in males in the 5,000-ppm group, but not in females. This indicates the sensitivity of sex differences both in knockout and wild-type mice.

Another study by the same authors performed in vivo micronucleus tests (on what appear to be the same set of animals), in addition to the DNA strand breaks, 8-hydroxyguanine DNA glycosylase 1 (8-hOGG1)-modified oxidative base modification, and 8-hydroxydeoxyguanosine (Weng et al., 2013). The mice (wild type and knockout, males and females) were exposed to 0, 500, 1,750, or 5,000 ppm ETBE for 6 hours/day, 5 days/week for 13 weeks. Peripheral blood samples

were obtained and processed to detect micronucleated reticulocytes (MNRETs) and micronuclei in the mature normochromatic erythrocyte population. The results indicate that ETBE significantly affected frequencies of MNRETs in male and female mice. In knockout male mice, the frequencies of MNRETs of the 1,750- and 5,000-ppm exposure groups were significantly increased when compared with the control group. In the wild-type male mice, however, only the 5,000-ppm group had a higher frequency of MNRETs than that of the control group. In female wild-type mice, there was no difference in the frequencies of MNRETs between exposure groups and the control group. In the same exposure group (5,000 ppm), the knockout mice had a higher frequency of MNRETs than the wild type. These results inform the influence of *Aldh2* and sex difference on genotoxicity as a result of exposure to ETBE.

In yet another study by the same authors (Weng et al., 2014), DNA strand breaks and 8-hydroxyguanine DNA glycosylase 1 (8-hOGG1)-modified oxidative base modification were measured in sperm collected from the left cauda epididymis. In addition to the 13-week protocol used in the other studies, Weng et al. (2014) included a 9-week study in which the male mice (wild type, knockout, and heterogeneous [HT]) were exposed to 0, 50, 200, or 500 ppm ETBE for 6 hours/day, 5 days/week. In the 13-week study, there were significant increases in damage in all three exposure groups in the knockout male mice, but only in the two highest dose groups in the wild-type males. In the 9-week study, there was no change in the wild-type mice, but both the heterogeneous and the knockout mice had significant increases in the two highest doses.

***Summary***

Limited studies have been conducted to understand the genotoxic potential of ETBE. Most studies indicate that ETBE does not induce genotoxicity in the systems tested. More recently, Weng and coauthors illustrated the influence of *Aldh2* on the genotoxic effects of ETBE. With respect to overall existing database, it should be noted that the array of genotoxic tests conducted are limited. The inadequacy of the database is two dimensional: (1) the coverage of the studies across the genotoxicity tests needed for proper interpretation of the weight of evidence of the data is sparse and (2) the quality of the available data is questionable. With respect to the array of types of genotoxicity tests available, ETBE has only been tested in one bacterial assay. Only two in vitro studies are available. The existing in vivo studies have tested only for the micronucleus assay, DNA strand breaks, or both. Key studies on chromosomal aberrations and DNA adducts are missing. Additionally, the few existing studies are unpublished reports lacking peer review. Given the above limitations (i.e., the significant deficiencies and sparse database both in terms of quality and quantity), the database is insufficient to draw a definitive conclusion on the genotoxic effects of ETBE.

# B.3. SUPPLEMENTAL ORGAN-WEIGHT DATA

## B.3.1. Relative Kidney-Weight Data

**Table B-18. Evidence pertaining to relative kidney-weight effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Fujii et al. (2010); JPEC (2008d)<br>Rat, S-D<br>Oral—gavage<br>P0, male (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 16 wk beginning 10 wk prior to mating<br>P0, female (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 17 wk beginning 10 wk prior to mating to LD 21 | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Relative weight (%)** | **Dose (mg/kg-d)** | **Relative weight (%)** |
| | 0 | – | 0 | – |
| | 100 | 8[a] | 100 | −3 |
| | 300 | 12[a] | 300 | −1 |
| | 1,000 | 26[a] | 1,000 | 2 |
| Gaoua (2004b)<br>Rat, S-D<br>Oral—gavage<br>P0, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups<br>P0, female (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until PND 21<br>F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Relative weight (%)** | **Dose (mg/kg-d)** | **Relative weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 11[a] | 250 | 9 |
| | 500 | 18[a] | 500 | 5 |
| | 1,000 | 28[a] | 1,000 | 3 |
| | **F1, Male** | | **F1, Female** | |
| | **Dose (mg/kg-d)** | **Relative weight (%)** | **Dose (mg/kg-d)** | **Relative weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 10[a] | 250 | 6 |
| | 500 | 19[a] | 500 | 6 |
| | 1,000 | 58[a] | 1,000 | 10[a] |
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0 or 1,000 mg/kg-d<br>Daily for 23 wk | **Male** | | | |
| | **Dose (mg/kg-d)** | **Relative weight (%)** | | |
| | 0 | – | | |
| | 1,000 | 25[a] | | |

**Table B-18. Evidence pertaining to relative kidney-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Miyata et al. (2013);JPEC (2008b)<br>Rat, Crl:CD(SD)<br>Oral—gavage<br>Male (15/group): 0, 5, 25, 100, or 400 mg/kg-d; female (15/group): 0, 5, 25, 100, or 400 mg/kg-d<br>Daily for 26 wk | Male | | Female | |
| | Dose (mg/kg-d) | Relative weight (%) | Dose (mg/kg-d) | Relative weight (%) |
| | 0 | – | 0 | – |
| | 5 | 8 | 5 | 7 |
| | 25 | 6 | 25 | 4 |
| | 100 | 12[a] | 100 | 11[a] |
| | 400 | 21[a] | 400 | 15[a] |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (50/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg-d);[b] female (50/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)[b]<br>Daily for 104 wk | Male | | Female | |
| | Dose (mg/kg-d) | Relative weight (%) | Dose (mg/kg-d) | Relative weight (%) |
| | 0 | – | 0 | – |
| | 28 | 0 | 46 | 13[a] |
| | 121 | 12[a] | 171 | 22[a] |
| | 542 | 31[a] | 560 | 37[a] |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$)<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Relative weight (%) | Dose (mg/m$^3$) | Relative weight (%) |
| | 0 | – | 0 | – |
| | 627 | 10 | 627 | 8 |
| | 2,090 | 9 | 2,090 | 7 |
| | 6,270 | 20[a] | 6,270 | 12[a] |
| | 20,900 | 24[a] | 20,900 | 20[a] |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$);[c] female (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk followed by a 28 d recovery period; generation method, analytical concentration and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Relative weight (%) | Dose (mg/m$^3$) | Relative weight (%) |
| | 0 | – | 0 | – |
| | 20,900 | 15[a] | 20,900 | 5 |

**Table B-18. Evidence pertaining to relative kidney-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Dose ($mg/m^3$) | Relative weight (%) | Dose ($mg/m^3$) | Relative weight (%) |
| Saito et al. (2013); JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 $mg/m^3$);[c] female (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 $mg/m^3$)[c]<br>Dynamic whole-body inhalation; 6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported | 0 | – | 0 | – |
| | 2,090 | 19[a] | 2,090 | 11[a] |
| | 6,270 | 26[a] | 6,270 | 16[a] |
| | 20,900 | 66[a] | 20,900 | 51[a] |

– = for controls, no response relevant; for other doses, no quantitative response reported; LD = lactation day; NR = not reported; PND = postnatal day.

[a]Result is statistically significant ($p < 0.05$) based on analysis of data by the study authors.
[b]Conversion performed by the study authors.
[c]4.18 $mg/m^3$ = 1 ppm.
Percentage change compared to controls calculated as 100 × [(treated value − control value) ÷ control value].

## B.3.2. Absolute Liver-Weight Data

**Table B-19. Evidence pertaining to absolute liver-weight effects in animals exposed to ETBE**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Fujii et al. (2010); JPEC (2008d)<br>Rat, S-D<br>Oral—gavage<br>P0, male (24/group): 0, 100, 300, or 1,000 mg/kg-d daily for 16 wk beginning 10 wk before mating<br>P0, female (24/group): 0, 100, 300, or 1,000 mg/kg-d<br>Daily for 17 wk beginning 10 wk before mating to LD 21 | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 100 | −3 | 100 | −1 |
| | 300 | −1 | 300 | 3 |
| | 1,000 | 13[a] | 1,000 | 14[a] |
| Gaoua (2004b)<br>Rat, S-D<br>Oral—gavage<br>P0, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups<br>P0, female (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>Daily for a total of 18 wk beginning 10 wk before mating until PND 21<br>F1, male (25/group): 0, 250, 500, or 1,000 mg/kg-d<br>P0 dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups<br>F1, female (24–25/group): 0, 250, 500, or 1,000 mg/kg-d<br>P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups | **P0, Male** | | **P0, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 2 | 250 | −1 |
| | 500 | 2 | 500 | 4 |
| | 1,000 | 17[a] | 1,000 | 6 |
| | **F1, Male** | | **F1, Female** | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | **Dose (mg/kg-d)** | **Absolute weight (%)** |
| | 0 | – | 0 | – |
| | 250 | 0 | 250 | 1 |
| | 500 | 14[a] | 500 | 3 |
| | 1,000 | 27[a] | 1,000 | 10[a] |
| Hagiwara et al. (2011); JPEC (2008c)<br>Rat, F344<br>Oral—gavage<br>Male (12/group): 0 or 1,000 mg/kg-d<br>Daily for 23 wk | **Male** | | | |
| | **Dose (mg/kg-d)** | **Absolute weight (%)** | | |
| | 0 | – | | |
| | 1,000 | 21[a] | | |

**Table B-19. Evidence pertaining to absolute liver-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Miyata et al. (2013); JPEC (2008b)<br>Rat, Crl:CD(SD)<br>Oral—gavage<br>Male (15/group): 0, 5, 25, 100, or 400 mg/kg-d; female (15/group): 0, 5, 25, 100, or 400 mg/kg-d<br>Daily for 26 wk | Male | | Female | |
| | Dose (mg/kg-d) | Absolute weight (%) | Dose (mg/kg-d) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 5 | −2 | 5 | −4 |
| | 25 | 7 | 25 | −1 |
| | 100 | 4 | 100 | 2 |
| | 400 | 19 | 400 | 9 |
| Suzuki et al. (2012); JPEC (2010a)<br>Rat, F344<br>Oral—water<br>Male (50/group): 0, 625, 2,500, or 10,000 ppm (0, 28, 121, or 542 mg/kg-d);[b] female (50/group): 0, 625, 2,500, or 10,000 ppm (0, 46, 171, or 560 mg/kg-d)[b]<br>Daily for 104 wk | Male | | Female | |
| | Dose (mg/kg-d) | Absolute weight (%) | Dose (mg/kg-d) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 28 | −11[a] | 46 | −5 |
| | 121 | −4 | 171 | −2 |
| | 542 | 2 | 560 | −10 |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (NR): 0, 150, 500, 1,500, or 5,000 ppm (0, 627, 2,090, 6,270, or 20,900 mg/m$^3$)<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 627 | 5 | 627 | −3 |
| | 2,090 | 6 | 2,090 | −8 |
| | 6,270 | 4 | 6,270 | −2 |
| | 20,900 | 2 | 20,900 | 5 |
| JPEC (2008a)<br>Rat, Crl:CD(SD)<br>Inhalation—vapor<br>Male (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$);[c] female (6/group): 0 or 5,000 ppm (0 or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk followed by a 28 d recovery period; generation method, analytical concentration, and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 20,900 | 13 | 20,900 | 11 |

**Table B-19. Evidence pertaining to absolute liver-weight effects in animals exposed to ETBE (continued)**

| Reference and study design | Results (percentage change compared to control) | | | |
|---|---|---|---|---|
| Saito et al. (2013); JPEC (2010b)<br>Rat, F344<br>Inhalation—vapor<br>Male (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m$^3$);[c] female (50/group): 0, 500, 1,500, or 5,000 ppm (0, 2,090, 6,270, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body inhalation; 6 h/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 1 | 2,090 | −3 |
| | 6,270 | 11[a] | 6,270 | −8 |
| | 20,900 | 10 | 20,900 | 1 |
| Medinsky et al. (1999); US EPA (1997)<br>Rat, F344<br>Inhalation—vapor<br>Male (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (48/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 6 | 2,090 | 2 |
| | 7,320 | 14[a] | 7,320 | 9 |
| | 20,900 | 32[a] | 20,900 | 26[a] |
| Medinsky et al. (1999); Bond et al. (1996)<br>Mice, CD-1<br>Inhalation—vapor<br>Male (40/group): 0, 500, 1,750, or 5,000 ppm (0, 2,090, 7,320, or 20,900 mg/m$^3$);[c] female (40/group): 0, 500, 1,750, or 5,000 ppm(0, 2,090, 7,320, or 20,900 mg/m$^3$)[c]<br>Dynamic whole-body chamber; 6 h/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method were reported | Male | | Female | |
| | Dose (mg/m$^3$) | Absolute weight (%) | Dose (mg/m$^3$) | Absolute weight (%) |
| | 0 | – | 0 | – |
| | 2,090 | 4 | 2,090 | 2 |
| | 7,320 | 13[a] | 7,320 | 19[a] |
| | 20,900 | 18[a] | 20,900 | 33[a] |

– = for controls, no response relevant; for other doses, no quantitative response reported; NR = not reported; PND = postnatal day.

[a]Result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
[b]Conversion performed by study authors.
[c]4.18 mg/m$^3$ = 1 ppm.
Percentage change compared to controls calculated as 100 × [(treated value − control value) ÷ control value].

# APPENDIX C. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

## C.1. BENCHMARK DOSE MODELING SUMMARY

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints were modeled using EPA's Benchmark Dose Software (BMDS, version 2.2). Section C.1.1 (noncancer) and Section C.1.2 (cancer) describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012). In some cases, it might be appropriate to use alternative methods based on statistical judgment; exceptions are noted as necessary in the summary of the modeling results.

### C.1.1. Noncancer Endpoints

#### *Evaluation of Model Fit*

For each dichotomous endpoint, BMDS dichotomous models[1] were fitted to the data using the maximum likelihood method. Each model was tested for goodness of fit using a chi-square goodness-of-fit test ($\chi^2$ $p$-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

For each continuous endpoint, BMDS continuous models[2] were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, the homogeneity of the variances was tested first using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected ($\chi^2$ $p$-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected ($\chi^2$ $p$-value < 0.10), the variance was modeled as a power function

[1]Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: for the Log-Logistic model, restrict slope ≥1; for the Gamma and Weibull models, restrict power ≥1.
[2]Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: for the Polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, Power, and Exponential models, restrict power ≥1.

of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with $\chi^2$ *p*-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

***Model Selection***

For each endpoint, the lower confidence limit of the benchmark dose or concentration (BMDL/BMCL), as estimated by the profile likelihood method and Akaike's information criterion (AIC) value, were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL/BMCL estimates were "sufficiently close," that is, differed by at most threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL/BMCL estimates were not sufficiently close, the lowest BMDLBMCL was selected as the POD.

**Table C-1. Noncancer endpoints selected for dose-response modeling for ETBE**

| Endpoint, study | Sex, strain, species | Doses/concentrations and effect data | | | | | |
|---|---|---|---|---|---|---|---|
| *Oral* | | | | | | | |
| Urothelial hyperplasia of the renal pelvis Suzuki et al. (2012); JPEC (2010a) | Male F344 rats | Dose (mg/kg-d) | 0 | 28 | 121 | 542 | |
| | | Incidence/total | 0/50 | 0/50 | 10/50 | 25/50 | |
| Increased absolute kidney weight Suzuki et al. (2012); JPEC (2010a) | Female F344 rats | Dose (mg/kg-d) | 0 | 46 | 171 | 560 | |
| | | No. of animals | 36 | 37 | 38 | 38 | |
| | | Mean ± SD | 1.81 ± 0.12 | 1.863 ± 0.14 | 1.988 ± 0.19 | 2.057 ± 0.26 | |
| Increased absolute kidney weight Miyata et al. (2013); JPEC (2008b) | Male Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 5 | 25 | 100 | 400 |
| | | No. of animals | 15 | 15 | 14 | 15 | 13 |
| | | Mean ± SD | 3.27 ± 0.34 | 3.29 ± 0.3 | 3.47 ± 0.32 | 3.42 ± 0.48 | 4.09 ± 0.86 |
| Increased absolute kidney weight Miyata et al. (2013); JPEC (2008b) | Female Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 5 | 25 | 100 | 400 |
| | | No. of animals | 15 | 15 | 15 | 15 | 15 |
| | | Mean ± SD | 1.88 ± 0.2 | 1.89 ± 0.16 | 1.88 ± 0.15 | 2.02 ± 0.21 | 2.07 ± 0.23 |
| Increased absolute kidney weight Gaoua (2004b) | P0 male Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 250 | 500 | 1,000 | |
| | | No. of animals | 25 | 25 | 25 | 25 | |
| | | Mean ± SD | 3.58 ± 0.413 | 3.96 ± 0.446 | 4.12 ± 0.624 | 4.34 ± 0.434 | |
| Increased absolute kidney weight Gaoua (2004b) | P0 female Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 250 | 500 | 1,000 | |
| | | No. of animals | 25 | 24 | 22 | 25 | |
| | | Mean ± SD | 2.24 ± 0.185 | 2.22 ± 0.16 | 2.29 ± 0.207 | 2.35 ± 0.224 | |

