PK/PBPK MODEL EVALUATION FOR THE IRIS ASSESSMENTS OF

ETHYL TERTIARY BUTYL ETHER
(CASRN 637-92-3)

AND

tert-BUTYL ALCOHOL
(CAS No. 75-65-0)

Prepared by the United States EPA Pharmacokinetics Working Group

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Introduction

Physiologically based pharmacokinetic (PBPK) models of the rat are used to perform route-to-route extrapolation of toxicological data for ethyl tertiary butyl ether (ETBE) and tert-Butyl Alcohol (*tert*-butanol). For ETBE, inhalation-to-oral extrapolation was performed using the ETBE metabolism rate as the internal dose metric. For TBA, oral-to-inhalation extrapolation was performed using the concentration of *tert*-butanol in blood as the internal dose metric. Overviews of ETBE and *tert*-butanol toxicokinetics, as well as the scientific rationale for selecting the internal dose metrics, are available in the respective toxicological assessments. Because the existing human PBPK model was not considered adequate (see below), default methodologies were applied to extrapolate toxicologically equivalent exposures from adult laboratory animals to adult humans. For inhalation exposures, the interspecies conversion was the ratio of animal/human blood:air partition coefficients (L_A/L_H), according to RfC guidelines for Category 3 gasses (U.S. EPA, 1994). For oral exposures, extrapolation is performed by body-weight scaling to the ¾ power ($BW^{3/4}$) (U.S. EPA, 2011).

All available PBPK models of ETBE and its principal metabolite *tert*-butanol were evaluated for potential use in the assessments. 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 measurements of blood concentrations of eight 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 were calculated from individual data on body weight and height. Additionally, to account for physical activity, blood flows to tissues were 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 use of the model in animal-to-human extrapolation for that route.

A number of PBPK models were developed previously for the related compound, methyl tertiary butyl ether (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 U.S. 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 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 2-methyl-1,2-propanediol (MPD) and hydroxyisobutyric acid (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 *ter*-butanol, the Leavens and Borghoff (2009) model has three features not included in the Blancato et al. (2007): (1) the alveolar ventilation was reduced during exposure, (2) the rate of *tert*-butanol metabolism increased over time due to account for induction of CYP enzymes, and (3) binding of MTBE and *tert*-butanol to α_{2u}-globulin was simulated in the kidney of male rats. The Blancato et al. (2007) model was configured through EPA's PBPK modeling framework, ERDEM (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 α_{2u}-globulin in the kidneys of male rats were incorporated in the PBPK model of MTBE by Leavens and Borghoff (2009). Binding to α_{2u}-globulin is one hypothesized mode of action for the observed kidney effects in MTBE-exposed animals. In the Leavens and Borghoff (2009) model, binding of MTBE to α_{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 α_{2u}-globulin binding. Moreover, as discussed below, the U.S. 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). 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.

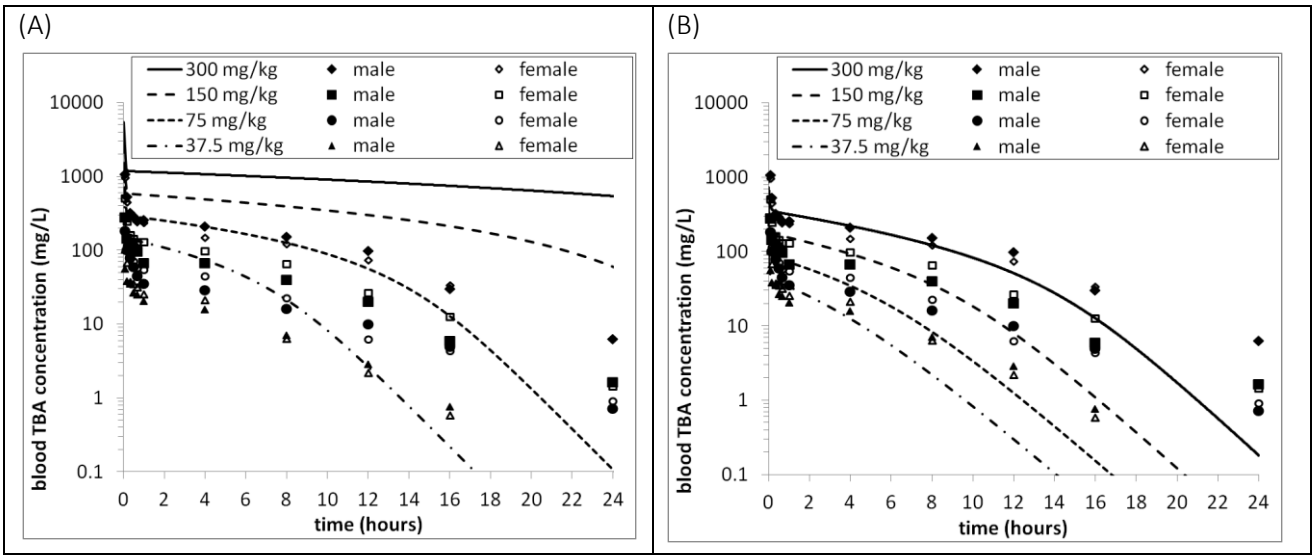


Figure 1. Comparison of the *tert*-butanol portions of existing MTBE models with *tert*-butanol blood concentrations from i.v. exposure by Poet et al. (1997). Neither the (A) Blancato et al. (2007) nor the (B) Leavens and Borghoff (2009) model adequately represents the measured tert-butanol blood concentrations.

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, which *tert*-butanol can enter reversibly, represented by a differential mass balance (see Figure 2). Other differences in model structure are that the brain was included in the other richly perfused tissues compartment and binding to α_{2u}-globulin was not included. Binding to α_{2u}-globulin was neglected since it was assumed to not significantly affect the blood concentration or metabolic rate of ETBE of 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 oral gavage exposures. The ETBE sub-model 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).

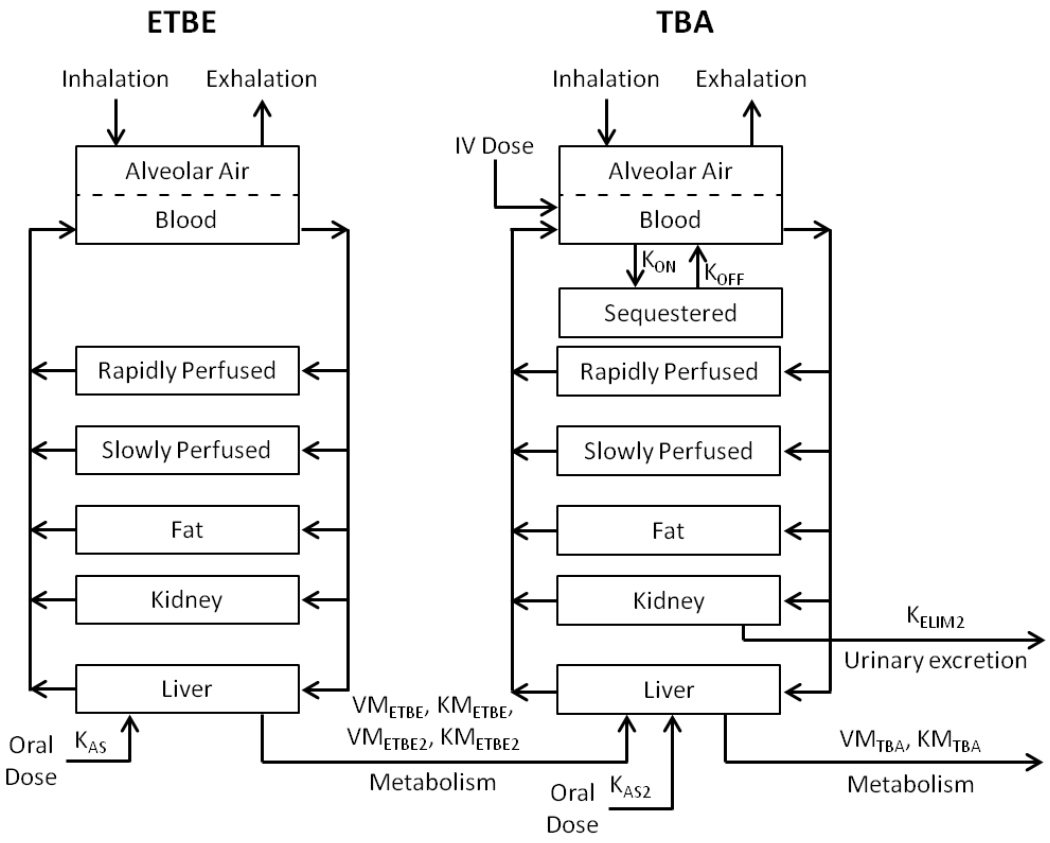


Figure 2. Schematic of the Salazar et al. (2015) PBPK model for ETBE and its major metabolite *tert*-butanol in rats. Exposure can be via multiple routes including inhalation, oral, or i.v. dosing. Metabolism of ETBE and tert-butanol occur in the liver and are described by Michaelis-Menten equations 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 α_{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 re-parameterized 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 Salazar et al. (2015) 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 metabolic parameters. Rate constants for binding of ETBE to α_{2u}-globulin and its dissociation were assumed to be the same as estimated for MTBE by Leavens and Borghoff (2009). Finally, unlike the Leavens and Borghoff (2009) model, 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, and k is the rate constant for the ascent to maximum induction. Because metabolic induction does not occur instantaneously, but involves a delay for induction of RNA transcription and translation, Borghoff et al. (2016) assumed that induction did not begin until 24 hour after the beginning of exposure. But the computational implementation then treated the effect as if the enzyme activity suddenly jumped each 24 h to the level indicated by the time-dependent equation shown in the paper. This step-wise increase in activity was not considered realistic. Therefore, in evaluating its impact, the U.S. EPA treated the induction as occurring continuously with time, but beginning at 12 h after the start of exposure. This change would not impact long-term steady-state or periodic simulations, in particular those used to characterize bioassay conditions, but has a modest effect on simulations at shorter times, used for model validation. However, as detailed below, with further review of the existing data on liver histology, which would also reflect metabolic induction if it occurs, and the pharmacokinetic data on which the induction sub-model was based, the U.S. 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.

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) claim that an allometric coefficient of 18.9 L/h/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/h 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/(100g BW). From the text immediately following this table, it is clear that this mean and range 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. Hence use of 18.9 L/h/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. It can be noted that 31.5 ml/min/(100g BW), the actual lower limit, equals 18.9 L/h/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 vs. data below, it appears that model fits to the data would be improved by further decreasing ventilation, which could now be justified. But the U.S. EPA has chosen to keep the value of QPC and absorption fraction as published by Borghoff et al. (2016) for current review purposes.

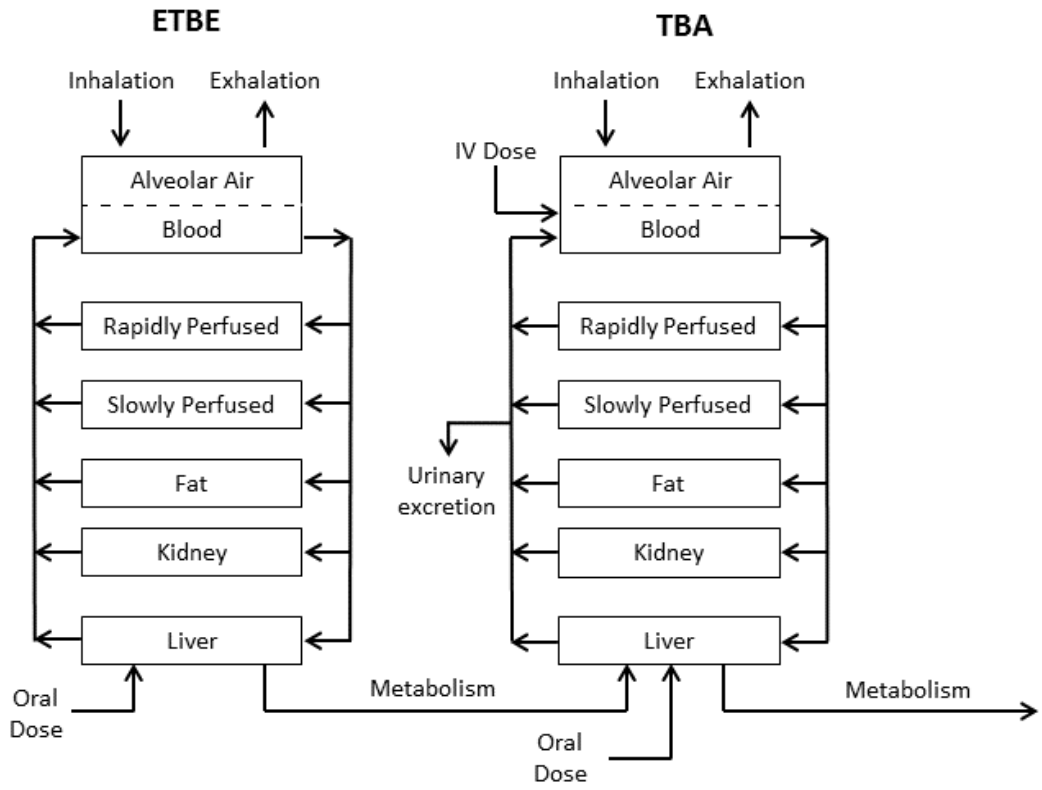


Figure 3. Schematic of the Borghoff et al. (2016) PBPK model for ETBE and its major metabolite *tert*-butanol in rats.