**Table C-1. Noncancer endpoints selected for dose-response modeling for ETBE(continued)**

| Endpoint, study | Sex, strain, species | Doses/concentrations and effect data | | | | | |
|---|---|---|---|---|---|---|---|
| Increased absolute kidney weight Gaoua (2004b) | F1 male Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 250 | 500 | 1,000 | |
| | | No. of animals | 24 | 25 | 24 | 25 | |
| | | Mean ± SD | 3.38 ± 0.341 | 3.73 ± 0.449 | 4.13 ± 0.64 | 5.34 ± 5.39 | |
| Increased absolute kidney weight Gaoua (2004b) | F1 female Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 250 | 500 | 1,000 | |
| | | No. of animals | 25 | 24 | 25 | 23 | |
| | | Mean ± SD | 2.24 ± 0.178 | 2.34 ± 0.242 | 2.3 ± 0.226 | 2.49 ± 0.284 | |
| Increased absolute kidney weight Fujii et al. (2010); JPEC (2008d) | Male Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 100 | 300 | 1,000 | |
| | | No. of animals | 24 | 24 | 24 | 24 | |
| | | Mean ± SD | 3.46 ± 0.57 | 3.62 ± 0.45 | 3.72 ± 0.35 | 4.07 ± 0.53 | |
| Increased relative kidney weight Fujii et al. (2010); JPEC (2008d) | Male Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 100 | 300 | 1,000 | |
| | | No. of animals | 24 | 24 | 24 | 24 | |
| | | Mean ± SD | 0.546 ± 0.059 | 0.592 ± 0.06 | 0.609 ± 0.042 | 0.689 ± 0.049 | |
| Increased absolute kidney weight Fujii et al. (2010); JPEC (2008d) | Female Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 100 | 300 | 1,000 | |
| | | No. of animals | 21 | 22 | 23 | 19 | |
| | | Mean ± SD | 2.17 ± 0.18 | 2.13 ± 0.14 | 2.17 ± 0.17 | 2.33 ± 0.24 | |
| Increased relative kidney weight Fujii et al. (2010); JPEC (2008d) | Female Sprague-Dawley rats | Dose (mg/kg-d) | 0 | 100 | 300 | 1,000 | |
| | | No. of animals | 24 | 24 | 24 | 24 | |
| | | Mean ± SD | 0.674 ± 0.053 | 0.656 ± 0.048 | 0.668 ± 0.057 | 0.687 ± 0.045 | |

**Table C-1. Noncancer endpoints selected for dose-response modeling for ETBE(continued)**

| Endpoint, study | Sex, strain, species | Doses/concentrations and effect data | | | | | |
|---|---|---|---|---|---|---|---|
| *Inhalation* | | | | | | | |
| Urothelial hyperplasia of the renal pelvis Saito et al. (2013); JPEC (2010b) | Male F344 rats | Exposure concentration ($mg/m^3$) | 0 | 2,090 | 6,270 | 20,900 | |
| | | Incidence/total | 2/50 | 5/50 | 16/49 | 41/50 | |
| Increased absolute kidney weight Saito et al. (2013); JPEC (2010b) | Female Sprague-Dawley rats | Exposure concentration (ppm) | 0 | 2,090 | 6,270 | 20,900 | |
| | | No. of animals | 37 | 39 | 29 | 30 | |
| | | Mean ± SD | 1.81 ± 0.18 | 1.90 ± 0.20 | 1.92 ± 0.13 | 2.13 ± 0.28 | |
| Increased absolute kidney weight JPEC (2008a) | Male Sprague-Dawley rats | Exposure concentration (ppm) | 0 | 150 | 500 | 1,500 | 5,000 |
| | | No. of animals | 10 | 10 | 10 | 10 | 10 |
| | | Mean ± SD | 3.15 ± 0.243 | 3.45 ± 0.385 | 3.49 ± 0.314 | 3.72 ± 0.365 | 3.64 ± 0.353 |
| Increased absolute kidney weight JPEC (2008a) | Female Sprague-Dawley rats | Exposure concentration (ppm) | 0 | 150 | 500 | 1,500 | 5,000 |
| | | No. of animals | 10 | 10 | 10 | 10 | 10 |
| | | Mean ± SD | 1.84 ± 0.129 | 1.85 ± 0.18 | 1.83 ± 0.118 | 1.92 ± 0.173 | 1.97 ± 0.16 |
| Increased absolute kidney weight Medinsky et al. (1999); US EPA (1997) | Male F344 rats | Exposure concentration (ppm) | 0 | 500 | 1,750 | 5,000 | |
| | | No. of animals | 11 | 11 | 11 | 11 | |
| | | Mean ± SD | 1.73 ± 0.155 | 1.85 ± 0.137 | 1.903 ± 0.1 | 2.067 ± 0.124 | |
| Increased absolute kidney weight | Female F344 rats | Exposure concentration (ppm) | 0 | 500 | 1,750 | 5,000 | |
| | | No. of animals | 10 | 11 | 11 | 11 | |

**Table C-1. Noncancer endpoints selected for dose-response modeling for ETBE(continued)**

| Endpoint, study | Sex, strain, species | Doses/concentrations and effect data | | | | | |
|---|---|---|---|---|---|---|---|
| Medinsky et al. (1999); US EPA (1997) | | Mean ± SD | 1.077 ± 0.069 | 1.125 ± 0.048 | 1.208 ± 0.076 | 1.306 ± 0.055 | |

No. = number; SD = standard deviation.

### *Modeling Results*

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

Oral exposure endpoints

**Table C-2. Summary of benchmark dose (BMD) modeling results for urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE in drinking water for 104 weeks (JPEC, 2010a) modeled with doses as mg/kg-day (calculated by the study authors); benchmark response (BMR) = 10% extra risk**

| Model[a] | Goodness of fit | | $BMD_{10}$ (mg/kg-d) | $BMDL_{10}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Gamma | 0.196 | 127.93 | 88.1 | 60.9 | Of the models that provided an adequate fit and a valid BMDL estimate, the Quantal-Linear model was selected based on lowest AIC. |
| Logistic | $1.00 \times 10^{-3}$ | 139.54 | 217 | 177 | |
| Log-Logistic | 0.264 | 127.28 | 85.3 | 49.5 | |
| Probit | 0.0015 | 138.30 | 197 | 162 | |
| Log-Probit | 0.374 | 126.14 | 85.8 | 51.3 | |
| Weibull | 0.202 | 128.00 | 85.7 | 60.7 | |
| Multistage (3 degree)[b] Multistage (2 degree)[c] | 0.395 | 126.07 | 79.3 | 60.5 | |
| **Quantal-Linear[c]** | **0.395** | **126.07** | **79.3** | **60.5** | |

[a]Selected model in bold; scaled residuals for selected model for doses 0, 28, 121, and 542 mg/kg-d were 0.000, −1.377, 1.024, and −0.187, respectively.

[b]For the Multistage (3 degree) model, the beta coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Multistage (2 degree) model.

[c]The Multistage (2 degree) model and Quantal-Linear models appear equivalent; however, differences exist in digits not displayed in the table.

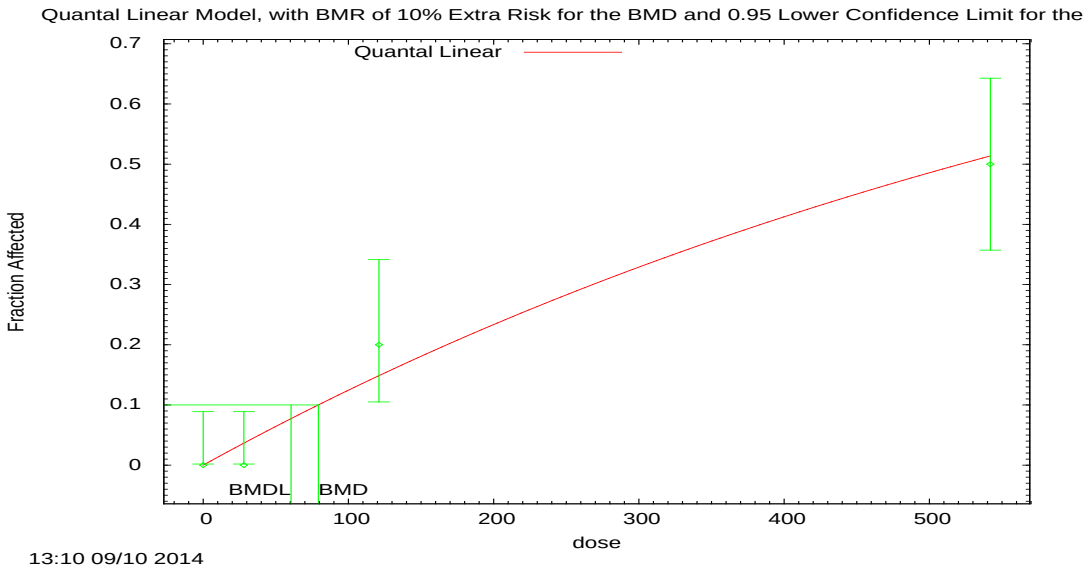



**Figure C-1. Plot of incidence rate by dose, with fitted curve for the Quantal Linear Model; dose shown in mg/kg-day.**

**Quantal Linear Model** using Weibull Model (Version: 2.16; Date: 2/28/2013)

The form of the probability function is:

P[response] = background + (1 − background) × [1 − exp(− slope × dose)]

**Benchmark Dose Computation.**

BMR = 10% extra risk

Benchmark dose (BMD) = 79.3147

BMDL at the 95% confidence level = 60.5163

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| Background | 0 | 0.0192308 |
| Slope | 0.00132839 | 0.00124304 |
| Power | N/A | 1 |

**Analysis of Deviance Table**

| Model | Log(likelihood) | No. parameters | Deviance | Test df | *p*-value |
|---|---|---|---|---|---|
| Full model | −59.6775 | 4 | | | |
| Fitted model | −62.0369 | 1 | 4.71891 | 3 | 0.1936 |
| Reduced model | −92.7453 | 1 | 66.1356 | 3 | <0.0001 |

AIC = 126.074

**Goodness-of-Fit Table**

| Dose | Estimated probability | Expected | Observed | Size | Scaled residual |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 50 | 0 |
| 28 | 0.0365 | 1.826 | 0 | 50 | −1.377 |
| 121 | 0.1485 | 7.424 | 10 | 50 | 1.024 |
| 542 | 0.5132 | 25.662 | 25 | 50 | −0.187 |

$\chi^2$ = 2.98; degrees of freedom (df) = 3; *p*-value = 0.3948

**Table C-3. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in female F344 rats exposed to ETBE in drinking water for 104 weeks (IPEC, 2010a); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.00752 | −339.97 | 385 | 290 | The Exponential (M4) model was selected as the only model with a goodness-of-fit *p*-value > 0.1. |
| **Exponential (M4)** | **0.621** | **−347.50** | **204** | **120** | |
| Exponential (M5) | N/A | −345.75 | 192 | 116 | |
| Hill | N/A | −345.75 | 195 | 107 | |
| Power[c]<br>Polynomial (3 degree)[d]<br>Polynomial (2 degree)[e]<br>Linear | 0.0115 | −340.82 | 367 | 272 | |

[a]Modeled variance case presented (BMDS Test 3 *p*-value = <0.8167), selected model in bold; scaled residuals for selected model for doses 0, 46, 171, and 560 mg/kg-d were 0.0259, −0.19, 0.474, and −0.289, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Power model, the power parameter estimate was 1 (boundary), and the model reduced to the Linear model.
[d]For the Polynomial (3 degree) model, the b3 and b2 coefficient estimates were 0 (boundary), and the model reduced to the Linear model.
[e]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary), and the model reduced to the Linear model.

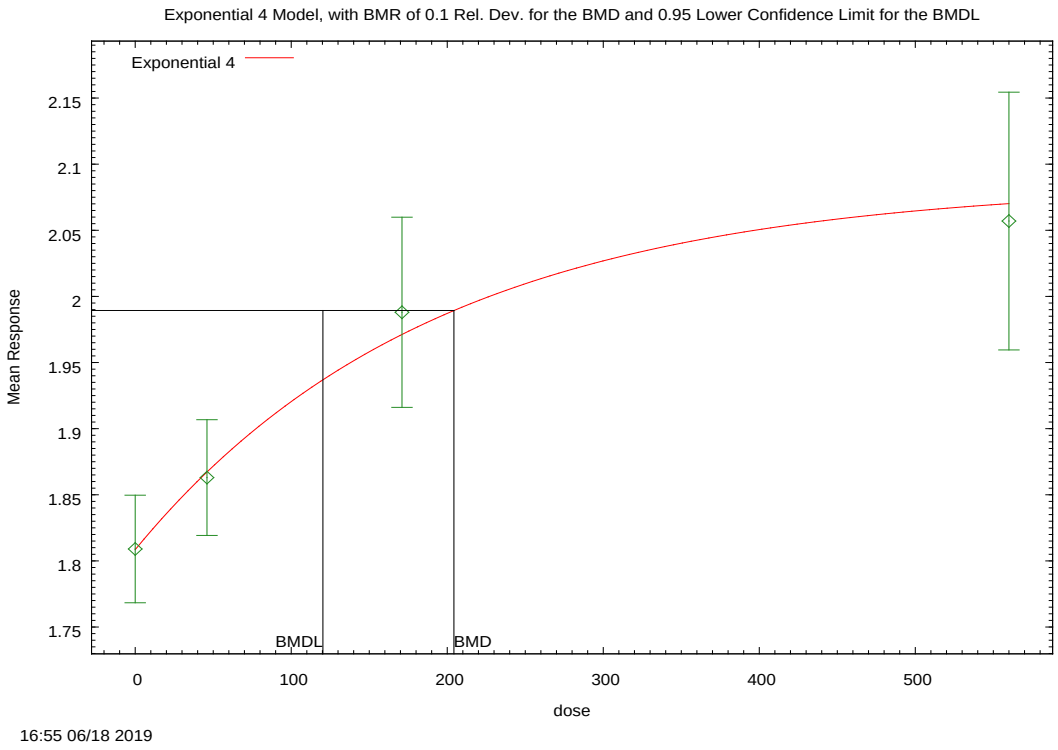



**Figure C-2. Plot of mean response by dose, with fitted curve for the Exponential 4 Model; dose shown in mg/kg-day.**

**Exponential 4 Model.** (BMDS Version 1.10; Date: 01/12/2015)
The form of the response function is: P[dose] = a × [c − (c − 1) × exp(−b × dose)]
A modeled variance is fit: Var[i] = exp(log-alpha + log[mean(i)] × rho)
Benchmark Dose Computation.
BMR = 10% relative deviation
BMD = 204 mg/kg-day
BMDL at the 95% confidence level = 120 mg/kg-day

**Parameter Estimates**

| Variable | Model 4 | Standard error |
|---|---|---|
| lnalpha | −11.0816 | 1.89029 |
| rho | 11.431 | 2.93477 |
| a | 1.80851 | 0.0173746 |
| b | 0.00518165 | 0.00207201 |
| c | 1.15314 | 0.0322089 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 37 | 1.809 | 1.809 | 0.122 | 0.116 | 0.02585 |
| 46 | 39 | 1.863 | 1.867 | 0.135 | 0.1392 | −0.1903 |
| 171 | 29 | 1.988 | 1.971 | 0.189 | 0.1898 | 0.4744 |
| 560 | 30 | 2.057 | 2.07 | 0.261 | 0.2511 | −0.2889 |

**Likelihoods of Interest**

| Model | Log(likelihood) | df | AIC |
|---|---|---|---|
| A1 | 166.6724 | 5 | −323.3449 |
| A2 | 179.0769 | 8 | −342.1539 |
| A3 | 178.8744 | 6 | −345.7488 |
| R | 148.74 | 2 | −293.4799 |
| 4 | 178.7521 | 5 | −347.5042 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 60.67 | 6 | <0.0001 |
| Test 2 | 24.81 | 3 | <0.0001 |
| Test 3 | 0.4051 | 2 | 0.8167 |
| Test 6a | 0.2446 | 1 | 0.6209 |

**Table C-4. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in male Sprague-Dawley rats exposed to ETBE by daily gavage for 26 weeks (Miyata et al., 2013; JPEC, 2008d); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.752 | −47.963 | 186 | 126 | The Linear model was selected based on lowest AIC. |
| Exponential (M4)<br>Exponential (M5)[c] | 0.603 | −46.156 | 157 | 67.7 | |
| Hill | 0.605 | −46.161 | 156 | 63.6 | |
| **Power[d]**<br>**Polynomial (2 degree)[e]**<br>**Linear[f]** | **0.774** | **−48.055** | **176** | **115** | |
| Polynomial (3 degree)[g] | 0.774 | −48.055 | 176 | 115 | |

[a]Modeled variance case presented (BMDS Test 3 *p*-value = <0), selected model in bold; scaled residuals for selected model for doses 0, 5, 25, 100, and 400 mg/kg-d were −0.421, −0.288, 1.29, −0.669, and 0.15, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.

[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

[e]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

[f]The Linear and Polynomial (3 degree) models appear equivalent; however, differences exist in digits not displayed in the table.

[g]The Linear model, Polynomial (2 degree and 3 degree) models and the Power models appear equivalent; however, differences exist in digits not displayed in the table.

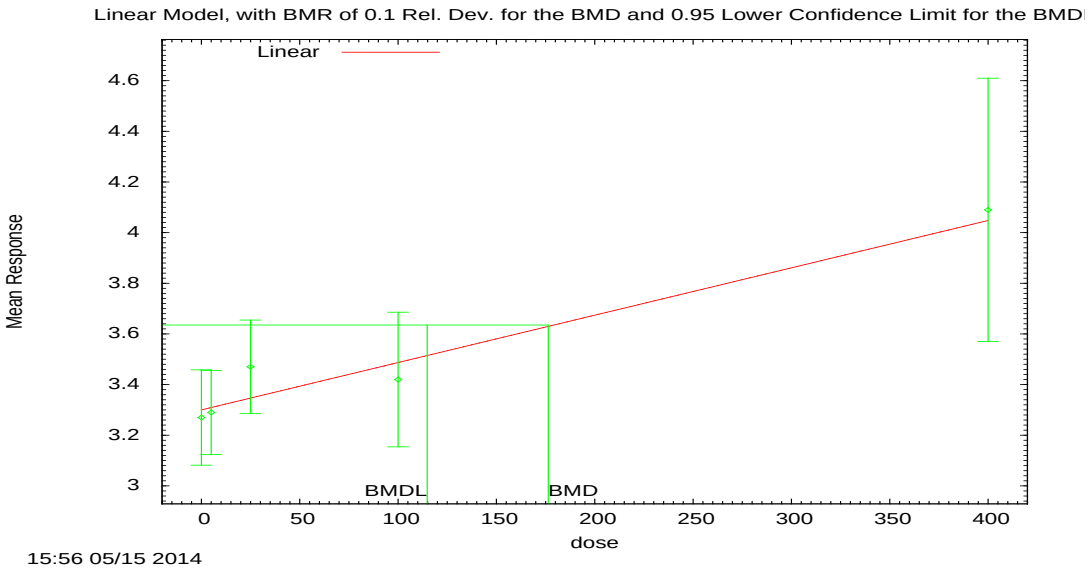



**Figure C-3. Plot of mean response by dose, with fitted curve for the Linear Model; dose shown in mg/kg-day.**

**Polynomial Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is: Y[dose] = beta_0 + beta_1 × dose

A modeled variance is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 176.354

BMDL at the 95% confidence level = 114.829

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | −13.8218 | −1.41289 |
| rho | 9.65704 | 0 |
| beta_0 | 3.30477 | 3.30246 |
| beta_1 | 0.00187393 | 0.00193902 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 15 | 3.27 | 3.3 | 0.34 | 0.32 | −0.421 |
| 5 | 15 | 3.29 | 3.31 | 0.3 | 0.325 | −0.288 |
| 25 | 14 | 3.47 | 3.35 | 0.32 | 0.343 | 1.29 |
| 100 | 15 | 3.42 | 3.49 | 0.48 | 0.418 | −0.669 |
| 400 | 13 | 4.09 | 4.05 | 0.86 | 0.859 | 0.15 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 17.455074 | 6 | −22.910149 |
| A2 | 29.755425 | 10 | −39.51085 |
| A3 | 28.583571 | 7 | −43.167142 |
| fitted | 28.027315 | 4 | −48.05463 |
| R | 6.041664 | 2 | −8.083328 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 47.4275 | 8 | <0.0001 |
| Test 2 | 24.6007 | 4 | <0.0001 |
| Test 3 | 2.34371 | 3 | 0.5042 |
| Test 4 | 1.11251 | 3 | 0.7741 |

**Table C-5. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in female Sprague-Dawley rats exposed to ETBE by daily gavage for 26 weeks (Miyata et al., 2013; JPEC, 2008d); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.369 | −168.25 | 406 | 271 | The Exponential (M4) model was selected based on lowest BMDL. |
| **Exponential (M4)** | **0.670** | **−168.60** | **224** | **56.9** | |
| Exponential (M5) | 0.865 | −167.37 | Error[c] | 0 | |
| Hill | 0.986 | −169.37 | Error[c] | Error[c] | |
| Power[d]<br>Polynomial (3 degree)[e]<br>Polynomial (2 degree)[f]<br>Linear | 0.382 | −168.34 | 402 | 263 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.425), selected model in bold; scaled residuals for selected model for doses 0, 5, 25, 100, and 400 mg/kg-d were 0.2257, 0.2206, −0.737, 0.3806, and −0.08999, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]BMD or BMDL computation failed for this model.

[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

[e]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.

[f]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

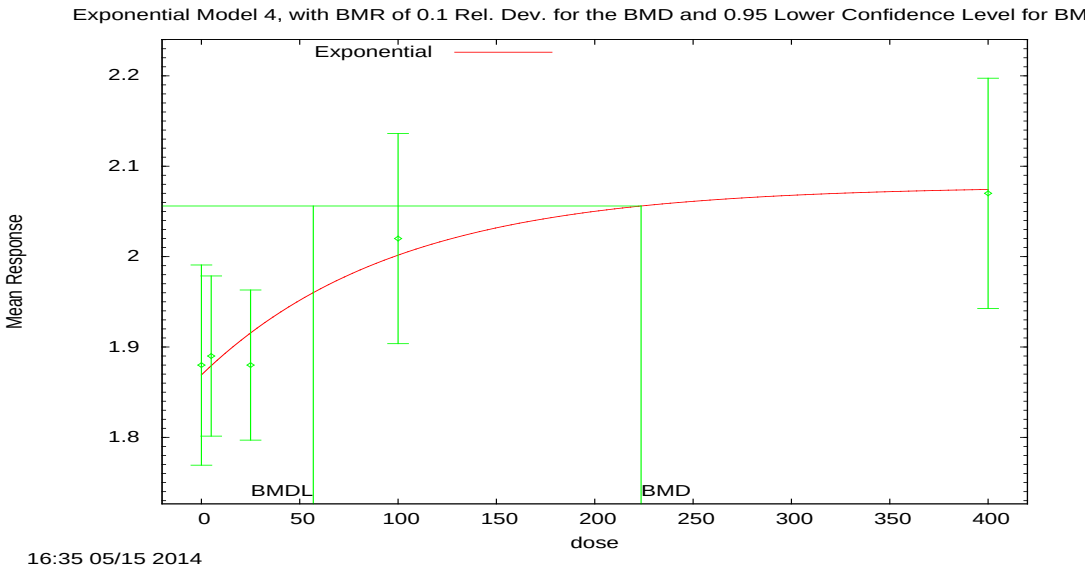



**Figure C-4. Plot of mean response by dose, with fitted curve for the Exponential Model 4; dose shown in mg/kg-day.**

**Exponential Model.** (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a × [c − (c − 1) × exp(−b × dose)]

A constant variance model is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 223.57

BMDL at the 95% confidence level = 56.8917

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| lnalpha | −3.35462 | −3.36529 |
| rho(S) | N/A | 0 |
| a | 1.86911 | 1.786 |
| b | 0.0100557 | 0.00368689 |
| c | 1.11181 | 1.21697 |
| d | 1 | 1 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 15 | 1.88 | 1.869 | 0.2 | 0.1869 | 0.2257 |
| 5 | 15 | 1.89 | 1.879 | 0.16 | 0.1869 | 0.2206 |
| 25 | 15 | 1.88 | 1.916 | 0.15 | 0.1869 | −0.737 |
| 100 | 15 | 2.02 | 2.002 | 0.21 | 0.1869 | 0.3806 |
| 400 | 15 | 2.07 | 2.074 | 0.23 | 0.1869 | −0.08999 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 88.69837 | 6 | −165.3967 |
| A2 | 90.62918 | 10 | −161.2584 |
| A3 | 88.69837 | 6 | −165.3967 |
| R | 82.20147 | 2 | −160.4029 |
| 4 | 88.29837 | 4 | −168.5967 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 16.86 | 8 | 0.03165 |
| Test 2 | 3.862 | 4 | 0.4251 |
| Test 3 | 3.862 | 4 | 0.4251 |
| Test 6a | 0.8 | 2 | 0.6703 |

**Table C-6. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in P0 male Sprague-Dawley rats exposed to ETBE by daily gavage for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups (Gaoua, 2004a); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.155 | −38.410 | 551 | 423 | The Hill model is selected based on lowest BMDL. |
| Exponential (M4)[c] | 0.727 | −40.012 | 255 | 123 | |
| Exponential (M5)[c] | 0.727 | −40.012 | 255 | 123 | |
| **Hill** | **0.811** | **−40.077** | **244** | **94.0** | |
| Power[d]<br>Polynomial (3 degree)[e]<br>Polynomial (2 degree)[f]<br>Linear | 0.199 | −38.902 | 517 | 386 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.119), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-d were −0.0247, 0.14, −0.181, and 0.0657, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]The Exponential (M4) model and the Exponential (M5) model appear equivalent; however, differences exist in digits not displayed in the table.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.
[f]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

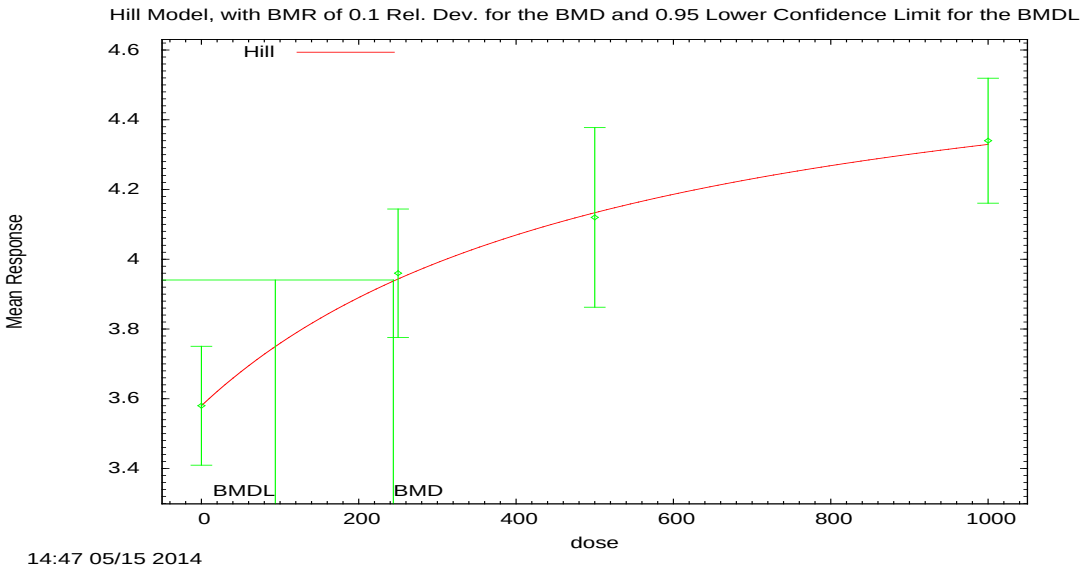



**Figure C-5. Plot of mean response by dose, with fitted curve for the Hill Model; dose shown in mg/kg-day.**

**Hill Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is: $Y[dose] = intercept + v \times dose^n/(k^n + dose^n)$

A constant variance model is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 243.968

BMDL at the 95% confidence level = 93.9617

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.227462 | 0.236804 |
| rho | N/A | 0 |
| intercept | 3.58236 | 3.58 |
| v | 1.16337 | 0.76 |
| n | 1 | 0.647728 |
| k | 548.322 | 250 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 25 | 3.58 | 3.58 | 0.413 | 0.477 | −0.0247 |
| 250 | 25 | 3.96 | 3.95 | 0.446 | 0.477 | 0.14 |
| 500 | 25 | 4.12 | 4.14 | 0.624 | 0.477 | −0.181 |
| 1,000 | 25 | 4.34 | 4.33 | 0.434 | 0.477 | 0.0657 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 24.067171 | 5 | −38.134342 |
| A2 | 26.992591 | 8 | −37.985183 |
| A3 | 24.067171 | 5 | −38.134342 |
| fitted | 24.038627 | 4 | −40.077253 |
| R | 9.48179 | 2 | −14.963581 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 35.0216 | 6 | <0.0001 |
| Test 2 | 5.85084 | 3 | 0.1191 |
| Test 3 | 5.85084 | 3 | 0.1191 |
| Test 4 | 0.057089 | 1 | 0.8112 |

**Table C-7. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in P0 female Sprague-Dawley rats exposed to ETBE by daily gavage for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups (Gaoua, 2004a); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| **Exponential (M2)** | **0.625** | **−214.58** | **1,734** | **1,030** | Exponential (M2) model is selected based on lowest AIC; however, BMDL is higher than the maximum dose. |
| Exponential (M3) | 0.416 | −212.86 | 1,458 | 1,040 | |
| Exponential (M4) | 0.327 | −212.56 | 1,774 | 1,032 | |
| Exponential (M5) | N/A[b] | −211.39 | Error[c] | 0 | |
| Hill | 0.715 | −213.39 | Error[c] | Error[c] | |
| Power | 0.418 | −212.87 | 1,470 | 1,041 | |
| Polynomial (3 degree) | 0.400 | −212.81 | 1,409 | 1,035 | |
| Polynomial (2 degree) | 0.400 | −212.81 | 1,409 | 1,037 | |
| Linear | 0.619 | −214.56 | 1,774 | 1,032 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.391), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-d were 0.5052, −0.7974, 0.1844, and 0.1033, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

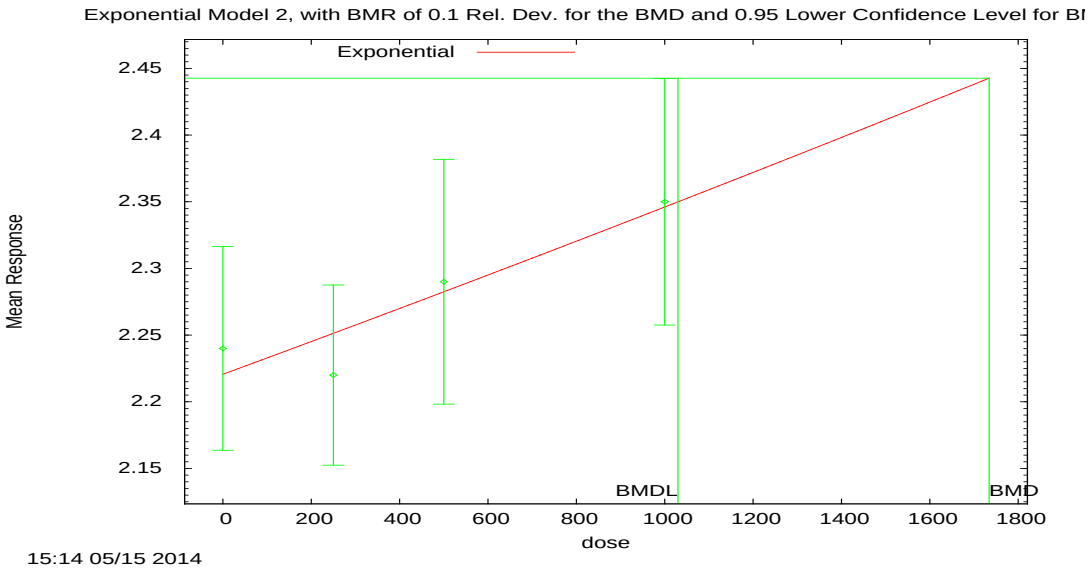



**Figure C-6. Plot of mean response by dose, with fitted curve for the Exponential Model 2; dose shown in mg/kg-day.**

**Exponential Model.** (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a × exp(sign × b × dose)

A constant variance model is fit

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 1,734.24

BMDL at the 95% confidence level = 1,030.08

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| lnalpha | −3.29773 | −3.30752 |
| rho(S) | N/A | 0 |
| a | 2.22057 | 2.22078 |
| b | 0.0000549578 | 0.0000546688 |
| c | 0 | 0 |
| d | 1 | 1 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 25 | 2.24 | 2.221 | 0.185 | 0.1923 | 0.5052 |
| 250 | 24 | 2.22 | 2.251 | 0.16 | 0.1923 | −0.7974 |
| 500 | 22 | 2.29 | 2.282 | 0.207 | 0.1923 | 0.1844 |
| 1,000 | 25 | 2.35 | 2.346 | 0.224 | 0.1923 | 0.1033 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 110.761 | 5 | −211.522 |
| A2 | 112.2635 | 8 | −208.5269 |
| A3 | 110.761 | 5 | −211.522 |
| R | 107.4777 | 2 | −210.9553 |
| 2 | 110.2909 | 3 | −214.5817 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 9.572 | 6 | 0.1439 |
| Test 2 | 3.005 | 3 | 0.3909 |
| Test 3 | 3.005 | 3 | 0.3909 |
| Test 4 | 0.9403 | 2 | 0.6249 |

**Table C-8. Summary of benchmark dose (BMD) modeling results for absolute kidney weight in F1 male Sprague-Dawley rats exposed to ETBE by gavage in a two-generation study (Gaoua, 2004b); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2) | $6.30 \times 10^{-4}$ | 89.912 | 232 | 175 | Of the models that provided an adequate fit and a valid BMDL estimate, the Polynomial (3 degree) model was selected based on lowest AIC. |
| Exponential (M3) | 0.129 | 79.474 | 335 | 256 | |
| Exponential (M4) | <0.0001 | 98.039 | 263 | 179 | |
| Exponential (M5) | N/A[b] | 82.504 | 347 | 267 | |
| Hill | N/A[b] | 82.509 | 347 | 267 | |
| Power | 0.0680 | 80.504 | 347 | 267 | |
| **Polynomial (3 degree)** | 0.374 | 77.965 | 318 | 235 | |
| Polynomial (2 degree) | 0.0943 | 79.973 | 330 | 251 | |
| Linear | <0.0001 | 96.039 | 263 | 179 | |

[a]Modeled variance case presented (BMDS Test 3 *p*-value = <0), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-d were −0.584, 0.717, 0.225, and −0.837, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.

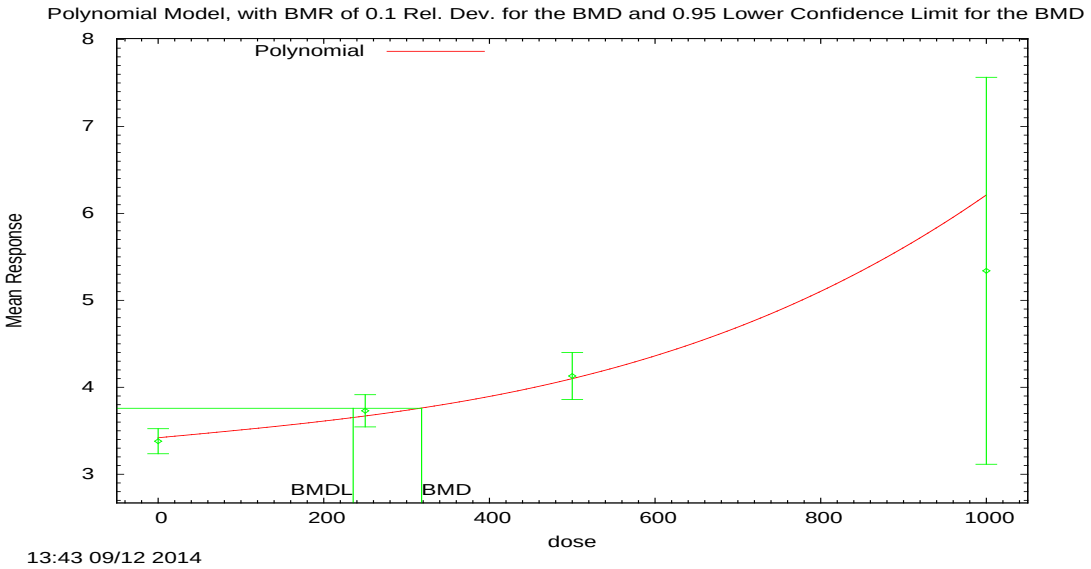



**Figure C-7. Plot of mean response by dose, with fitted curve for the Polynomial Model; dose shown in mg/kg-day.**

**Polynomial Model.** (Version: 2.19; Date: 06/25/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1 × dose + beta_2 × dose$^2$ + …
A modeled variance is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation
BMD = 318.084
BMDL at the 95% confidence level = 235.491

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| lnalpha | −13.8779 | 2.02785 |
| rho | 9.40248 | 0 |
| beta_0 | 3.41732 | 3.38 |
| beta_1 | 0.000881597 | 0.00138667 |
| beta_2 | $2.232 \times 10^{-28}$ | 0 |
| beta_3 | $1.90507 \times 10^{-9}$ | $6.93333 \times 10^{-9}$ |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 24 | 3.38 | 3.42 | 0.341 | 0.313 | −0.584 |
| 250 | 25 | 3.73 | 3.67 | 0.449 | 0.436 | 0.717 |
| 500 | 24 | 4.13 | 4.1 | 0.64 | 0.734 | 0.225 |
| 1,000 | 25 | 5.34 | 6.2 | 5.39 | 5.16 | −0.837 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | −146.32249 | 5 | 302.644981 |
| A2 | −32.521507 | 8 | 81.043013 |
| A3 | −33.58656 | 6 | 79.17312 |
| Fitted | −33.982384 | 5 | 77.964768 |
| R | −149.897277 | 2 | 303.794554 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 234.752 | 6 | <0.0001 |
| Test 2 | 227.602 | 3 | <0.0001 |
| Test 3 | 2.13011 | 2 | 0.3447 |
| Test 4 | 0.791648 | 1 | 0.3736 |

**Table C-9. Summary of benchmark dose (BMD) modeling results for absolute kidney weight in F1 female Sprague-Dawley rats exposed to ETBE by gavage in a two-generation study (Gaoua, 2004b); benchmark response (BMR) = 10% relative deviation**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| **Exponential (M2)** | **0.311** | **−180.23** | **978** | **670** | Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential (M2) model was selected based on lowest AIC. |
| Exponential (M3) | 0.147 | −178.46 | 1,016 | 679 | |
| Exponential (M4) | 0.121 | −178.16 | 980 | 654 | |
| Exponential (M5) | N/A[b] | −176.44 | 1,019 | 613 | |
| Hill | N/A[b] | −176.44 | 1,019 | 611 | |
| Power | 0.145 | −178.44 | 1,019 | 666 | |
| Polynomial (3 degree) | 0.184 | −178.80 | 1,001 | 684 | |
| Polynomial (2 degree) | 0.159 | −178.58 | 1,002 | 673 | |
| Linear | 0.301 | −180.16 | 980 | 654 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.159), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-d were −0.05426, 0.8898, −1.173, and 0.3711, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.