Table 1. PBPK model physiologic parameters and partition coefficients*

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	0.35xBW + 0.00205	Brown et al. (1997)
Richly perfused (total)	0.136	Brown et al. (1997)
Richly perfused	0.0177	[a]
Poorly perfused (total)	0.757	Brown et al. (1997)
Poorly perfused	0.75495 - 0.35xBW	
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/hr-kg)	18.9	Brown et al. (1997)[b]
Alveolar ventilation (L/hr-kg)	18.9	Brown et al. (1997)[b]
Liver	0.174	Brown et al. (1997)[c]
Kidney	0.141	Brown et al. (1997)
Fat	0.07	Brown et al. (1997)
Richly perfused (total)	0.47	[d]
Richly perfused	0.155	[e]
Poorly perfused (total)	0.53	Brown et al. (1997)
Poorly perfused	0.46	[f]
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	[g]
Kidney:blood	2.9	[h]
Partition coefficients for *tert*-butanol		
Blood:air	481	Borghoff et al. (1996)
Liver:blood	0.83	Borghoff et al. (1996)
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)

*Values have been updated to incorporate corrections from a QA review and to include values to the number of digits used in the model code.

[a]0.165 - Σ(kidney,liver.blood)

[b]Lower limit of alveolar ventilation for rat reported in Brown et al. (1997); alveolar ventilation is set equal to cardiac output. Note: Borghoff et al. (2016) contains an allometric scaling error (see text).

[c]sum of liver and gastrointestinal (GI) blood flows.

[d] Brown et al. (1997) only accounts for 94% of the blood flow. This assumes unaccounted 6% is richly perfused.

[e] 0.47- Σ(kidney, liver)

[f] 0.53- fat

Table 2. 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)
Michelis–Menten constant (µmol/L)	379	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/day)[1]	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)
Michelis–Menten constant for ETBE (µmol/L)	1248	Rao and Ginsberg (1997)
α2u-globulin binding parameters		
Steady-state free kidney α2u-globulin (µmol/L)	550[2]	Leavens and Borghoff (2009)
First order constant for hydrolysis of free α2u (1/h)	0.31	Leavens and Borghoff (2009)
First order constant for hydrolysis of bound α2u (1/h)	0.11	Leavens and Borghoff (2009)
Second order binding constant for TBA to α2u (L/µmol/h)	1.3	Leavens and Borghoff (2009)
α2u dissociation constant for TBA (µmol/L)	120	Leavens and Borghoff (2009)
First order constant for unbinding of TBA from α2u (1/h)	calculated[3]	
Second order binding constant for ETBE to α2u (L/µmol/h)	0.15	Leavens and Borghoff (2009)
α2u dissociation constant for ETBE (µmol/L)	1	Leavens and Borghoff (2009)
First order constant for unbinding of ETBE from α2u (1/h)	calculated[4]	

[1]Note: model revised from a daily stepwise induction change to a continuous change (with a 12-hour time lag), while still maintaining the default parameters.
[2]Based on values ranging from ~160 to 1000 µmol/L (Carruthers et al., 1987; Charbonneau et al., 1987; Olson et al., 1987; Stonard et al., 1986).
[3]Product of α2u dissociation constant for TBA and second order binding constant for TBA to α2u.
[4]Product of α2u dissociation constant for ETBE and second order binding constant for ETBE to α2u.

Evaluation of evidence for induction of liver enzymes following ETBE or TBA exposure in rodents

Induction of liver cytochrome p450 (CYP) mixed-function oxidase (MFO) enzymes in rats has been reported following exposure to high ETBE concentrations, while exposure to lower concentrations was associated with more limited or transient effects. Four days of exposure to 200 or 400 mg/kg-d via i.p. administration did not significantly increase liver microsomal monooxygenase activity in male Sprague-Dawley rats, while 2 days of 2 ml (in 50% corn oil solution)/kg-d increased CYP2B1/2 (7-pentoxyresorufin-O¬-dealkylase [PROD], 16β-testosterone hydroxylase) and CYPE1 (p-nitrophenol hydroxylase) activity, and appeared to elevate total liver CYP450 content (~60% increase, not statistically significant: Turini et al. (1998). Consistent with these observations, 1 week of exposure to 300 mg/kg-d ETBE via gavage increased CYP2B1/2 and CYP2C6 mRNA expression in male F344 rats, and increased liver CYP450 content

by 66%; 1 week of exposure to 2000 mg/kg-d increased liver mRNA and/or protein levels of CYP1A1, CYP2B1/2, CYP2C6, CYP2E1, CYP3A1, and increased liver CYP450 content by nearly 3-fold (Kakehashi et al., 2013). Furthermore, while only CYP2C6 mRNA expression remained elevated after 2 weeks of exposure to 300 mg/kg-d, the pattern of liver enzyme induction was maintained after 2 weeks of exposure to 2000 mg/kg-d (Kakehashi et al., 2013). Similar studies in mice were not identified. While no studies were identified reporting rat liver microsomal enzyme expression levels following subchronic or longer durations of exposure, sustained induction of liver microsomal enzyme activity would be expected to manifest some manner of liver histopathology (Maronpot et al., 2010; U.S. EPA, 1998; NTP, 1995). Following exposure to ETBE, incidence of centrilobular hepatocellular hypertrophy incidence was increased in both sexes of rats after 13-week inhalation and 26-week oral exposures, but typically only in the highest exposure groups also experiencing increased relative liver weights, and not in any groups following 2-years of oral or inhalation ETBE exposure, suggesting a sustained albeit resolving, high-dose effect. Of the enzymes described above, CYP2B1 contributes the most activity to ETBE metabolism in rats, with CYP2E1 unlikely to contribute significantly to ETBE metabolism in humans, rats or mice.