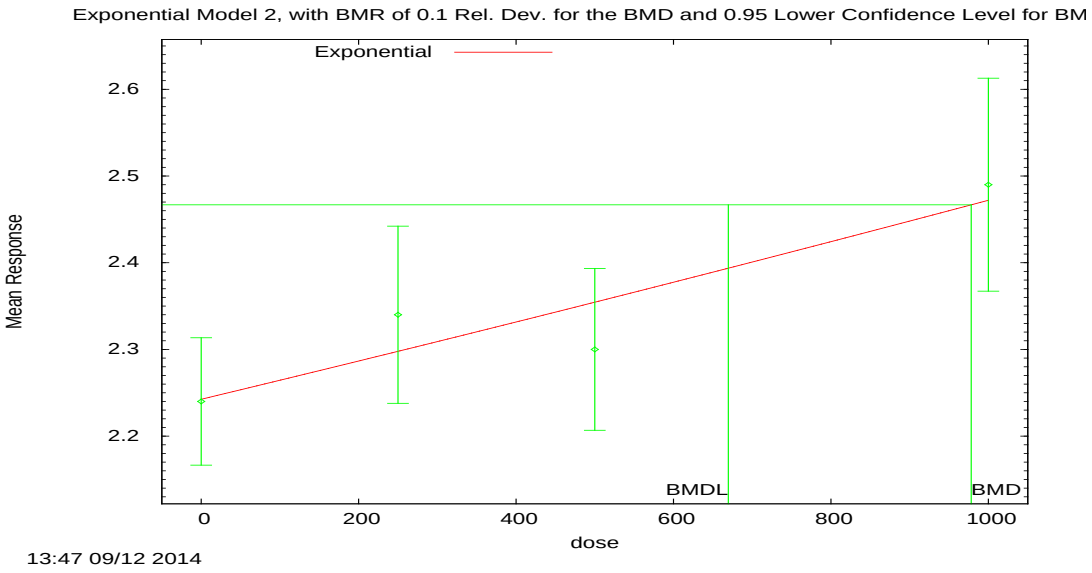



**Figure C-8. Plot of mean response by dose, with fitted curve for the Exponential Model 2; dose shown in mg/kg-day.**

**Exponential Model.** (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a × exp(sign × b × dose)

A constant variance model is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 978.157

BMDL at the 95% confidence level = 669.643

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| lnalpha | −2.91989 | −2.94397 |
| rho(S) | N/A | 0 |
| a | 2.24252 | 2.24321 |
| b | 0.0000974385 | 0.000096475 |
| c | 0 | 0 |
| d | 1 | 1 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 25 | 2.24 | 2.243 | 0.178 | 0.2322 | −0.05426 |
| 250 | 24 | 2.34 | 2.298 | 0.242 | 0.2322 | 0.8898 |
| 500 | 25 | 2.3 | 2.354 | 0.226 | 0.2322 | −1.173 |
| 1,000 | 23 | 2.49 | 2.472 | 0.284 | 0.2322 | 0.3711 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 94.28268 | 5 | −178.5654 |
| A2 | 96.87585 | 8 | −177.7517 |
| A3 | 94.28268 | 5 | −178.5654 |
| R | 87.16418 | 2 | −170.3284 |
| 2 | 93.11474 | 3 | −180.2295 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 19.42 | 6 | 0.003505 |
| Test 2 | 5.186 | 3 | 0.1587 |
| Test 3 | 5.186 | 3 | 0.1587 |
| Test 4 | 2.336 | 2 | 0.311 |

**Table C-10. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in P0 male Sprague-Dawley rats exposed to ETBE by daily gavage for 16 weeks beginning 10 weeks prior to mating (Fujii et al., 2010); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.668 | −41.247 | 648 | 479 | The Hill model was selected based on lowest BMDL. (BMDLs were greater than threefold difference.) |
| Exponential (M4)<br>Exponential (M5)[c] | 0.600 | −39.779 | 438 | 163 | |
| **Hill** | **0.613** | **−39.799** | **435** | **139** | |
| Power[d]<br>Polynomial (3 degree)[e]<br>Polynomial (2 degree)[f]<br>Linear | 0.700 | −41.342 | 625 | 448 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.102), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1,000 mg/kg-d were −0.202, 0.399, −0.232, and 0.0344, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model. For the Polynomial (3 degree) model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

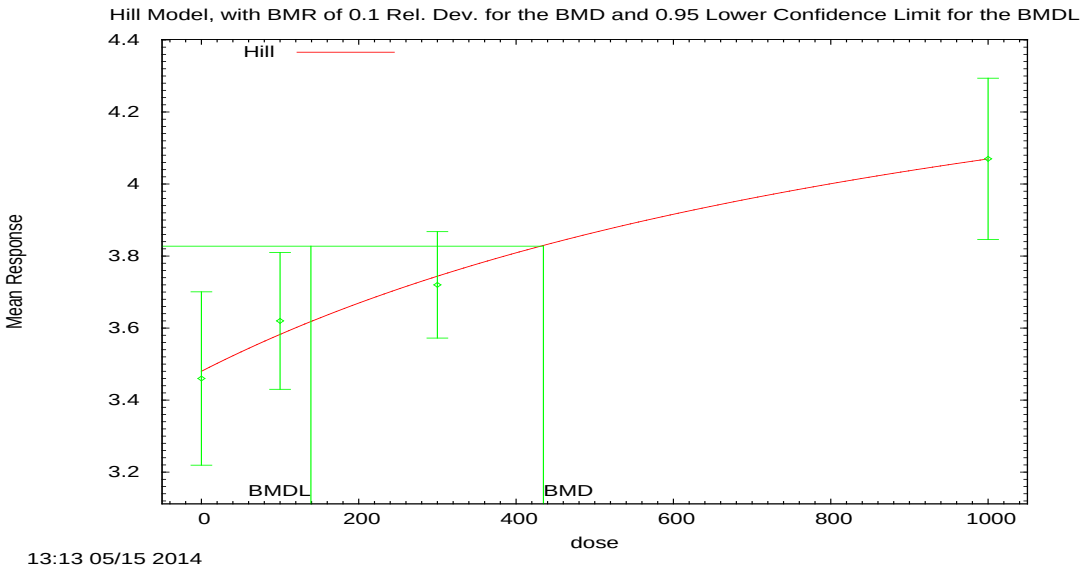



**Figure C-9. Plot of mean response by dose, with fitted curve for the Hill Model; dose shown in mg/kg-day.**

**Hill Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is: $Y[dose] = intercept + v \times dose^n/(k^n + dose^n)$

A constant variance model is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 434.715

BMDL at the 95% confidence level = 139.178

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.223598 | 0.2327 |
| rho | N/A | 0 |
| intercept | 3.47949 | 3.46 |
| v | 1.24601 | 0.61 |
| n | 1 | 0.27452 |
| k | 1,122 | 1,610 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 24 | 3.46 | 3.48 | 0.57 | 0.473 | −0.202 |
| 100 | 24 | 3.62 | 3.58 | 0.45 | 0.473 | 0.399 |
| 300 | 24 | 3.72 | 3.74 | 0.35 | 0.473 | −0.232 |
| 1,000 | 24 | 4.07 | 4.07 | 0.53 | 0.473 | 0.0344 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 24.027112 | 5 | −38.054223 |
| A2 | 27.13071 | 8 | −38.26142 |
| A3 | 24.027112 | 5 | −38.054223 |
| Fitted | 23.899392 | 4 | −39.798783 |
| R | 14.313578 | 2 | −24.627156 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 25.6343 | 6 | 0.0002604 |
| Test 2 | 6.2072 | 3 | 0.102 |
| Test 3 | 6.2072 | 3 | 0.102 |
| Test 4 | 0.25544 | 1 | 0.6133 |

**Table C-11. Summary of benchmark dose (BMD) modeling results for increased absolute kidney weight in P0 female Sprague-Dawley rats exposed to ETBE by daily gavage for 17 weeks beginning 10 weeks prior to mating until Lactation Day 21 (Fujii et al., 2010); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMD_{10RD}$ (mg/kg-d) | $BMDL_{10RD}$ (mg/kg-d) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2) | 0.483 | −199.73 | 1,135 | 781 | Polynomial (2 degree) is selected based on most parsimonious model with lowest AIC. |
| Exponential (M3) | 0.441 | −198.60 | 1,089 | 826 | |
| Exponential (M4) | 0.217 | −197.67 | 1,144 | 771 | |
| Exponential (M5) | N/A[b] | −196.66 | Error[c] | 0 | |
| Hill | N/A[b] | −196.66 | Error[c] | Error[c] | |
| Power | 0.441 | −198.60 | 1,092 | 823 | |
| Polynomial (3 degree)[d] **Polynomial (2 degree)** | **0.743** | **−200.60** | **1,094** | **905** | |
| Linear | 0.467 | −199.67 | 1,144 | 771 | |

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.103), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1,000 mg/kg-d were 0.499, −0.579, 0.0914, and −0.00282, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.
[d]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.

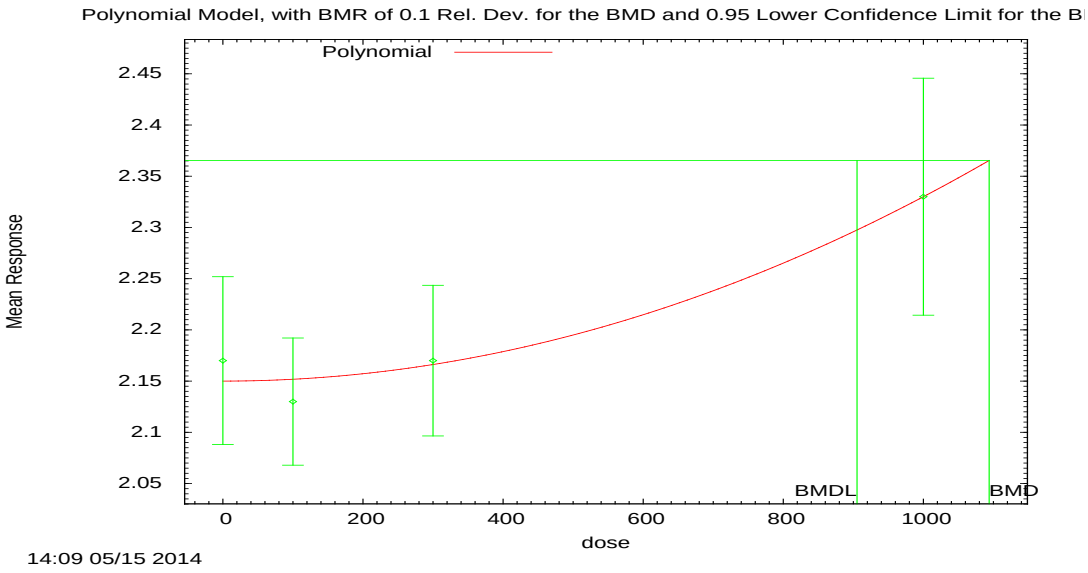



**Figure C-10. Plot of mean response by dose, with fitted curve for the Polynomial Model; dose shown in mg/kg-day.**

**Polynomial Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is: Y[dose] = beta_0 + beta_1 × dose + beta_2 × dose$^2$ + …

A constant variance model is fit.

**Benchmark Dose Computation.**

BMR = 10% relative deviation

BMD = 1,093.86

BMDL at the 95% confidence level = 905.267

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.0323691 | 0.0337309 |
| rho | N/A | 0 |
| beta_0 | 2.1504 | 2.15624 |
| beta_1 | $7.16226 \times 10^{-28}$ | 0 |
| beta_2 | $1.79719 \times 10^{-6}$ | 0 |

**Table of Data and Estimated Values of Interest**

| Dose | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 21 | 2.17 | 2.15 | 0.18 | 0.18 | 0.499 |
| 100 | 22 | 2.13 | 2.15 | 0.14 | 0.18 | −0.579 |
| 300 | 23 | 2.17 | 2.17 | 0.17 | 0.18 | 0.0914 |
| 1,000 | 19 | 2.33 | 2.33 | 0.24 | 0.18 | −0.00282 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 103.595625 | 5 | −197.191249 |
| A2 | 106.684319 | 8 | −197.368637 |
| A3 | 103.595625 | 5 | −197.191249 |
| fitted | 103.298361 | 3 | −200.596722 |
| R | 96.89324 | 2 | −189.78648 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 19.5822 | 6 | 0.003286 |
| Test 2 | 6.17739 | 3 | 0.1033 |
| Test 3 | 6.17739 | 3 | 0.1033 |
| Test 4 | 0.594528 | 2 | 0.7428 |

Inhalation exposure endpoints

**Table C-12. Summary of benchmark concentration (BMC) modeling results for urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks (JPEC, 2010b); benchmark response (BMR) = 10% extra risk**

| Model[a] | Goodness of fit | | $BMC_{10}$ ($mg/m^3$) | $BMCL_{10}$ ($mg/m^3$) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| **Gamma** | **0.874** | **164.37** | **2,734** | **1,498** | Of the models that provided an adequate fit and a valid BMCL estimate, the Gamma model was selected based on lowest AIC. |
| Logistic | 0.146 | 166.30 | 4,329 | 3,522 | |
| Log-Logistic | 0.814 | 164.40 | 3,010 | 1,831 | |
| Probit | 0.202 | 165.59 | 4,059 | 3,365 | |
| Log-Probit | 0.633 | 164.57 | 3,050 | 1,896 | |
| Weibull | 0.758 | 164.44 | 2,623 | 1,478 | |
| Multistage (3 degree) | 0.565 | 164.69 | 2,386 | 1,412 | |
| Multistage (2 degree) | 0.565 | 164.69 | 2,386 | 1,422 | |
| Quantal-Linear | 0.269 | 165.16 | 1,541 | 1,227 | |

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Selected model in bold; scaled residuals for selected model for concentrations 0, 2,089, 6,268, and 20,893 $mg/m^3$ were 0.036, −0.107, 0.104, and −0.040, respectively. Exposure concentrations were converted from 0, 500, 1,500, and 5,000 ppm to $mg/m^3$ using the calculation $mg/m^3$ = (102.17, molecular weight of ETBE) × ppm ÷ 24.45.

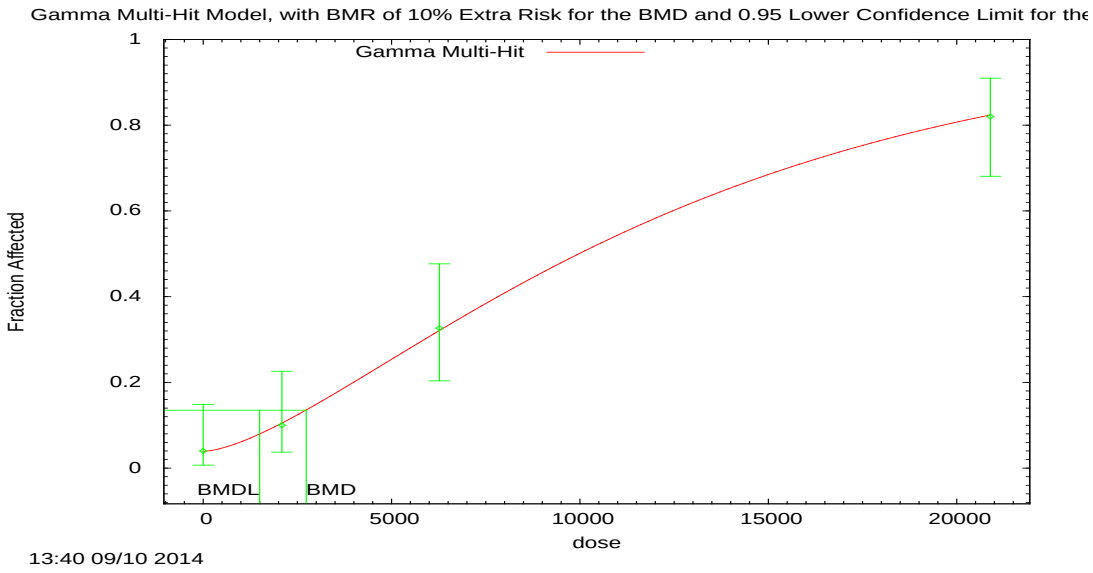



**Figure C-11. Plot of incidence rate by concentration, with fitted curve for the Gamma Model; concentration shown in mg/m³.**

**Gamma Model.** (Version: 2.16; Date: 2/28/2013)

The form of the probability function is:

P[response] = background + (1 − background) × CumGamma[slope × concentration,power], where CumGamma(.) is the cumulative Gamma distribution function.

Power parameter is restricted as power ≥1.

**Benchmark Concentration Computation.**

BMR = 10% extra risk

BMC = 2,734.41

BMCL at the 95% confidence level = 1,497.7

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| Background | 0.0390054 | 0.0576923 |
| Slope | 0.000121504 | 0.000132454 |
| Power | 1.59019 | 1.84876 |

**Analysis of Deviance Table**

| Model | Log(likelihood) | No. parameters | Deviance | Test df | *p*-value |
|---|---|---|---|---|---|
| Full model | −79.1741 | 4 | | | |
| Fitted model | −79.1867 | 3 | 0.0253512 | 1 | 0.8735 |
| Reduced model | −124.987 | 1 | 91.626 | 3 | <0.0001 |

AIC = 164.373

**Goodness-of-Fit Table**

| Concentration | Estimated probability | Expected | Observed | Size | Scaled residual |
|---|---|---|---|---|---|
| 0 | 0.039 | 1.95 | 2 | 50 | 0.036 |
| 2,089 | 0.1046 | 5.231 | 5 | 50 | −0.107 |
| 6,268 | 0.3196 | 15.659 | 16 | 49 | 0.104 |
| 20,893 | 0.8222 | 41.109 | 41 | 50 | −0.04 |

$\chi^2$ = 0.03; df = 1; *p*-value = 0.8737

**Table C-13. Summary of benchmark concentration (BMC) modeling results for increased absolute kidney weight in female F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks (IPEC, 2010b), benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMC_{10RD}$ ($mg/m^3$) | $BMCL_{10RD}$ ($mg/m^3$) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| **Exponential (M2)** Exponential (M3)[b] | **0.1482** | **−293.77** | **13,422.9** | **10,431.3** | Of the models that provided an adequate fit and a valid BMCL estimate, the Exponential (M2) model was selected based on lowest AIC. |
| Exponential (M4) | 0.04944 | −291.73 | 13,028.1 | 7,023.54 | |
| Exponential (M5) | 0.04944 | −291.73 | 13,027.3 | 7,023.54 | |
| Hill | 0.04939 | −291.73 | 13,027.3 | 9,893.86 | |
| Polynomial (2 degree)[c] | 0.05124 | −291.79 | 13,959.9 | 9,936.46 | |
| Polynomial (3 degree)[d] | 0.05454 | −291.89 | 14,857.4 | 9,985.31 | |
| Power[e] Linear | 0.1451 | −293.73 | 13,029.1 | 9,909.08 | |

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Selected model in bold; modeled variance case presented. For this data set variance was modeled as a power function of the mean, but the *p*-value (BMDS Test 3 *p*-value = <0.018) was below the threshold criterion for variance testing of 0.1.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

[d]For the Polynomial (3 degree) model, the b3 and b2 coefficient estimates were 0 (boundary), and the model reduced to the Linear model.

[e]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

**Table C-14. Summary of benchmark concentration (BMC) modeling results for increased absolute kidney weight in male Sprague-Dawley rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week for 13 weeks (IPEC, 2008b); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMC_{10RD}$ (ppm) | $BMCL_{10RD}$ (ppm) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2) Exponential (M3)[b] | 0.168 | −43.014 | 1,105 | 750 | Of the models that provided an adequate fit and a valid BMCL estimate, the Hill model was selected based on lowest BMCL (BMCLs differed by more than 3). |
| Exponential (M4) | 0.200 | −42.943 | 380 | 1.73 | |
| Exponential (M5) | 0.200 | −42.943 | 380 | 2.61 | |
| **Hill** | **0.294** | **−43.484** | **264** | **15.4** | |
| Power[c] Polynomial (3 degree)[d] Polynomial (2 degree)[e] Linear | 0.178 | −43.133 | 1,071 | 703 | |

ppm = 4.18 $mg/m^3$
BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.506), selected model in bold; scaled residuals for selected model for concentrations 0, 150, 500, and 1,500 ppm were −0.13, 0.54, −0.8, 0.38, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[d]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model. For the Polynomial (3 degree) model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[e]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

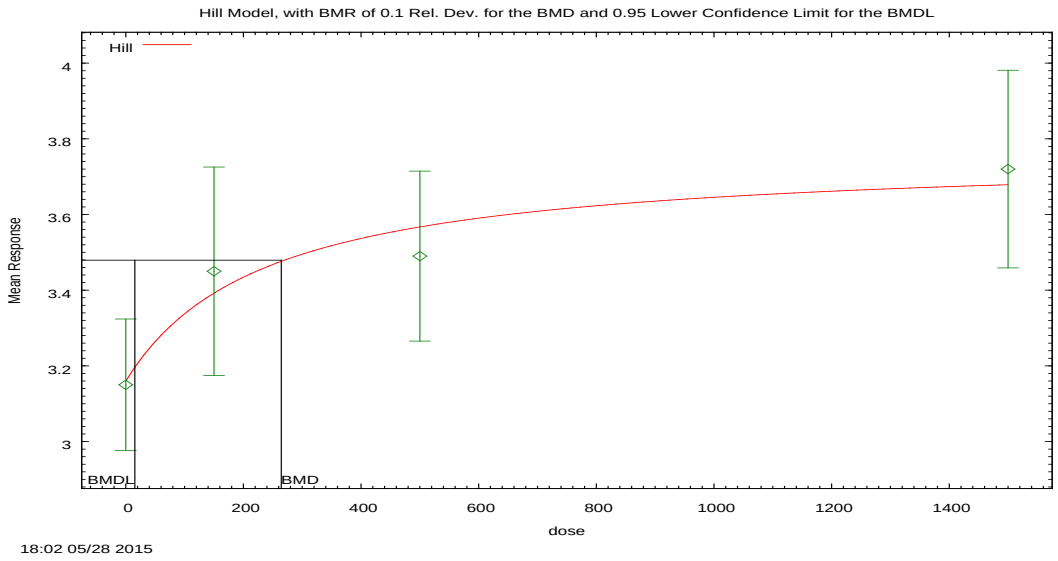



**Figure C-12. Plot of mean response by concentration, with fitted curve for the Hill Model; concentration shown in ppm.**

**Hill Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is:

Y[concentration] = intercept + v × concentration$^n$/(k$^n$ + concentration$^n$)

A constant variance model is fit.

**Benchmark Concentration Computation.**

BMR = 10% relative deviation

BMC = 264.371

BMCL at the 95% confidence level = 15.4115

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.101559 | 0.109774 |
| rho | N/A | 0 |
| intercept | 3.16295 | 3.15 |
| v | 0.600878 | 0.57 |
| n | 1 | 0.169179 |
| k | 237.864 | 157.5 |

**Table of Data and Estimated Values of Interest**

| Concentration | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 10 | 3.15 | 3.16 | 0.24 | 0.32 | −0.129 |
| 150 | 10 | 3.45 | 3.4 | 0.38 | 0.32 | 0.542 |
| 500 | 10 | 3.49 | 3.57 | 0.31 | 0.32 | −0.795 |
| 1,500 | 10 | 3.72 | 3.68 | 0.36 | 0.32 | 0.381 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 26.293887 | 5 | −42.587775 |
| A2 | 27.46147 | 8 | −38.922941 |
| A3 | 26.293887 | 5 | −42.587775 |
| Fitted | 25.742228 | 4 | −43.484456 |
| R | 19.334386 | 2 | −34.668772 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 16.2542 | 6 | 0.01245 |
| Test 2 | 2.33517 | 3 | 0.5058 |
| Test 3 | 2.33517 | 3 | 0.5058 |
| Test 4 | 1.10332 | 1 | 0.2935 |

**Table C-15. Summary of benchmark concentration (BMC) modeling results for increased absolute kidney weight in female Sprague-Dawley rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week for 13 weeks (IPEC, 2008b); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMC_{10RD}$ (ppm) | $BMCL_{10RD}$ (ppm) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2) Exponential (M3)[b] | 0.8 | −135.38 | 6,790 | 4,046 | The Linear model is selected based on lowest AIC; however, the BMC is higher than the maximum concentration. |
| Exponential (M4) | 0.731 | −133.76 | Error[c] | 0 | |
| Exponential (M5) | 0.760 | −132.29 | Error[c] | 0 | |
| Hill | 0.760 | −132.29 | Error[c] | Error[c] | |
| Power[d] Polynomial (3 degree)[e] Polynomial (2 degree)[f] **Linear** | **0.806** | **−135.40** | **6,840** | **3,978** | |

ppm = 4.18 $mg/m^3$

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.623), selected model in bold; scaled residuals for selected model for concentrations 0, 150, 500, 1,500, and 5,000 ppm were −0.0742, 0.0535, −0.578, 0.774, and −0.176, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]BMC or BMCL computation failed for this model.

[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

[e]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.

[f]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

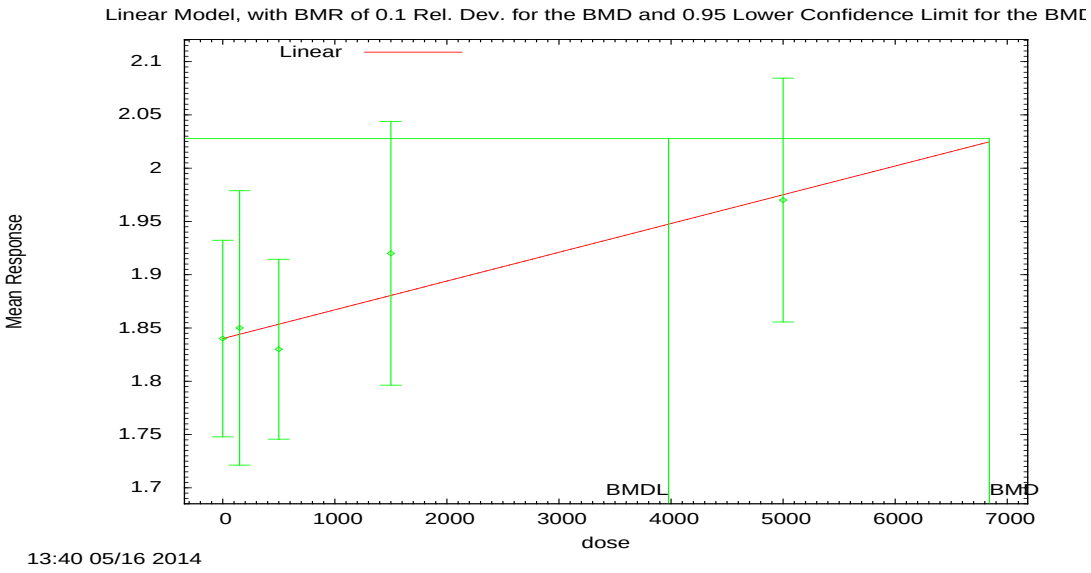



**Figure C-13. Plot of mean response by concentration, with fitted curve for the Linear Model; concentration shown in ppm.**

**Polynomial Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is: Y[concentration] = beta_0 + beta_1 × concentration

A constant variance model is fit.

**Benchmark Concentration Computation.**

BMR = 10% relative deviation

BMC = 6,840.02

BMCL at the 95% confidence level = 3,978.09

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.021752 | 0.0236988 |
| rho | N/A | 0 |
| beta_0 | 1.84346 | 1.84346 |
| beta_1 | 0.0000269511 | 0.0000269511 |

**Table of Data and Estimated Values of Interest**

| Concentration | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 10 | 1.84 | 1.84 | 0.129 | 0.147 | −0.0742 |
| 150 | 10 | 1.85 | 1.85 | 0.18 | 0.147 | 0.0535 |
| 500 | 10 | 1.83 | 1.86 | 0.118 | 0.147 | −0.578 |
| 1,500 | 10 | 1.92 | 1.88 | 0.173 | 0.147 | 0.774 |
| 5,000 | 10 | 1.97 | 1.98 | 0.16 | 0.147 | −0.176 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 71.192285 | 6 | −130.384569 |
| A2 | 72.502584 | 10 | −125.005168 |
| A3 | 71.192285 | 6 | −130.384569 |
| Fitted | 70.701239 | 3 | −135.402478 |
| R | 67.96809 | 2 | −131.93618 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 9.06899 | 8 | 0.3365 |
| Test 2 | 2.6206 | 4 | 0.6232 |
| Test 3 | 2.6206 | 4 | 0.6232 |
| Test 4 | 0.982091 | 3 | 0.8056 |

**Table C-16. Summary of benchmark concentration (BMC) modeling results for increased absolute kidney weight in male F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 13 weeks (Medinsky et al., 1999; US EPA, 1997); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMC_{10RD}$ (ppm) | $BMCL_{10RD}$ (ppm) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.184 | −129.97 | 3,107 | 2,439 | The Hill model was selected based on lowest BMCL. |
| Exponential (M4)<br>Exponential (M5)[c] | 0.199 | −129.71 | 1,798 | 808 | |
| **Hill** | **0.224** | **−129.89** | **1,667** | **603** | |
| Power[d]<br>Polynomial (3 degree)[e]<br>Polynomial (2 degree)[f]<br>Linear | 0.208 | −130.22 | 2,980 | 2,288 | |

ppm = 4.18 $mg/m^3$

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.540), selected model in bold; scaled residuals for selected model for concentrations0, 500, 1,750, and 5,000 ppm were −0.441, 0.91, −0.635, and 0.166, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.

[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

[e]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.

[f]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

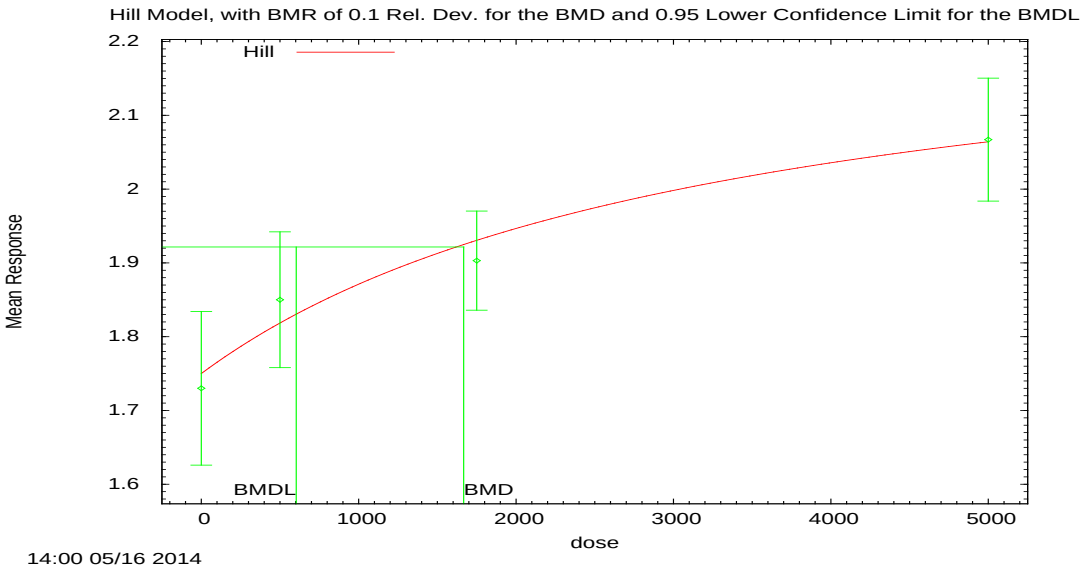



**Figure C-14. Plot of mean response by concentration, with fitted curve for the Hill Model; concentration shown in ppm.**

**Hill Model.** (Version: 2.17; Date: 01/28/2013)

The form of the response function is:

Y[concentration] = intercept + v × concentration$^n$/(k$^n$ + concentration$^n$)

A constant variance model is fit.

**Benchmark Concentration Computation.**

BMR = 10% relative deviation

BMC = 1,666.92

BMCL at the 95% confidence level = 603.113

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| alpha | 0.0160221 | 0.0170425 |
| rho | N/A | 0 |
| intercept | 1.74684 | 1.73 |
| v | 0.521534 | 0.337 |
| n | 1 | 0.225826 |
| k | 3,309.8 | 1,856.13 |

**Table of Data and Estimated Values of Interest**

| Concentration | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 11 | 1.73 | 1.75 | 0.155 | 0.127 | −0.441 |
| 500 | 11 | 1.85 | 1.82 | 0.137 | 0.127 | 0.91 |
| 1,750 | 11 | 1.9 | 1.93 | 0.1 | 0.127 | −0.635 |
| 5,000 | 11 | 2.07 | 2.06 | 0.124 | 0.127 | 0.166 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 69.681815 | 5 | −129.36363 |
| A2 | 70.76062 | 8 | −125.521241 |
| A3 | 69.681815 | 5 | −129.36363 |
| Fitted | 68.943332 | 4 | −129.886663 |
| R | 55.026208 | 2 | −106.052416 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 31.4688 | 6 | <0.0001 |
| Test 2 | 2.15761 | 3 | 0.5403 |
| Test 3 | 2.15761 | 3 | 0.5403 |
| Test 4 | 1.47697 | 1 | 0.2242 |

**Table C-17. Summary of benchmark concentration (BMC) modeling results for increased absolute kidney weight in female F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 13 weeks (Medinsky et al., 1999; US EPA, 1997); benchmark response (BMR) = 10% relative deviation from the mean**

| Model[a] | Goodness of fit | | $BMC_{10RD}$ (ppm) | $BMCL_{10RD}$ (ppm) | Basis for model selection |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Exponential (M2)<br>Exponential (M3)[b] | 0.0630 | −187.67 | 2,706 | 2,275 | The Exponential (M4) model was selected as the most parsimonious model of adequate fit. |
| **Exponential (M4)**<br>Exponential (M5)[c] | **0.956** | **−191.20** | **1,342** | **816** | |
| Hill | N/A[d] | −189.20 | 1,325 | 741 | |
| Power[e]<br>Polynomial (3 degree)[f]<br>Polynomial (2 degree)[g]<br>Linear | 0.0928 | −188.45 | 2,552 | 2,111 | |

ppm = 4.18 $mg/m^3$

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.428), selected model in bold; scaled residuals for selected model for concentrations 0, 500, 1,750, and 5,000 ppm were −0.0252, 0.043, −0.02385, and 0.004872, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.

[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.

[d]No available degrees of freedom to calculate a goodness-of-fit value.

[e]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.

[f]For the Polynomial (3 degree) model, the b3 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Polynomial (2 degree) model.