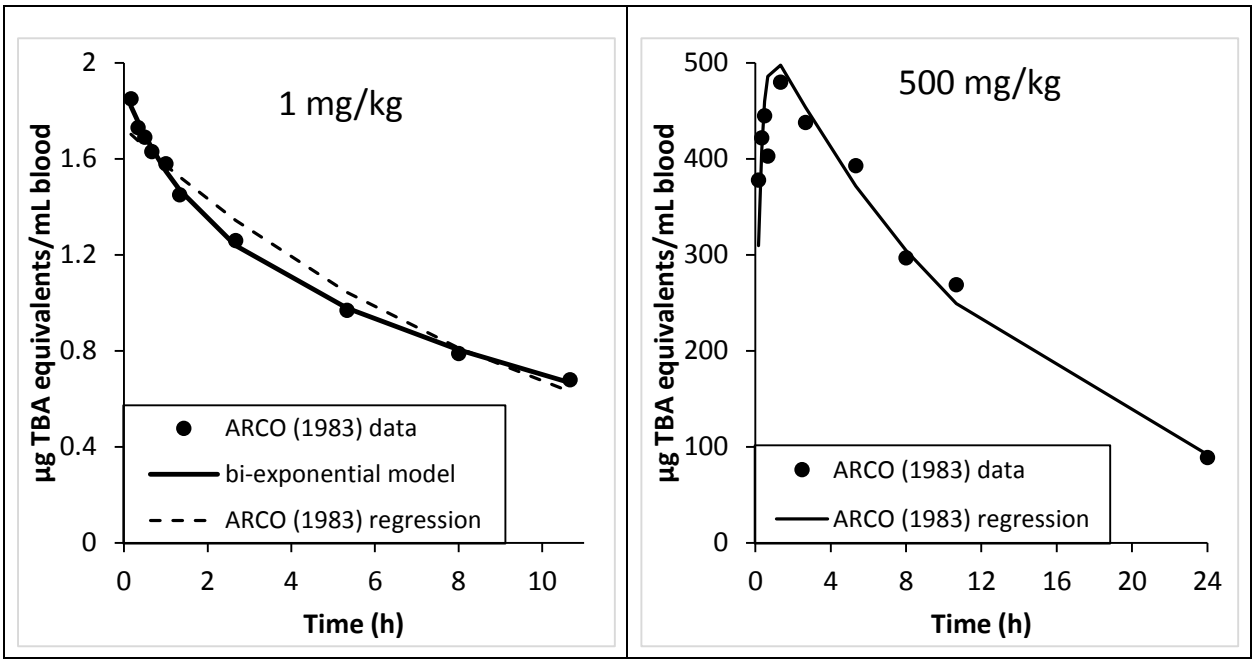
Unlike ETBE, no studies identified have reported induction of rat liver microsomal monooxygenase activity following exposure to tert-butanol. Similar to ETBE, four days of exposure to 200 or 400 mg/kg-d via i.p. administration did not significantly increase liver microsomal monooxygenase activity in male Sprague-Dawley rats, but unlike with ETBE, a higher concentration (i.e. 2 days of 2 ml/kg-d) was not evaluated (Turini et al., 1998). Following subchronic and chronic exposure to tert-butanol in toxicology bioassays, the relative liver weights of male and female F344 rats were increased following 13 weeks of exposure to 290 – 3620 mg/kg-d, and were significantly increased after 15 months of exposure to 420 – 650 mg/kg-d; however, there were no liver histopathological effects reported (NTP, 1995), unlike the increase in centrilobular hepatocellular hypertrophy observed following exposure to ETBE. A single study was identified reporting liver microsomal enzyme activity in female B6C3F1 mice. Three days of drinking water exposure to 344 or 818 mg/kg-d did not affect total CYP protein levels, CYP activity, or expression, while 14 days of drinking water exposure to 418 mg/kg-d modestly increased 7-benzoxyresorufin-O-debenzylase (BROD) activity and CYP2B10 expression, and exposure to 1616 mg/kg-d increased total liver CYP450 content, BROD and PROD activity, as well as CYP2B9/10 expression (Blanck et al., 2010). No changes were reported in CYP1A1 activity, unlike the that reported in rat livers following a similar duration of exposure to 2000 mg/kg-d ETBE, and further direct comparisons are not possible because the expression of CYP2B1/2, CYP2C6, CYP2E1, and CYP3A1 were not evaluated in mice (Blanck et al., 2010). Similar to rats following subchronic exposure to ETBE, centrilobular hepatocellular hypertrophy was reported in 2/5 high-dose mice exposed for 14 days (Blanck et al., 2010); however, incidence of this lesion was not increased in any other rodent study, and the only liver histopathological effect reported following extended exposure was increased incidence of fatty liver in male mice exposed to ~2110 mg/kg-d for 104 weeks (NTP, 1997, 1995). Furthermore, liver weight was not increased in male

or female mice exposed for 2.5 – 104 weeks to ETBE concentrations up to 3000 mg/kg-d (NTP, 1997, 1995). Although liver enzyme levels and activity were not specifically evaluated following subchronic to chronic exposure, the lack of liver pathology suggests a comparable lack of enzyme induction. Therefore, the available evidence suggests that tert-butanol induces a fairly limited set of liver microsomal enzymes in mice, which may be a transient, high-dose effect. While continuous exposure has been reported to increase tert-butanol elimination in mice (McComb and Goldstein, 1979), unlike ETBE, no studies have identified specific liver microsomal enzymes responsible for biotransforming tert-butanol (TBA Supplemental Information, Section B.1.3). Further, McComb and Goldstein (1979) used a continuous exposure pattern starting ~ 1200 ppm (50 µmol/L) which was increased each day to ~ 2800 ppm tert-butanol to achieve the increased elimination rate. Hence the effect was only observed at concentrations an order of magnitude higher than those at which the PBPK model will be used for route-to-route extrapolation.

Given these observations, it appears that inclusion of metabolic induction in the ETBE/tert-butanol PBPK model (for elimination of tert-butanol) is not sufficiently supported. Further, the impact of this mechanism on model fits to repeated dose data, as shown in the scoping document, are only modest for tert-butanol inhalation exposure, and if anything degrade model fits to repeated ETBE oral gavage.

Toxicokinetic data extraction and adjustments

The ARCO (1983) study reported *tert*-butanol blood levels after oral 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 were also reported, although not at identical time-points. Therefore, empirical bi-exponential curves (Figure 3) were used to interpolate between the time-points when total *tert*-butanol equivalents were measured to estimate total equivalents at other times. The total equivalents calculated this way were then multiplied by the fraction of TBA reported at 0.5, 3, 6, and 12 h for 1 mg/kg (ARCO (1983), Table 24) and 500 mg/kg (ARCO (1983), Table 25) to obtain the data used for PBPK modeling (Table 4).



Time-course data and empirical regressions for TBA 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).

Figure 4. TBA PK Data for 1 and 500 mg/kg Oral Exposures from ARCO (1983).

The single-dose data from JPEC (2008b) 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/d, 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)*(1000 mL/L)*(10^{-6} mg/ng)*(74.12 [MW *tert*-butanol])/(102.17 [MW ETBE]) = 0.302 mg *tert*-butanol-eq/L. Likewise the data for the repeated dose study JPEC (2008a), 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,b) 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,b), the concentration of *tert*-butanol in plasma is approximately 130% of the concentration in whole blood (Table 5). 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.

1 Table 3. Summary of pharmacokinetic data used for model calibration and evaluation

Exposure		Measured		Data source	Fig. # in Salazar et al. (2015)	Conversion	Notes
Chemical	Route	Chemical	Medium				
TBA	iv	TBA	blood	Poet et al. (1997) Fig. 1 & 2	3A	µM to mg/L	digitized from the figure
	inhalation	TBA	blood	Leavens and Borghoff (2009) Fig. 8A-B	3B	µM to mg/L	digitized from the figure, showing only 1 day of exposure
	oral gavage	TBA	blood	ARCO (1983), % total TBA, Tables 24-25; TBA equivalents, Fig 6	3C	TBA equivalents to TBA concentration	
ETBE	oral gavage	TBA	blood	JPEC (2008b) Appendix 12	4A	ETBE equivalents to mg/L TBA	"P5" is TBA
		TBA	urine	JPEC (2008b) 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 Fig 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) Fig 8B	5A-B	µM to mg/L	digitized from the figure
		TBA	blood	Leavens and Borghoff (2009) Fig 8A	5C-D	µM to mg/L	digitized from the figure
ETBE	oral gavage	TBA	blood	JPEC (2008b) Appendix 12	5E	ETBE equivalents to mg/L TBA	"P5" is TBA

2

Table 4. Conversion of ARCO (1983) total TBA equivalents and serum fraction data to TBA concentrations

Time (h)	% TBA[1]	Total TBA equivalents interpolated (µg/ml)[2]	TBA concentration using interpolated equivalents (µg/mL = mg/L)[3]	Total TBA equivalents measured at nearest time-point (time measured)[4]	TBA concentration using nearest time-point (mg/L)[5]
			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

[1] From Table 24, p. 48 of ARCO (1983) (1 mg/kg) and Table 25, p. 49 of ARCO (1983) (500 mg/kg)
[2] Using bi-exponential functions given in the legend of Figure B-new
[3] Values used in PBPK modeling; %TBA × total TBA equivalents interpolated
[4] From Table 14, p. 32 of ARCO (1983) (1 mg/kg) and Table 11, p. 27 of ARCO (1983) (500 mg/kg)
[5] %TBA × total TBA equivalents at nearest time-point

1 Table 5. Ratio of ^{14}C activity in blood vs. plasma after ^{14}C-ETBE exposures in rats (JPEC 2008a,b)

Time (h)	Animal #	Plasma (ng ^{14}C-eq/mL)	Blood (ng ^{14}C-eq/mL)	Plasma/Blood (%)
Single dose, JPEC (2008b) Appendix Table 5, p. 94				
8	97	78133	40667	192.1%
	98	95533	80000	119.4%
	99	89367	64667	138.2%
	100	72400	62333	116.2%
24	37	10900	8800	123.9%
	38	19133	14433	132.6%
	39	19433	15400	126.2%
	40	30767	22967	134.0%
72	41	2133	1600	133.3%
	42	2833	3033	93.4%
	43	4033	3200	126.0%
	44	3167	2333	135.7%
			Mean ± SD	**130.9 ± 22.8%**
Single dose, JPEC (2008b) Appendix Table 3, p. 91				
8	17	2853	1784	159.9%
	18	2850	1802	158.2%
	19	2629	1568	167.7%
	20	3918	2718	144.2%
24	21	1692	1255	134.8%
	22	846.7	642.9	131.7%
	23	1048	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%
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, JPEC (2008a), Appendix Table 3, p. 49				
8 (7 days dosing)		3789	3029	125.1%
		5041	3988	126.4%
		4914	3938	124.8%
		5608	4638	120.9%
24 (7 days dosing)		2740	1908	143.6%

		3433	2575	133.3%
		2488	1888	131.8%
		963.3	812.5	118.6%
8 (14 days dosing)		5665	4546	124.6%
		5175	4075	127.0%
		3889	3058	127.2%
		5090	3858	131.9%
24 (14 days dosing)		2003	1508	132.8%
		2121	1692	125.4%
		1948	1354	143.9%
		1037	804.2	128.9%
72 (14 days dosing)		1378	1138	121.1%
		301.3	245.8	122.6%
		110	N.D.	
		421.3	337.5	124.8%
			Mean ± SD	**128.1 ± 6.85%**

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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, Alabama).

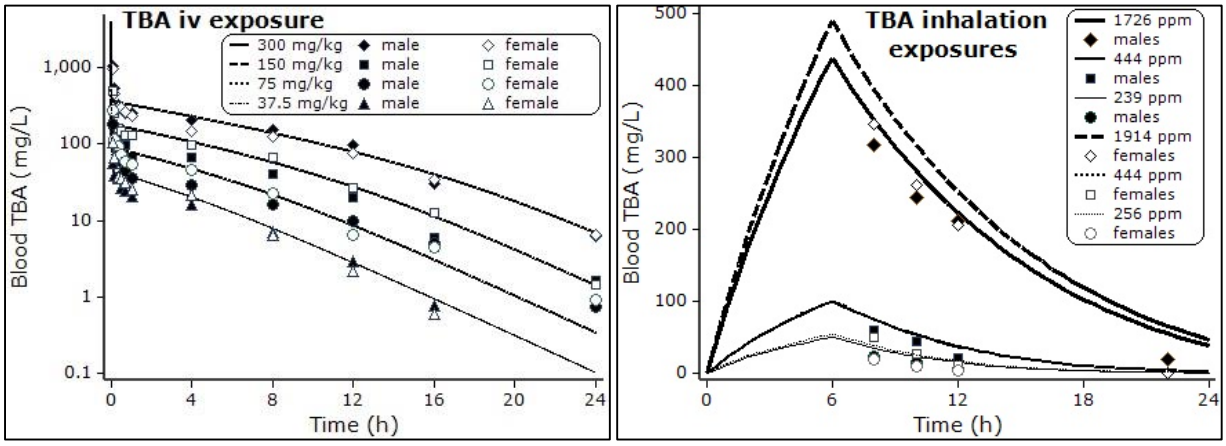
The following modifications were made:

1- Periodic drinking water pathway was incorporated into the 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 h.
3- Otherwise enzyme induction was not used (set to zero).
4- Tissue volumes and the rate of hydrolysis of free α2u-globulin were corrected (slightly) to values shown in Table 1.
5- 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.
6- Digitized data from Amberg et al. (2000) were updated subsequent to a QA review.
7- Tabulated data from Borghoff and Asgharian (1996) were updated subsequent to a QA review.