[g]For the Polynomial (2 degree) model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

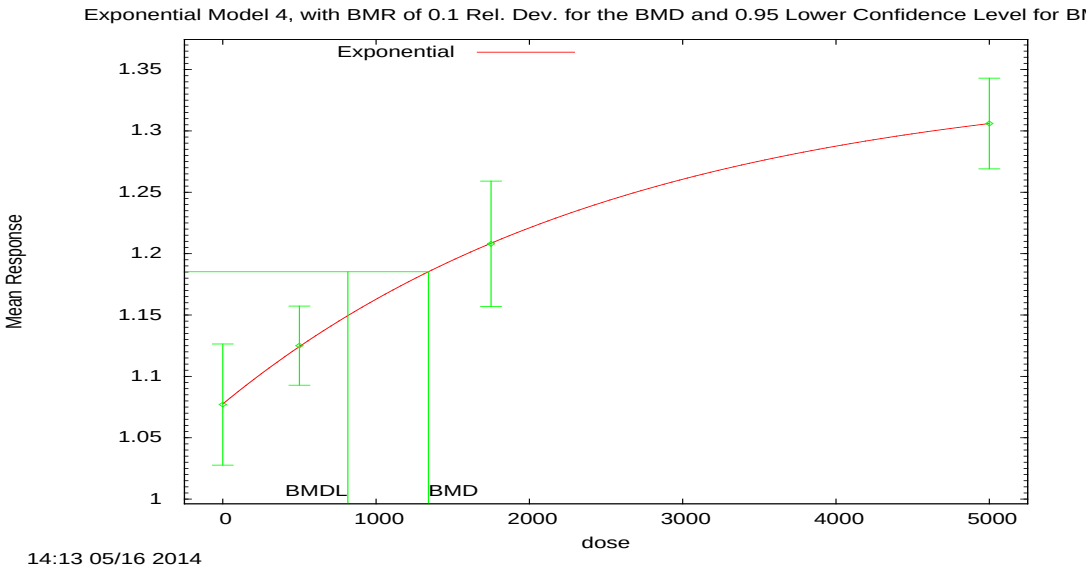



**Figure C-15. Plot of mean response by concentration, with fitted curve for the Exponential Model 4; concentration shown in ppm.**

**Exponential Model.** (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[concentration] = a × [c − (c − 1) × exp(−b × concentration)]

A constant variance model is fit.

**Benchmark Concentration Computation.**

BMR = 10% relative deviation

BMC = 1,341.66

BMCL at the 95% confidence level = 815.742

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| lnalpha | −5.63259 | −5.63266 |
| rho(S) | N/A | 0 |
| a | 1.07748 | 1.02315 |
| b | 0.000383798 | 0.000348471 |
| c | 1.24847 | 1.34027 |
| d | 1 | 1 |

**Table of Data and Estimated Values of Interest**

| Concentration | *N* | Observed mean | Estimated mean | Observed standard deviation | Estimated standard deviation | Scaled residual |
|---|---|---|---|---|---|---|
| 0 | 10 | 1.077 | 1.077 | 0.069 | 0.05983 | −0.0252 |
| 500 | 11 | 1.125 | 1.124 | 0.048 | 0.05983 | 0.043 |
| 1,750 | 11 | 1.208 | 1.208 | 0.076 | 0.05983 | −0.02385 |
| 5,000 | 11 | 1.306 | 1.306 | 0.055 | 0.05983 | 0.004872 |

**Likelihoods of Interest**

| Model | Log(likelihood) | No. parameters | AIC |
|---|---|---|---|
| A1 | 99.60217 | 5 | −189.2043 |
| A2 | 100.9899 | 8 | −185.9798 |
| A3 | 99.60217 | 5 | −189.2043 |
| R | 75.30605 | 2 | −146.6121 |
| 4 | 99.60063 | 4 | −191.2013 |

**Tests of Interest**

| Test | −2 × log(likelihood ratio) | Test df | *p*-value |
|---|---|---|---|
| Test 1 | 51.37 | 6 | <0.0001 |
| Test 2 | 2.775 | 3 | 0.4276 |
| Test 3 | 2.775 | 3 | 0.4276 |
| Test 6a | 0.003077 | 1 | 0.9558 |

### C.1.2. Cancer Endpoints

For the Multistage Cancer models, the coefficients were restricted to be nonnegative (beta ≥0). For each endpoint, Multistage Cancer models were fitted to the data using the maximum likelihood method. Each model was tested for goodness of fit using a chi-square goodness-of-fit test ($\chi^2$ *p*-value < 0.05[3] indicates lack of fit). Other factors were used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each endpoint, the BMDL/BMCL estimate (95% lower confidence limit on the BMD/BMC, as estimated by the profile likelihood method) and AIC value were used to select a

[3]A significance level of 0.05 is used for selecting cancer models because the model family (Multistage) is selected a priori; see *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012).

best-fit model from among the models exhibiting adequate fit. If the BMDL/BMCL estimates were "sufficiently close," that is, differed by more than threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL/BMCL estimates were not sufficiently close, the lowest BMDL/BMCL was selected as the POD.

The incidence of liver tumors in male F344 rats was found to be statistically significantly increased following a 2-year inhalation exposure; hepatocellular adenomas and a single hepatocellular carcinoma in the high-dose group were combined in modeling the data set. The data were modeled using two different exposure metrics: administered concentration as ppm as $mg/m^3$.

**Table C-18. Cancer endpoints selected for dose-response modeling for ETBE**

| Species/sex endpoint | Concentration and effect data | | | | |
|---|---|---|---|---|---|
| Hepatocellular adenomas and carcinomas in male rats; JPEC (2010b) | Exposure concentration (ppm) | 0 | 500 | 1,500 | 5,000 |
| | Exposure concentration ($mg/m^3$) | 0 | 2,089 | 6,268 | 20,893 |
| | Incidence/total | 0/50 | 2/50 | 1/49 | 10/50 |

### *Modeling Results*

Below are tables summarizing the modeling results for the cancer endpoints modeled.

**Table C-19. Summary of benchmark concentration (BMC) modeling results for hepatocellular adenomas and carcinomas in male F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks; modeled with concentrations as administered, exposure concentration in ppm (JPEC, 2010b); benchmark response (BMR) = 10% extra risk**

| Model[a] | Goodness of fit | | | $BMC_{10}$ (ppm) | $BMCL_{10}$ (ppm) | Basis for model selection |
|---|---|---|---|---|---|---|
| | *p*-value | Scaled residuals | AIC | | | |
| 3 degree | 0.0991 | −0.030, 1.382, −0.898, and 0.048 | 84.961 | 2,942 | 1,735 | Multistage (1 degree) was selected based on lowest AIC. |
| 2 degree | 0.264 | 0.000, 1.284, −1.000, and 0.137 | 83.093 | 2,756 | 1,718 | |
| **1 degree** | **0.490** | **0.000, 1.009, −1.144, and 0.309** | **81.208** | **2,605** | **1,703** | |

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Selected model in bold.

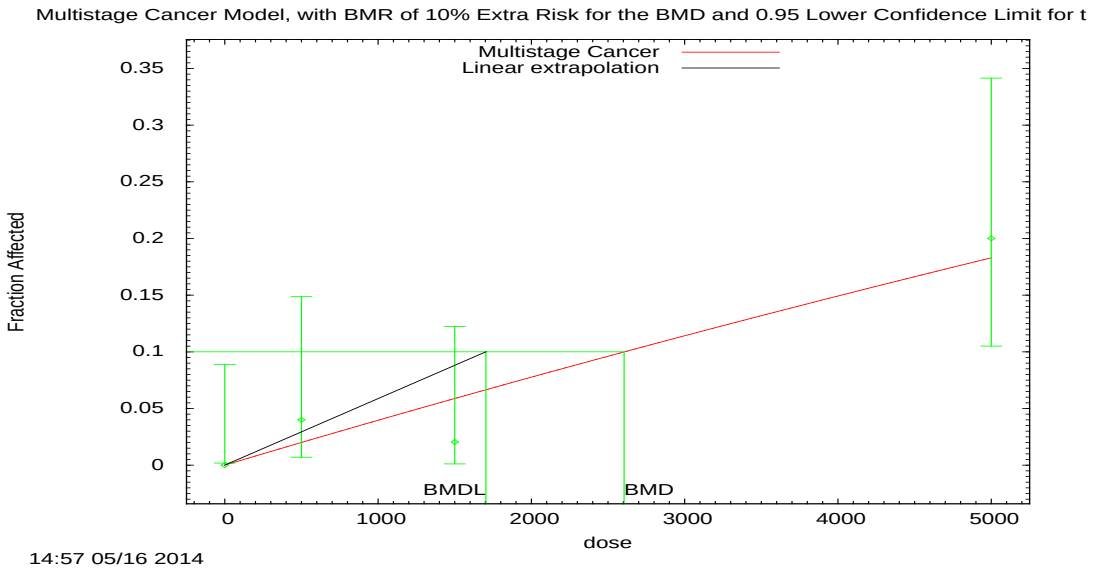



**Figure C-16. Plot of incidence rate by concentration, with fitted curve for the Multistage Model; concentration shown in ppm.**

**Multistage Model.** (Version: 3.4; Date: 05/02/2014)

The form of the probability function is:

P[response] = background + (1 − background) × [1 − exp(−beta1 × concentration$^1$−beta2 × concentration$^2$...)]

The parameter betas are restricted to be positive.

**Benchmark Concentration Computation.**

BMR = 10% extra risk

BMC = 2,604.82

BMCL at the 95% confidence level = 1,703.47

Benchmark concentration upper confidence limit (BMCU) at the 95% confidence level = 4,634.52

Collectively, (1,703.47, 4,634.52) is a 90% two-sided confidence interval for the BMC.

Multistage cancer slope factor = error

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| Background | 0 | 0 |
| Beta(1) | $4.04483 \times 10^{-4}$ | $4.38711 \times 10^{-4}$ |

**Analysis of Deviance Table**

| Model | Log(likelihood) | No. parameters | Deviance | Test df | *p*-value |
|---|---|---|---|---|---|
| Full model | −38.2989 | 4 | | | |
| Fitted model | −39.6042 | 1 | 2.61063 | 3 | 0.4556 |
| Reduced model | −48.0344 | 1 | 19.4711 | 3 | 0.0002184 |

AIC = 81.2084

**Goodness-of-Fit Table**

| Concentration | Estimated probability | Expected | Observed | Size | Scaled residual |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 50 | 0 |
| 500 | 0.02 | 1.001 | 2 | 50 | 1.009 |
| 1,500 | 0.0589 | 2.885 | 1 | 49 | −1.144 |
| 5,000 | 0.1831 | 9.155 | 10 | 50 | 0.309 |

$\chi^2$ = 2.42; df = 3; *p*-value = 0.4898

**Table C-20. Summary of benchmark concentration (BMC) modeling results for hepatocellular adenomas and carcinomas in male F344 rats exposed to ETBE by whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks; modeled with concentrations as mg/m³ (JPEC, 2010b); benchmark response (BMR) = 10% extra risk**

| Model[a] | Goodness of fit | | | $BMC_{10}$ (mg/m³) | $BMCL_{10}$ (mg/m³) | Basis for model selection |
|---|---|---|---|---|---|---|
| | *p*-value | Scaled residuals | AIC | | | |
| 3 degree | 0.0991 | −0.040, 1.382, −0.897, and 0.048 | 84.961 | 12,300 | 7,251 | Multistage (1 degree) was selected based on lowest AIC |
| 2 degree | 0.264 | 0.000, 1.284, −1.000, and 0.137 | 83.093 | 11,514 | 7,179 | |
| **1 degree** | **0.490** | **0.000, 1.009, −1.144, and 0.309** | **81.209** | **10,884** | **7,118** | |

BMC = benchmark concentration; BMCL = benchmark concentration lower confidence level.

[a]Selected model in bold.

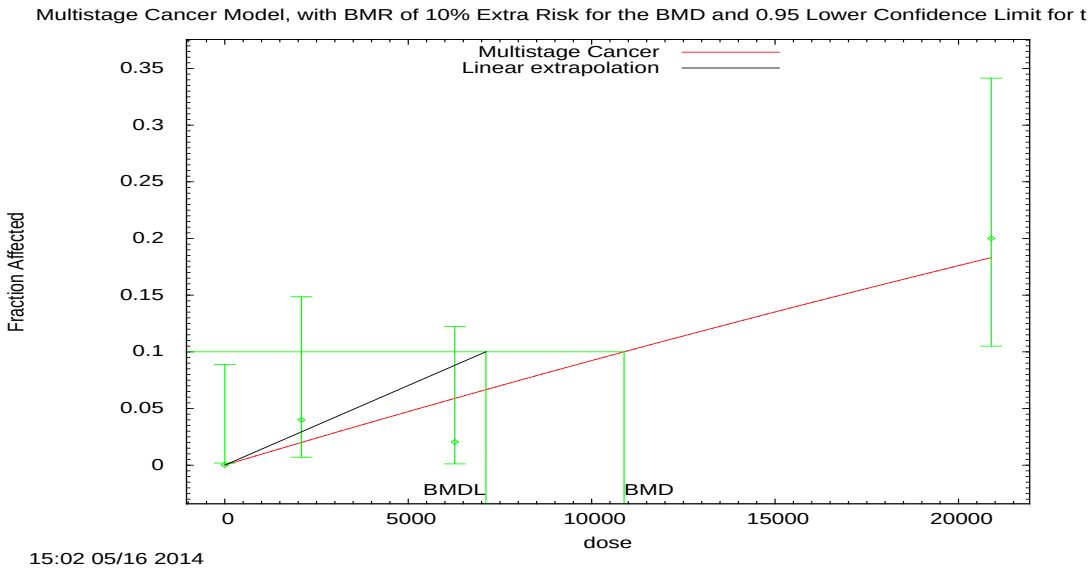



**Figure C-17. Plot of incidence rate by concentration, with fitted curve for the Multistage Model; concentration shown in mg/m³.**

**Multistage Model.** (Version: 3.4; Date: 05/02/2014)

The form of the probability function is:

P[response] = background + (1 − background) × [1 − exp(−beta1 × concentration$^1$− beta2 × concentration$^2$...)]

The parameter betas are restricted to be positive.

**Benchmark Concentration Computation.**

BMR = 10% extra risk

BMC = 10,884.4

BMCL at the 95% confidence level = 7,118.08

BMCU at the 95% confidence level = 19,366.3

Collectively, (7,118.08, 19,366.3) is a 90% two-sided confidence interval for the BMC.

Multistage cancer slope factor = error

**Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|---|---|---|
| Background | 0 | 0 |
| Beta(1) | $9.6799 \times 10^{-6}$ | $1.04989 \times 10^{-4}$ |

**Analysis of Deviance Table**

| Model | Log(likelihood) | No. parameters | Deviance | Test df | *p*-value |
|---|---|---|---|---|---|
| Full model | −38.2989 | 4 | | | |
| Fitted model | −39.6044 | 1 | 2.61098 | 3 | 0.4556 |
| Reduced model | −48.0344 | 1 | 19.4711 | 3 | 0.0002184 |

AIC = 81.2087

**Goodness-of-Fit Table**

| Concentration | Estimated probability | Expected | Observed | Size | Scaled residual |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 50 | 0 |
| 2,089 | 0.02 | 1.001 | 2 | 50 | 1.009 |
| 6,268 | 0.0589 | 2.885 | 1 | 49 | −1.144 |
| 20,893 | 0.1831 | 9.155 | 10 | 50 | 0.309 |

$\chi^2$ = 2.42; df = 3; *p*-value = 0.4897

# APPENDIX D. PATHOLOGY CONSULT FOR ETHYL TERTIARY BUTYL ETHER (ETBE) AND *TERT*-BUTANOL

**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**

WASHINGTON, D.C. 20460

November 28, 2018

**To: John Bucher, NTP**

**From: Kristina Thayer, NCEA-IRIS**

**Subject: Pathology consult for ETBE and tBA**

**Purpose**

The purpose of this memo is to request a consult for pathology-related issues discussed in the ethyl tertiary butyl ether (ETBE) and tert-butyl alcohol (tBA) draft IRIS assessments. This request is being conducted under the existing MOU between EPA NCEA and the National Toxicology Program (NTP) that covers cooperation and communication in the development of human health toxicological assessments.

**Background**

The draft IRIS assessments identify kidney effects as a potential human hazard of ETBE and its metabolite tBA, primarily based on evidence in rats (ETBE and tBA Sections 1.2.1, 1.3.1). EPA evaluated the evidence, including the role of α2u – globulin (in accordance with EPA guidance [U.S. EPA, 1991]) and chronic progressive nephropathy (CPN; for which no formal guidance is available). tBA was determined to induce α2u -globulin mediated nephrotoxicity, however, for ETBE, although increased hyaline droplets of α2u -globulin were observed, data were insufficient to conclude that ETBE induces α2u-globulin nephropathy (only one of the five steps in the pathological sequence, linear mineralization, was consistently observed). Both chemicals show dose-related exacerbation of CPN (increased incidence and/or severity), as well as lesions that are not specifically defined as CPN (increased urothelial hyperplasia of the renal pelvis and suppurative inflammation) but are reported to be associated with late stages of CPN (Frazier et al., 2012). Thus, EPA selected urothelial hyperplasia/transitional epithelial hyperplasia of the renal pelvis as the basis for the reference values for both ETBE and tBA.

The SAB committee reviewing ETBE and tBA was unable to reach a consensus with respect to how the EPA interpreted the ETBE and tBA databases for noncancer kidney effects. There was disagreement within the SAB as to whether any noncancer kidney effects for ETBE and tBA should be considered a hazard relevant to humans. Specifically, the difference in opinion was related to the extent of confidence in the roles that CPN and/or α2u-globulin-based mechanisms played in the development of the renal effects seen with tBA and ETBE.

**Charge Questions**

In this pathology consult, IRIS is seeking additional input on the role that α2u-globulin and CPN play in the observed kidney toxicity. Please consider the following questions and provide references, as applicable, with your responses. Please also comment on any sex-related aspects that are pertinent to these questions.

- Is the etiology of CPN in rats known?
- Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?
- Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u -globulin nephropathy?
- CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2u-globulin related processes. Please comment on the strength of the above proposition.
- It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology?
- Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

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**Attachments**

JPEC (Japan Petroleum Energy Center). (2010a). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Drinking water study). (Study No: 0691).

JPEC (Japan Petroleum Energy Center). (2010b). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Inhalation study). (Study No: 0686).