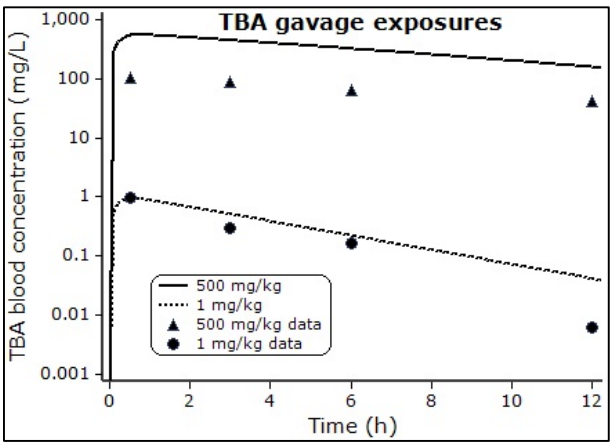
The PBPK acslX model code is available electronically through EPA's Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2016). The model contains workspaces for the EPA implementation of Salazar et al. (2015) model, the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

Selected model outputs compared to experimental datasets are provided below.

(A) (B)



(C)



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

Figure 5. Comparison of the Borghoff et al. (2016) model predictions with measured *tert*-butanol blood concentrations for i.v., inhalation, and oral gavage exposure to *tert*-butanol.

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 over-predicts 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.

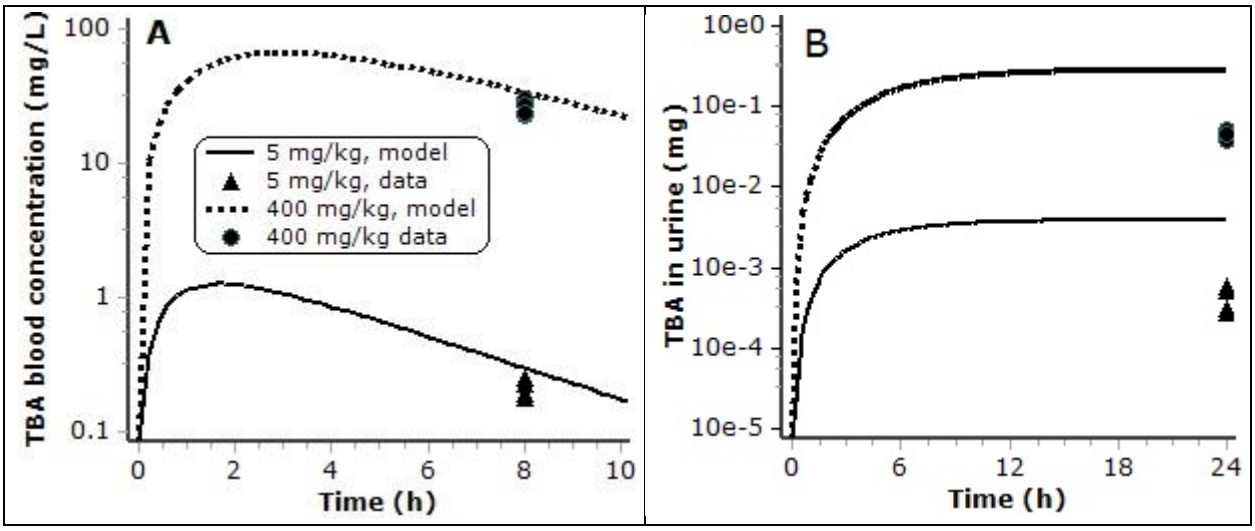


Figure 6. Comparison of Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol after oral gavage of ETBE.

The data points show the measurements from the four individual rats in the JPEC (2008b) 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 over-prediction of *tert*-butanol in urine (B) is by a factor 3-10-fold.

The predictions of the model are compared with amounts measured by Amberg et al. (2000) after ETBE inhalation in Figure 6-A. The prediction of the *tert*-butanol blood concentrations are slightly higher than was measured. The *tert*-butanol blood concentration would be reduced if exposed animals were reducing their breathing rate or other breathing parameters but the exposure concentration of ETBE exposure 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 elimination of *tert*-butanol is significantly overestimated (~ 3-10-fold) by the *tert*-butanol submodel (Figure 6-B)