Kristina Thayer

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February 13, 2019

Kristina Thayer, Ph.D.
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Dear Dr. Thayer,

With respect to your November 28, 2018 request for a pathology consult under the NTP/NCEA Memorandum of Understanding, I asked Dr. Robert Sills, Chief, Cellular and Molecular Pathology Branch to provide responses reflecting the current NTP perspective on the issues you raise. Dr. Sills worked with John Curtis Seely, DVM Diplomate, ACVP Senior Pathologist Experimental Pathology Laboratories, Inc, an internationally recognized expert in rodent renal pathology, to provide answers to your questions.

1. Is the etiology of CPN known?

   The etiology of CPN is unknown (Peter et al., 1986; Hard and Khan, 2004; Hard et al., 2013). Although several theories have been postulated to be the etiology of CPN none have been recognized as the absolute cause of CPN. Factors which have been suggested to be associated with the etiology of CPN include genetics, increased glomerular permeability and dysfunction due to hyperfiltration and functional overload, high renal protein levels, and hemodynamic changes. All of these may influence the progression of CPN but do not appear to initiate renal CPN disease (Baylis, 1984; Barthold, 1998; Abrass, 2000; Hard and Khan, 2004). CPN is a spontaneous and complex degenerative/regenerative disease process influenced by age (incidence and severity increases with age), sex (males affected more than females), and strain (in order of highest to lowest CPN incidence: Sprague-Dawley →Fischer 344 →Wistar rats). It can be modified by diet (increased protein and high caloric intake), hormones (testosterone, estrogen), and many other factors (Seely et al., 2018).

2. Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?

   Yes, the older terminology of "transitional epithelium hyperplasia, renal pelvis" is being updated and replaced by the newer terminology of "urothelial hyperplasia, renal pelvis". Urothelium is recognized as the correct terminology of the epithelium lining the renal pelvis, ureter, urinary bladder and a portion of the urethra (Frazier and Seely, 2018). However, in advanced stages of CPN a type of epithelial proliferation/hyperplasia may be observed along the epithelial lining of the

renal papilla which in some older studies was designated as "urothelial hyperplasia". Recently, the epithelial lining of the renal papilla has been unequivocally demonstrated to represent a type of epithelium different from the urothelium lining the renal pelvis. The difference between urothelium (uroplakin positive) and the epithelium lining the renal papilla (uroplakin negative) was confirmed by immunostaining for uroplakin (a distinct cell marker for urothelium) (Souza et al., 2018).

3. Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al., 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u-globulin nephropathy?

   Renal inflammation is not uncommon in the laboratory rat and can be observed throughout all portions of the kidney. Within the pelvis, inflammation tends to result in a reactive hyperplasia of the urothelium (Seely et at., 2018). Most cases of suppurative inflammation and urothelial hyperplasia are observed as spontaneous changes of undetermined origin. Interstitial mononuclear cell infiltrates are commonly observed in advanced stages of CPN (Frazier and Seely, 2018). However, suppurative inflammation and urothelial hyperplasia are typically unrelated to CPN or, at most, occasionally noted as an uncommon secondary change to CPN. Therefore, CPN does not directly result in suppurative inflammation or urothelial hyperplasia of the renal pelvis in its advanced stages. Cases of suppurative inflammation and urothelial hyperplasia are more likely to be associated with the presence of renal pelvic mineralization, pelvic calculi, or from an ascending bacterial infection or pyelonephritis (Seely et al., 2018). Furthermore, mineralization has been reported to be associated with an increased incidence and severity of spontaneous inflammation and urothelial hyperplasia in the renal pelvis of female rats (Tomonari et al., 2016). In addition, there is no information that appears to support that suppurative inflammation and pelvic urothelial hyperplasia are directly associated with the spectrum of morphological changes associated with α2u-globulin nephropathy (Frazier et al., 2012; Frazier and Seely, 2018).

4. CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2-globulin related processes. Please comment on the strength of the above proposition

   According to the IARC Scientific Publication No. 147 (1999), chemicals which cause α2u-globulin nephropathy are often associated with an accelerated onset and severity (exacerbation) of the cortical changes typical of chronic progressive nephropathy seen in older male rats (Alden et al., 1984; Swenberg and Lehman-McKeenan, 1999; Travlos et al., 2011; Frazier et al., 2012). However, studies on 2-ethoxy-2 methylpropene (ethyl tertiary butyl ether; inhalation and drinking water studies) confirmed the presence of exacerbated CPN in both male and female rats at the highest dose levels (Japan Industrial Safety and Health Association/Japan Bioassay Research Center, 2010[a] 2010[b]). Because of "urothelial hyperplasia" and linear pelvic (papillary) mineralization noted in the male rats from these studies, it was proposed that α2u-globulin nephropathy contributed to the exacerbation of CPN in the males although no pathogenesis of the exacerbated CPN in females was given. Additionally, in these studies, "urothelial hyperplasia" was apparently and according to its description more likely to represent a proliferation of the papillary lining epithelium and not representative of true "urothelial hyperplasia". This proliferative epithelial finding is often observed

as part of advanced cases of rat CPN and has no similarity to any human renal papillary finding (Seely et al., 2018; Souza et al., 2018). Long term exposures to methyl tertiary -butyl ether also resulted in an α2u-globulin nephropathy and exacerbated CPN in both male and female rats (Cruzan et al., 2007). The etiology of exacerbated CPN in females is not known since α2u-globulin nephropathy is regarded as a male only condition. Therefore, although α2u-globulin nephropathy may account for cases of chemically exacerbated CPN, other undetermined factors contributing to CPN exacerbation cannot be discounted (Doi et al., 2007).

5. It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology.

   Yes, the publication by Hard, Johnson, and Cohen makes a very strong case that the renal development, biological behavior, and morphological spectrum of CPN have no analog in the human kidney and that CPN is a distinct entity in the rat. (Hard et al., 2009). Overall, CPN has prominent protein filled dilated tubules, no vascular changes, no immunological or autoimmune basis, and little inflammation which distinguishes CPN from most human nephropathies (Hard et al., 2009). There appears to be nothing in the literature that counters this assumption.

6. Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

   The etiology of CPN is unknown and represents a complex disease process in rats. Given the fact that there is no definitive pathogenesis for this multifactorial disease process, it cannot be fully ruled out that chemicals which exacerbate CPN in rats may have the potential to exacerbate disease processes in the human kidney.

   Please let me know if you have additional questions or wish further clarification of any of these responses.

   Sincerely,

   John Bucher

   John Bucher, Ph.D.
   National Toxicology Program,
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# APPENDIX E. SUMMARY OF EXTERNAL PEER-REVIEW COMMENTS AND EPA'S DISPOSITION

The Toxicological Review of ethyl tertiary butyl ether (ETBE), dated June 2017, underwent a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2015). This peer review was conducted by the Chemical Assessment Advisory Committee (CAAC) augmented for review of the draft Integrated Risk Information System (IRIS) ETBE assessment (SAB-CAAC ETBE panel) of EPA's Science Advisory Board (SAB). An external peer review workshop was held on August 15–17, 2017. Public teleconferences of the SAB-CAAC ETBE panel were held on July 11, 2017, March 22, 2018, March 27, 2018, and June 6, 2018. The Chartered SAB held a public meeting on September 26, 2018 to conduct a quality review of the draft SAB-CAAC peer review report[4]. The final report of the SAB was released on February 27, 2019.

The SAB-CAAC was tasked with providing feedback in response to charge questions that addressed scientific issues related to the hazard identification and dose-response assessment of ETBE. Key recommendations of the SAB[5] and EPA's responses to these recommendations, organized by charge question, follow. Editorial changes and factual corrections offered by SAB were incorporated in the document as appropriate and are not discussed further.

## 1. Literature Search/Study Selection and Evaluation

***Charge Question 1. The section on Literature Search Strategy | Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.***

Key Recommendation: The SAB recommended EPA should provide clarification on the rationales for several decisions that impacted how the literature search was conducted. This includes (1) the

[4]During the quality review by the Chartered SAB, 2 of the 44 members provided dissenting comments related to the cancer weight of evidence descriptors and the quantitative cancer risk estimates for ETBE and tBA. These comments were included as an appendix to the final SAB report and are summarized and addressed following the disposition of the SAB-CAAC recommendations below.

[5]The SAB provided tiered recommendations: Tier 1 (key recommendations), Tier 2 (suggestions), and Tier 3 (future considerations).

rationale for the selection of some synonyms of ETBE as key search words and not others; (2) the rationale for imposing limitations on sources in the first stage of the scientific literature search (i.e., PubMed, Web of Science); and (3) the rationale for limiting the search for additional citations to only some of the publications available in peer-reviewed literature and secondary sources, but not others.

Response: The literature search was developed and executed in consultation with information specialists and librarians through EPA's Health and Environmental Research Online (HERO) database. The process includes developing, testing, and implementing a comprehensive literature search strategy in an iterative and collaborative manner. Responses to the above enumerated SAB concerns follow. (1) The most common synonyms and trade names were used as the keywords in the literature search. This included the preferred IUPAC name of 2-Methoxy-2-methylpropane. (2) PubMed, Web of Science, and Toxline are the core sources that IRIS uses for published studies. Past experience has demonstrated searching of PubMed, Web of Science and Toxline generally provides sufficient coverage for literature pertinent to human health assessments. The TSCATS2 database was included to capture submissions of health and safety data submitted to the EPA either as required or voluntarily under certain sections of TSCA. Based on the attributes of the chemical, along with input from HERO, EPA did not include supplemental databases (e.g., databases for pesticides, U.S. Department of Agriculture -related compounds or inhalation values). (3) To ensure no key studies were missed, a manual search of citations was performed on published reviews and studies identified from public comments, as well as reviews previously conducted by other international and federal agencies. Table LS-2 lists the approach and sources used in the manual searching of citations.

Key Recommendation: The SAB recommended that the EPA address why not all databases were updated through December 2016.

Response: All databases were updated through December 2016 in the external peer review draft assessment; text has been edited to ensure clarity. In addition, a literature update following SAB review was conducted through July 2019. Information on this literature update has been added to the Literature Search Section of the document.

Key Recommendation: The SAB recommended that the EPA address the discrepancy in the number of health effects studies reported in Table LS-1 and in the text.

Response: The number of studies identified has been corrected.

Key Recommendation: The SAB recommended that the EPA clarify why ecological/non-mammalian studies were apparently excluded from any consideration (despite the footnote in Table LS-3).

Response: Regarding the ETBE search strategy, ecological studies and non-mammalian studies were not "excluded" but were instead considered supplemental data. The footnote in Table LS-3 was revised to be more transparent.

## 2. Chemical Properties and Toxicokinetics

***Charge Question 2a. Chemical properties. Is the information on chemical properties accurate?***

Key Recommendation: The SAB offered several specific recommendations for improvement of the chemical properties table generally focused on increasing confidence and transparency in the values presented. The SAB also recommended the use of a template focusing on the chemical properties most relevant to the chemical and the assessment. Several recommendations focused on a preference for the citation of chemical properties from primary sources, for vetting the data in cases in which more than one value is published, and for presenting rationales for the selected values.

Response: In response to SAB comments, EPA has revised the ETBE chemical properties table (Table 1-1) to present average experimental and predicted chemical properties from high quality databases as curated by EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard). The CompTox Chemicals Dashboard aggregates and presents both experimental and predicted chemical property data, with links to the source and/or model data [for details see Williams et al. (2017)]. The experimental data are sourced from publicly available databases as well PHYSPROP downloadable files (Mansouri et al., 2016). Predicted chemical property data are obtained from EPISuite, OPERA models (Mansouri et al., 2016), NICEATM models (Zang et al., 2017), and the Toxicity Estimation Software Tool (TEST) Models (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test).

A key benefit of this aggregation of chemical properties over reporting an individual measurement is a more robust estimate than is possible from an individual study, with each study reporting measurements that are expected to have some degree of error.

***Charge Question 2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?***

Key Recommendation**:** The SAB recommended that the EPA revise the model code to describe metabolism as a function of the free liver concentration, CVL, and that metabolic parameters (e.g., Km or first order rate constants) be re-estimated. Metabolism based upon total liver concentration, CL, is not scientifically correct.

Response: Model code has been revised to describe metabolism as a function of the free liver concentration and the metabolic parameters have been re-estimated. The revised final code is available on HERO (U.S. EPA, 2016).

Key Recommendation: The SAB recommended that the overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and should provide essential information, assumptions, results, and conclusions. The SAB recommended that the text of the PBPK model evaluation report (U.S. EPA, 2017) be included in the Supplemental Information, in which case it would benefit from adding a conclusions section.

Response: PBPK model evaluation for the IRIS assessments of ETBE and *tert*-butanol has been added to the Supplemental Information to the Toxicological Review (See Appendix B.1.7).

***Charge Question 2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?***

Key Recommendation: The SAB recommended not implementing route extrapolation for the oral cancer dose-response analysis for ETBE. Therefore, there was no need for the Agency to select a dose metric.

Response: Route-to-route extrapolation is not implemented, in accordance with SAB recommendation. Consequently, a dose metric wasn't warranted. See the response to Charge Question 4d for further details.

**3. Hazard Identification and Dose–Response Assessment**

***Charge Question 3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.***

Key Recommendation: The SAB was unable to reach consensus on whether noncancer kidney effects should be considered a hazard relevant to humans based on the presented information in the assessment. The SAB recommended EPA strengthen the justification regarding the decision to consider the observed kidney effects as a hazard relevant to humans.

Response: In response to concerns regarding the human relevance of the observed kidney endpoints following ETBE exposure in rats, further expert consultation was requested from the National Toxicology Program (see Appendix D). With this additional expert consultation, the assessment has been revised to strengthen the justification regarding the human relevance of the observed kidney effects. Briefly, dose-related increases in kidney effects (increased kidney weight, increased severity of chronic progressive nephropathy, CPN) were observed in both male and female rats treated with ETBE. While ETBE binds to alpha 2u-globulin and meets some but not all the criteria in the EPA and International Agency for Research on Cancer (IARC) alpha 2u-globulin frameworks [Capen et al. (1999); U.S. EPA (1991); see Section 1.2.1], U.S. EPA (1991) noted that" [i]f a compound induces $\alpha_{2u}$-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occurring in humans" (Section XVIII, p. 89). However, as alpha 2u-globulin nephropathy is strictly a male rat phenomenon, dose-related kidney effects in female rats are not confounded by alpha 2u-globulin nephropathy. With respect to CPN, this condition has no known analogue in the aging human kidney (NIEHS, 2019; Hard et al., 2009) and its etiology is unknown (NIEHS, 2019; Frazier et al., 2012; Hard and Khan, 2004; Peter et al., 1986). However, many of the same lesions observed in CPN (e.g., thickening of tubule basement membranes, tubule atrophy, tubule dilation, and glomerular sclerosis) are also observed in chronic kidney disease in humans (Lusco et al., 2016; Zoja et al., 2015; Frazier et al., 2012; Abrass, 2000). As summarized in the NTP pathology consultation, given that there is no definitive pathogenesis for CPN, it cannot be ruled out that a chemical which exacerbates CPN in rats could also exacerbate existing disease processes in the human kidney (NIEHS, 2019). Therefore, increased incidence of kidney effects with ETBE exposure in the female rat (including increased kidney weight and increased severity of CPN) are considered relevant to humans. See revised text in Sections 1.2.1 and 1.3.1 of the Toxicological Review.

Key Recommendation: The SAB acknowledged the role of alpha 2u-globulin in ETBE-induced nephropathy in male rats was thoroughly considered according to the EPA 1991 criteria, however, the SAB recommended also applying the criteria published by IARC in 1999 (Capen et al., 1999).

Response: The U.S. EPA (1991) and IARC criteria (Capen et al., 1999) are very similar with the IARC criteria having specific criteria pertaining to lack of genotoxicity of parent compound/metabolite and male rat specificity for nephropathy and renal tumorigenicity whereas the EPA framework considers these data as supplemental information (see *Part 4, XVII B. Additional Information Useful*

*for the Analysis*). The assessment was revised in Section 1.2.1 (see *Mode of Action Analysis for Kidney Effects*) to provide additional discussion of the IARC criteria not explicitly required in the EPA 1991 alpha 2u-globulin framework.

***Charge Question 3b. Noncancer toxicity at other sites (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.***

- Liver effects: Suggestive evidence
- Developmental toxicity: Inadequate evidence
- Male and female reproductive toxicity: Inadequate evidence

Key Recommendation: The SAB had suggestions for better describing the overall evidence for male reproductive toxicity as "minimal effects at otherwise toxic dose levels," rather than "inadequate evidence," since the SAB concludes there is an adequate amount of evidence that shows minimal effects, at least in populations with normal ALDH2 function. The SAB also recommended female reproductive toxicity be characterized as "no effects even at otherwise toxic dose levels," rather than "inadequate evidence," since the SAB concludes there is an adequate amount of evidence, which shows minimal effects.

Response: The description of male and female reproductive effects in section 1.2.3 has been revised to be responsive to the SAB's suggested language. Regarding the overall evidence for male reproductive toxicity, although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify male reproductive effects as a potential human hazard of ETBE exposure and male reproductive effects are not carried forward as a hazard. While the ALDH2 knock out data suggest a potential sensitive subpopulation for male reproductive effects, this evidence is considered preliminary (see discussion of these findings in Section 1.2.3, and response to Key Recommendation below).

Regarding the overall evidence for female reproductive effects, although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify female reproductive effects as a potential human hazard of ETBE exposure and therefore, female reproductive effects are not carried forward as a hazard.

Key Recommendation: The SAB recommended that male reproductive effects in genetically susceptible mice be clearly acknowledged in the assessment rather than treated as inconsistent results. The SAB also suggested that an RfC be calculated for the male reproductive effects of ETBE in ALDH2 KO mice, as this may be the most sensitive target for a sensitive subgroup.

Response: Based on the available mechanistic study indicating potentially increased susceptibility for reproductive effects in male mice with impaired acetaldehyde metabolism, text in Sections 1.2.3 and 1.3.3 has been revised to clarify that effects observed in the studies in ALDH2 KO mice may be indicative of increased susceptibility in a small percentage of the US population with inactive *ALDH2* variations. However, these findings are considered to be preliminary due to the small sample size (n=5), single species, and the unconvincing magnitude of many of the statistically significant effects, including the observation that the heterozygotes exhibited more robust changes than the knockout mice. Thus, EPA did not calculate an RfC for these effects.

***Charge Question 3c. Oral reference dose for noncancer outcomes. Section 2.1 presents an oral reference dose of $5x10^{-1}$ mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012; JPEC, 2010a). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.***

Key Recommendation: The SAB recommended that EPA carefully examine the question of the validity and applicability of the endpoints chosen and analyzed for the oral RfD, including the potential for CPN to serve as the mechanism of the kidney effects.