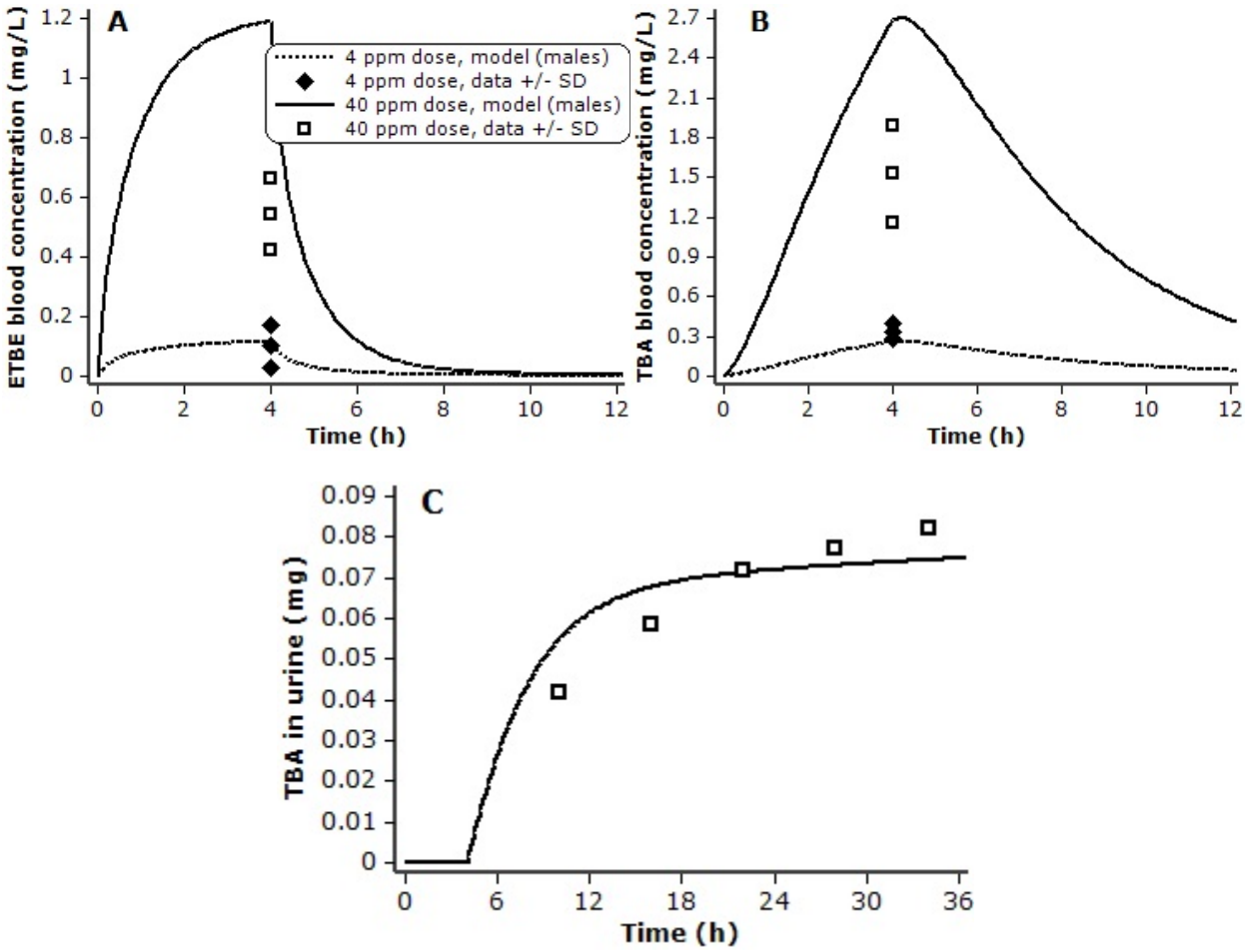


Figure 7. Comparison of Borghoff et al. (2016) model predictions with measured amounts after a 4-hour inhalation exposure to 4 and 40 ppm ETBE.

Concentrations in blood are shown in A) for ETBE, 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).

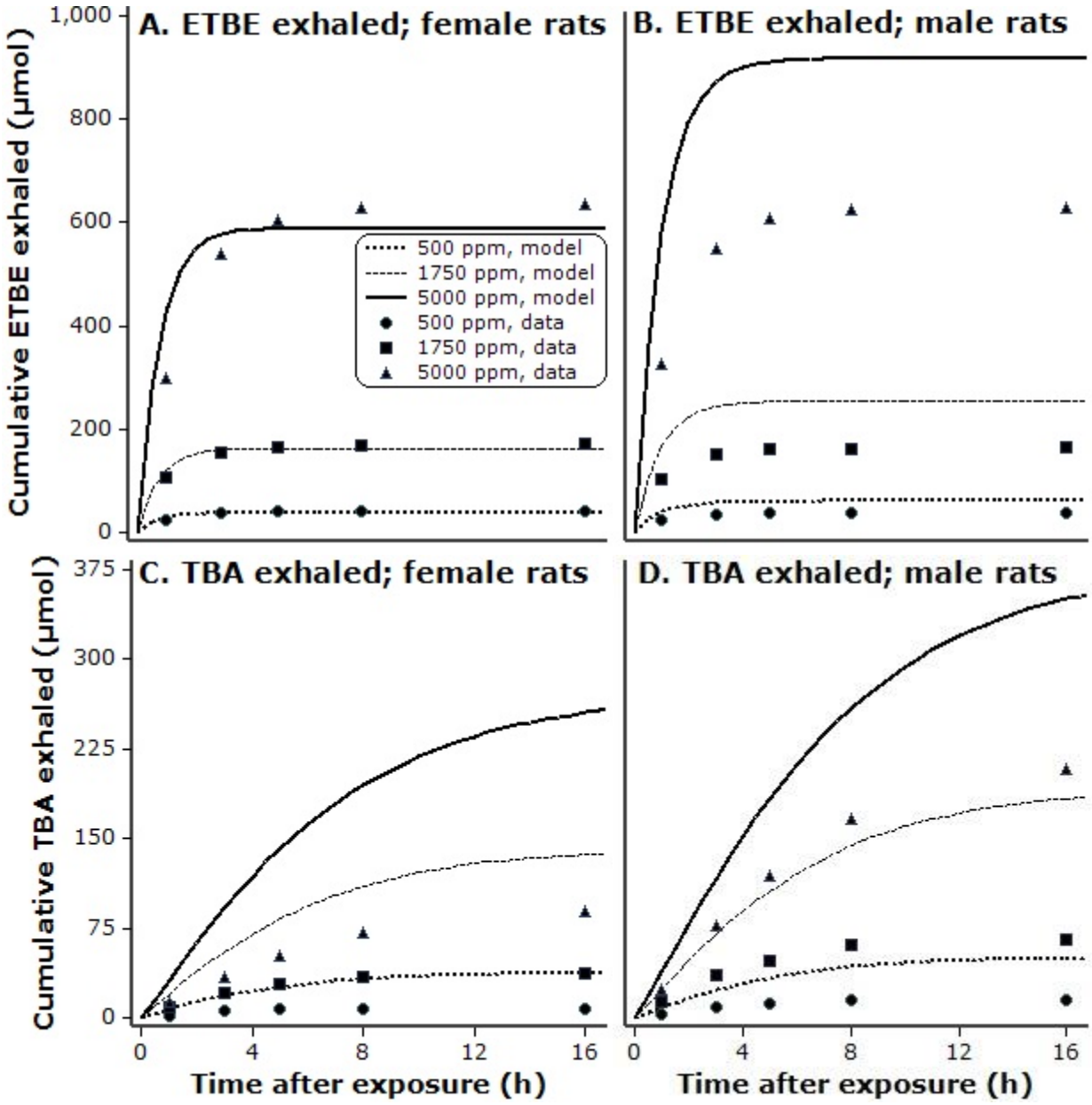
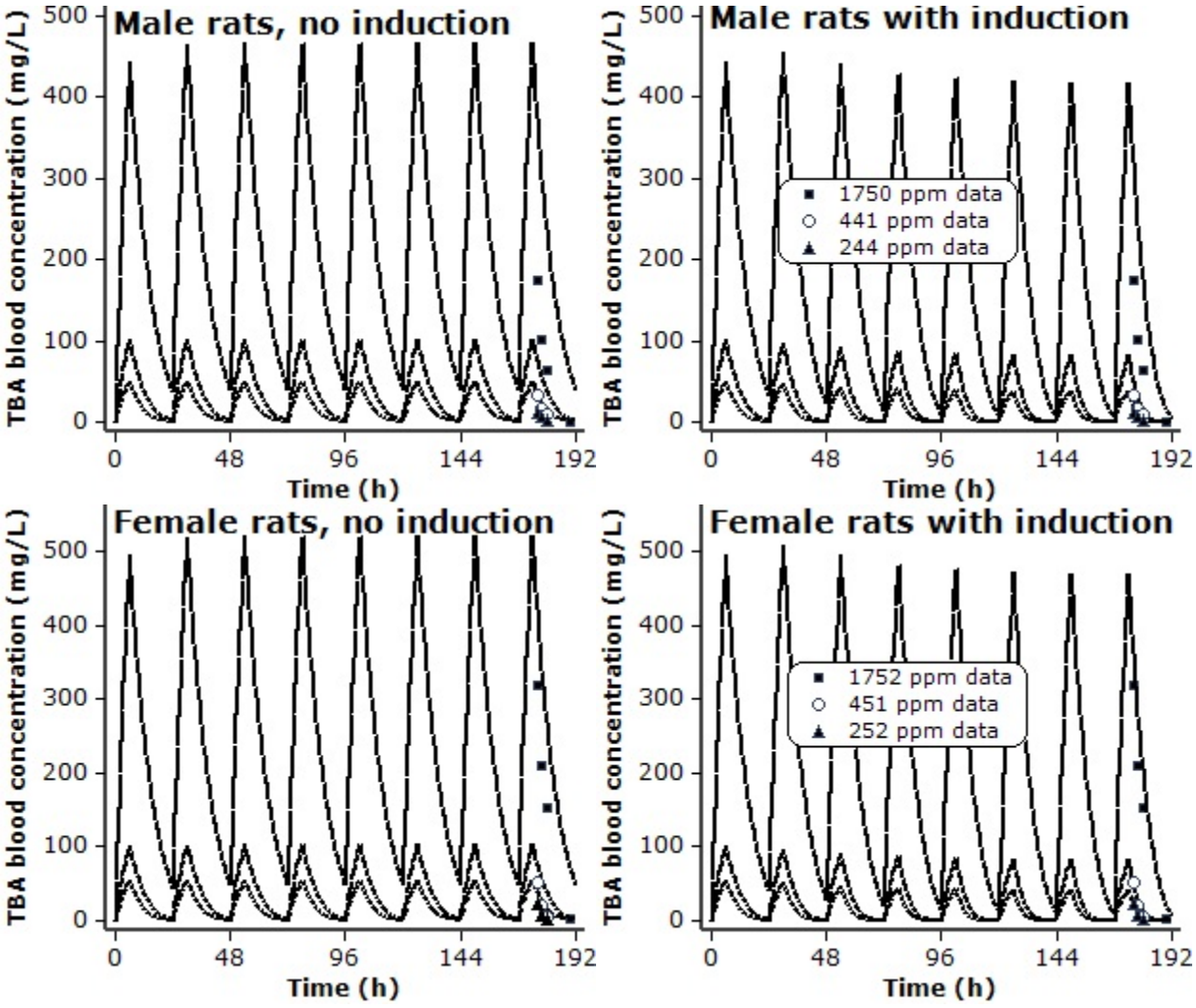


Figure 8. Comparison of Borghoff et al. (2016) model predictions with measured amounts of A) ETBE and B) *tert*-butanol in exhaled breath after a 6-hour inhalation exposure to 500, 1750, and 5,000 ppm ETBE.

The data points are from the Borghoff and Asgharian (1996)) study. The model significantly over predicted exhaled breath of both ETBE and *tert*-butanol following ETBE inhalation exposure for male rats and the exhaled *tert*-butanol for female rats. 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.



Male rats were exposed to 239, 444, or 1726 ppm and female rats were exposed to 256, 444, or 1914 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) compared to without enzyme induction (left panels).

Figure 9. Comparison of the Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol in blood after repeated inhalation exposure to *tert*-butanol.

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 9. 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.

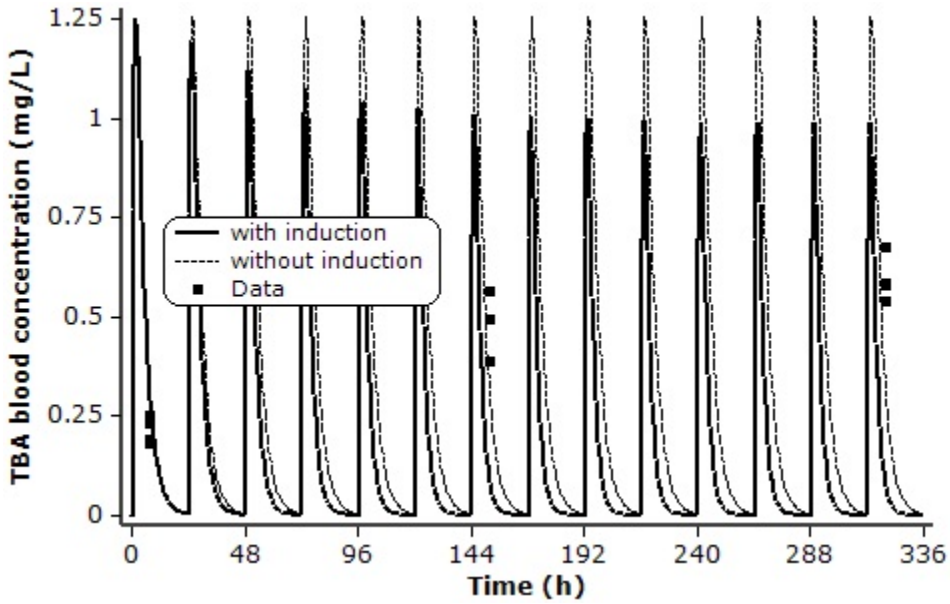


Figure 10. Comparison of EPA model predictions with measured amounts of *tert*-butanol in blood after 5 mg/kg-day ETBE oral gavage for up to 14 days in male rats.

The data show the individual measurements of the four rats in the JPEC (2008a, 2008b) 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.

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