Response: CPN is not a defined mechanism or a more general MOA, rather it is a group of age-related lesions observed in rats and of unknown etiology. However, many of the lesions observed in CPN are also observed in chronic kidney disease in humans (Lusco et al., 2016; Zoja et al., 2015; Frazier et al., 2012; Abrass, 2000). In response to comments regarding the human relevance of the observed kidney endpoints following ETBE exposure in rats (e.g., related to an alpha 2u-globulin mechanism or exacerbation of CPN), further expert consultation was requested from the National Toxicology Program [(NIEHS, 2019); see also Appendix D]. With this additional expert consultation, EPA evaluated the validity and applicability of the observed kidney effects and revised the assessment to strengthen the discussion regarding the human relevance of the various kidney effects. Furthermore, to avoid the uncertainty and confounding by alpha 2u-globulin-related processes in male rats, the assessment has been revised to rely on data sets for kidney toxicity from female rats. See revised text in Sections 1.2.1, 1.3.1, 2.1.4, and 2.2.8 of the Toxicological Review. See also response to Charge Question 3a.

Key Recommendation: SAB recommended that the tables within this section include units for completeness and interpretability and that EPA consider a more integrated presentation of the current text, tables, and graph so as to facilitate better tracking of information without much page-flipping.

Response: Units have been added to the tables where missing, however, endpoints which display changes as "% change relative to control" are unitless. A more integrated presentation of text, tables, and figures is being implemented in future IRIS assessment templates.

***Charge Question 3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 9 x $10^0$ mg/$m^3$, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.***

Key Recommendation: SAB recommended that EPA provide statistical analysis to make clear the rationale for including or excluding studies. The SAB elaborated in the body of the final report that "there does not seem to be any reporting of statistical analysis of individual studies (trend tests or pair wise significance tests, and other statistical tests determined to be appropriate), and this omission hampers consideration of the appropriateness of inclusion and use of studies."

The SAB also recommended the EPA provide statistical analysis to help elucidate differences in response based on sex, further observing that sex differences in response appear more marked for inhalation than for oral exposures. The SAB suggested that an evaluation of possible reasons for this (including mere statistical fluctuation which, if responsible, would suggest averaging endpoint values across sexes) would be informative.

Response: As discussed in the Preamble of this assessment, data analysis is part of the evaluation of study quality of the available literature. Statistical analysis of data (including pairwise tests and trend tests) is predominantly performed and reported by the study authors. Data informative for EPA evaluation of organ/system findings are reported in the evidence tables and data arrays in Section 1 (Hazard Identification) with positive statistical findings highlighted (for example, see Table 1-2, Figures 1-4 and 1-5 for kidney histological effects). When additional data analysis is deemed informative and/or is missing from the publication, further statistical tests will be conducted by EPA and noted in the assessment. For example see "average severity of CPN" from JPEC (2010a, 2010b) denoted in Table 1-2. However, it is important to note that while endpoints

with statistically significant findings (especially those with significant dose response trends) inform the selection of endpoints for the derivation of candidate values, the biological significance and coherence of an outcome is more important (U.S. EPA, 2002). Decisions regarding the rationale for endpoint and study inclusion for dose response assessment for ETBE are discussed in detail in Section 1.3.1 and in Section 2.1.1 and 2.2.1 of the Toxicological Review.

Regarding the SAB recommendation for additional statistical analysis to inform averaging kidney endpoints across sex, pooling kidney endpoints across sexes is not considered appropriate due to biological considerations, specifically, the apparent increased susceptibility of male rats to ETBE-induced kidney effects, potentially related to alpha 2u-globulin binding with ETBE in male, but not female rats. Additional consideration of the human relevance of the kidney effects observed in male and female rats has been added to the assessment (see response to Charge Question 3a). Therefore, to avoid the uncertainty and confounding by alpha 2u-globulin-related processes in male rats, the assessment has been revised to rely on data sets for kidney toxicity from female rats. Please see the revised text in Sections 1.3.1, 2.2.4, and 2.2.8.

***Charge Question 4a. Cancer modes-of-action in the liver. As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPARα, PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.***

Key Recommendation: The SAB recommended that EPA should clarify the evidence needed to conclude that a PPARα, CAR, and/or PXR MOA is operative and to indicate that liver tumors may not be relevant to humans. The SAB suggested that examples, if provided, would be helpful to illustrate the types of studies/information needed to satisfy each criterion, and that EPA should revisit the evaluation of information available for ETBE using these criteria.

Response: Text has been added to clarify additional data gaps (see Section 1.2.2). Briefly, several gaps in the receptor mediated effects data were explicitly noted such as evidence in only one species, lack of any studies in receptor knock-out or humanized mice, lack of dose response concordance between receptor mediated gene changes and tumors, and lack of any receptor mediated data outside of the 1 and 2 week time points, which preclude establishing temporal associations.

Key Recommendation: The SAB recommended that EPA may want to reconsider statements about transient hypertrophy.

Response: Statements regarding transient hypertrophy have been revised and additional information on the observation of related endpoints, such as increased liver weight, has been added for context.

Key Recommendation: The SAB recommended that EPA should revise Table 1-13 and accompanying narrative to be more descriptive regarding availability of information for each MOA and indicate whether studies relevant to the MOA exist, and where results are positive or negative, instead of saying "no positive studies identified".

Response: The table and narrative text have been clarified to indicate the categories under which no pertinent studies were identified.

Key Recommendation: The SAB commented that while acetaldehyde was proposed as a strong candidate MOA for male rat liver tumors in the assessment, the plausibility of this MOA was not well explored. The SAB recommended that evidence for this MOA should be developed and presented more thoroughly; or, alternatively, encouraged the Agency to reduce emphasis on this MOA in the final assessment.

Response: The data for an acetaldehyde based MOA for the observed liver tumors has been evaluated in the Toxicological Review in Section 1.2.2. Although the available evidence suggests a potential role for acetaldehyde in the increased liver tumor response observed in male rats exposed to ETBE, the data are inadequate to conclude that ETBE induces liver tumors via acetaldehyde-mediated mutagenicity. Therefore, emphasis on this MOA and its effect on the assessment conclusions has been reduced throughout the document.

***Charge Question 4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure.*** *Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported*[6]***. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes***

[6]This unbolded segment of the charge question was added by the SAB-CAAC

***of exposure. If another cancer descriptor should be selected, please outline how it might be supported.***

Key Recommendation: The SAB recommended the use of the descriptor "Inadequate Information" for oral ETBE, and "Suggestive Evidence" for inhaled ETBE.

Response: The EPA agrees with the SAB recommendation and has implemented these descriptors in the revised assessment.

Key Recommendation: The SAB recommended that the EPA not use the initiation-promotion assay as key evidence to support a conclusion of carcinogenic potential.

Response: Section 1.2.5 of the Toxicological Review has been revised to clarify that initiation-promotion assays are included only as supplemental studies informing carcinogenicity. Regarding the animal database for carcinogenicity, EPA considers chronic bioassays as key evidence, and other types of studies (including initiation promotion studies, co-carcinogenicity studies, studies in genetically modified animals, etc.) as supplemental lines of information which can aid in the interpretation of more standard toxicological evidence, especially regarding potential modes of action (U.S. EPA, 2005).

Key Recommendation: The SAB recommended that the EPA explain within the assessment that the assigned cancer classifications are an EPA Cancer Guidelines policy-based decision.

Response: "Cancer classifications" or cancer weight of evidence descriptors, are used as part of the hazard narrative to express the conclusion regarding the weight of evidence for carcinogenic hazard potential. Choosing a descriptor is a matter of scientific judgement, not policy, guided by examples and considerations discussed in EPA's Cancer Guidelines (U.S. EPA, 2005).

***Charge Question 4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (U.S. EPA, 2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."***

***Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.***

Key Recommendation: The SAB recommended that the EPA refrain from conducting a quantitative analysis for ETBE carcinogenicity or explain the limitations of the analysis and clearly state that the intended purpose is to simply provide some sense of the magnitude of potential risks.

Response: No quantitative analysis of cancer risk is carried out for oral ETBE exposure. For inhalation exposure, additional text has been added to the assessment to discuss the strengths and limitations of a quantitative analysis of the tumor data and to clarify that the purpose is to provide a sense of the magnitude of a potential cancer risk (this is useful because when no information on the potential magnitude of risk is provided, it generally implies zero risk). See Section 1.3.2 and Section 2.4.1. The assessment also notes the increased uncertainty in this risk estimate because of the suggestive nature of the tumorigenic response (U.S. EPA, 2005).

***Charge Question 4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x $10^{-3}$ per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.***

Key Recommendation: The SAB recommended that since the only available ETBE inhalation cancer bioassay (Saito et al., 2013; JPEC, 2010b) is not suitable for developing an oral cancer slope factor, the EPA should not derive an oral slope factor by route extrapolation absent pharmacokinetic/pharmacodynamic modeling that demonstrates consistency between the oral and inhalation study results. The SAB indicated the following concerns about the use of Saito for route to route extrapolation for developing an oral slope factor: (1) Oral studies did not demonstrate cancer (2) EPA analysis indicated that a consistent dose response relationship could not be observed when comparing across oral and inhalation exposures on the basis of any internal dose measures.

Response: In response to the SAB recommendation, EPA is not carrying out a route to route extrapolation for the derivation of the oral slope factor. Furthermore, there is uncertainty as to whether the liver tumors observed in male rats following inhalation would be reasonably expected following oral exposure as one high confidence oral cancer bioassay (Suzuki et al., 2012; JPEC,

2010a), and a lower confidence chronic oral cancer bioassay (Maltoni et al., 1999) did not observe elevated liver tumors.

***Charge Question 4e. Inhalation unit risk for cancer. Section 2.4 presents an inhalation unit risk of 8 x $10^{-5}$ per mg/$m^3$, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.***

Key Recommendation: The SAB had no specific recommendations; the SAB-CAAC did not reach consensus on this charge question. Some members supported use of the (Saito et al., 2013) study for dose-response assessment recognizing it as a well-designed, well-conducted, and well-reported study and also noting the liver metabolism of ETBE to acetaldehyde, a genotoxic carcinogen. Other members believed the ETBE concentration which induced liver tumors to be excessively high, with significantly elevated tumors only in one sex, at one dose, and questioned whether modeling a single positive concentration would produce a meaningful inhalation unit risk.

Response: Text has been added to the assessment to more clearly denote the strengths and the uncertainties in the data used to derive the inhalation unit risk (see Section 1.3.2, *Biological Considerations for Dose-Response*). Briefly, while liver adenomas were primarily observed at the highest dose in male rats, three liver adenomas were also observed at the lower two concentrations, resulting in a significant positive exposure-response trend ($p < 0.001$ with Peto's test). The Saito et al. (2013) study was considered appropriate for the basis of a quantitative cancer estimate as it is a well-designed, conducted and reported study which included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results. Decreased body weight gain and survival was noted in the high dose males and females; however, the study authors did not detect changes to the animals' general condition (e.g., abnormal behavior or clinical signs) associated with ETBE. Similar decreases in body weight were observed in male (75% of control) and female animals (78% of control), although significantly increased liver tumors were only observed in male rats. Given the lack of overt toxicity and no apparent alterations in toxicokinetics, the ETBE concentrations were not considered to be excessively high. Additionally, text describing the suitability of the study, its utilization in deriving a cancer risk estimate, and the characterization of a cancer value considering the suggestive nature of the cancer potential are further discussed in Section 2.4.

***Charge Question 5. Question on Susceptible Populations and Lifestages***
***Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.***

Key Recommendation**:** The SAB recommends that the Agency clearly describe the uncertainty between oral exposure and other routes of exposure in the ETBE assessment and provide relevant positions with respect to differences in expected outcomes.

Response: Text has been added to this section to highlight the uncertainty pertaining to the fact that the available database to inform early life susceptibility of ETBE is limited to the oral route of exposure.

Key Recommendation: The SAB recommended the Agency identify susceptible populations and incorporate information about them into the ETBE assessment to improve the scientific concepts of the assessment. Specifically, the SAB recommended discussing individuals with noncoding region variants in *adlh2*, which could potentially affect gene expression, as well as discussing individuals with other variants in alcohol metabolism who may be affected by ETBE exposure.

Response: Increased discussion of these additional potentially susceptible populations has been added to the document.

Key Recommendation: The SAB recommends that information regarding lifestages should be included in the assessment.

Response: Discussion of data informing potential early life susceptibility to ETBE has been added to this section.

***Charge Question 6. Question on the Executive Summary***
***The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.***

Key Recommendation: The SAB advises that it will be important for the final Executive Summary to highlight the consequences of alternative choices for the final assessment, especially when these hinge on decisions made about the interpretation and relevance of key toxicity endpoints that have been contested.

Response: Text has been added to the Executive Summary to more clearly highlight the context around the interpretation and relevance of key endpoints such as the human relevance of the observed kidney effects (see *Key Issues Addressed in Assessment*).

**Comments from two members of the Chartered SAB during the QA Review of the SAAB CAAC Peer Review Report**

The Chartered SAB is tasked with conducting quality reviews of draft SAB reports to determine if they are ready for transmittal to the Administrator, reviewing whether the charge questions were adequately addressed by the CAAC, whether the report has technical errors or omissions, if the report is clear and logical, and if the CAAC recommendations in the report are supported by the body of the draft report. During this quality review of the draft SAB-CAAC report on the Draft IRIS assessments of ETBE and *tert*-butanol, two members of the chartered SAB (44 total members) disagreed with the CAAC regarding the recommendation for the cancer weight of evidence descriptors for ETBE and tBA. These two members provided additional comments which were included as Appendix C of the Final SAB report. A summary and response to their comments, as they pertain to ETBE, are included below.

Comment: Two members of the chartered SAB disagreed with the SAB-CAAC's support of EPA's cancer weight of evidence descriptor of "suggestive evidence" for ETBE. They stated ETBE should be characterized as "insufficient evidence" (presumably analogous to EPA's cancer weight of evidence descriptor for "inadequate evidence") because liver tumors were observed only in male rats at the highest exposure concentration in the JPEC (2010b) inhalation bioassay, a concentration they characterize as beyond the maximum tolerated dose (MTD) due to a 25% reduction in body weight. In addition, two chronic oral bioassays were negative for liver tumors.

Response: The SAB-CAAC agreed with the cancer weight of evidence descriptor of "suggestive evidence" (See Charge Question 4b) for the inhalation route of exposure, as the database was consistent with this descriptor as illustrated in the Cancer Guidelines, based on the occurrences of tumors in one sex of one species. Briefly, a statistically significant increase in liver tumors was observed in male rats exposed to ETBE by inhalation (primarily, but not exclusively at the high dose) with the incidence of combined adenomas and carcinomas of 0/50, 2/50, 1/50 and 10/50 at 0, 2,090, 6,270, 20,900 mg/m$^3$, resulting in a statistically significant, positive exposure-response trend (Peto's test $p < 0.001$).

Regarding the assertion that the highest inhalation dose in the Saito et al. (2013) study exceeded the MTD, EPA's 2005 Cancer Guidelines discuss the determination of an "excessively high dose" and

describe the process as one of expert judgment which requires that "...adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent." In the case of the Saito et al. (2013) inhalation study, the study authors did not report any overt toxicity or altered toxicokinetics at the high dose. In addition, the high-dose female rats had a similar reduction in body weight (22%) and no liver tumors were observed (see discussion in Section 1.2.2). Discussion regarding the cancer descriptor for the inhalation route of exposure, the rationale for deriving the inhalation unit risk (including consideration of potential excessively high dose), and the characterization of the cancer risk estimate can be found in Sections 1.3.2 and 2.4.1, and in response to comments under Charge Questions 4b, 4c, and 4e.

With regard to the comments on the cancer descriptor and the oral cancer studies, the SAB-CAAC recommended EPA's cancer weight of evidence descriptor of "inadequate evidence of carcinogenic potential" for the oral route of exposure. EPA agreed and revised the assessment accordingly. Thus, a cancer risk estimate for the oral route was not derived. See Sections 1.3.2 and 2.3, and responses under Charge Questions 4b and 4d.

# APPENDIX F. QUALITY ASSURANCE (QA) FOR THE IRIS TOXICOLOGICAL REVIEW OF ETHYL TERTIARY BUTYL ETHER

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.1) and follows the specifications outlined in EPA Order CIO 2105.1.

As required by CIO 2105.1, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using Guidance for Developing Quality Systems for Environmental Programs (QA/G-1). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of Ethyl-Tertiary Butyl Ether has been designated as Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA Guidance for Quality Assurance Project Plans (QA/G-5), and the latest approved version is dated March 2020. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

| Title | Document number | Date |
|---|---|---|
| Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program | L-CPAD-0030729-QP-1-4 | April 2021 |
| An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry | L-CPAD-0032188-QP-1-2 | December 2020 |

| | | |
|---|---|---|
| and Mechanism-Based Models (PBPK) | | |
| Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS) | L-HEEAD-0032189-QP-1-2 | September 2020 |
| Contractor QAPP 1 | B-IRISD-0030538 | |
| Contractor QAPP 2 | B-IRISD-0030622 | |

During assessment development, this project underwent three quality audits during assessment development including:

| **Date** | **Type of audit** | **Major findings** | **Actions taken** |
|---|---|---|---|
| August 2020 | Technical System Audit | None | None |
| August 2019 | Technical System Audit | None | None |
| June 2018 | Technical System Audit | None | None |

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review is subjected to external reviews by other federal agency partners, including the Executive Offices of the White House. Comments during these IRIS process steps are available in the docket EPA-HQ-ORD-2009-0229 on regulations.gov.

During Step 4 of assessment development, the IRIS Toxicological Review of Ethyl-Tertiary-Butyl Ether underwent public comment from May 16, 2016 to July 15, 2016. Following this comment period, the toxicological review underwent external peer review by SAB in June 2017. The peer review report is available on the U.S. EPA website (https://yosemite.epa.gov/sab/sabproduct.nsf/0/8e4436d62da1fd2d85257e38006a3131!OpenDocument&TableRow=2.3#2.). All public and peer-review comments are available in the Docket EPA-HQ-ORD-2009-0229 on regulations.gov.

Prior to release (Step 7 of the IRIS Process), the final toxicological review is submitted to management and QA clearance. During this step, the CPHEA QA Director and QA Managers review the project QA documentation and ensure that EPA QA requirements have been met.

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