CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- Inhaled or ingested ethylene oxide is readily absorbed through the lung and by the gastrointestinal tract.
- Following absorption, ethylene oxide and its metabolites are rapidly distributed by the blood to a wide variety of tissues.
- As a reactive epoxide, ethylene oxide forms hydroxyethyl adducts with proteins (including hemoglobin) and deoxyribonucleic acid (DNA).
- Metabolism of ethylene oxide occurs by two separate pathways; hydrolysis and glutathione conjugation.
- Ethylene oxide metabolites are rapidly excreted, predominantly in urine.

Endogenous production of ethylene oxide occurs in the body via oxidation of ethylene; biological processes that produce endogenous ethylene include lipid peroxidation, methionine and heme oxidation, and metabolic activity of intestinal bacteria (Thier and Bolt 2000). Isotope labeling is one way to differentiate between absorbed ethylene oxide from exogenous sources and endogenously-produced ethylene oxide. The toxicokinetics of exogenous ethylene oxide is commonly measured using a radiolabeled isotope (typically ^{14}C-ethylene oxide). In such studies, the radiolabel is monitored to determine the extent of absorption, distribution, and excretion related to exogenous exposure to ^{14}C-ethylene oxide.

Levels of endogenously produced ethylene oxide in humans have not been quantified. Kirman and Hays (2017) compared levels of the hemoglobin adduct, N-(2 hydroxyethyl)-valine (HEV), in occupationally exposed workers and smokers (both unique groups described as representing significant exogenous ethylene oxide exposed human populations) with a low-exposure group (described as representing nonexposed human populations) that included nonsmokers and passive smoke-exposed subjects who had no occupational exposures to ethylene oxide. In studies on smokers and studies on occupationally exposed workers, mean HEV values ranged from 19.2 to 15,472 pmol/g hemoglobin. In the low-exposure group, mean HEV values ranged from 12.9 to 117 pmol/g hemoglobin. Based on an observed linear relationship between air exposure levels and HEV levels in workers (slope: 10.9 pmol HEV/g Hgb per ppb ethylene oxide) (Angerer et al. 1998), Kirman and Hays (2017) predicted air ethylene oxide levels

that could result in the HEV levels observed in the low-exposure group. The median was 1.6 ppb (5^{th}–95^{th} percentile range: 0.56, 4.5).

3.1.1 Absorption

In a study of hospital workers exposed to ethylene oxide in the workplace air at concentrations ranging from 0.2 to 24.1 mg/m^3 (0.11–13.2 ppm), approximately 75% of the inhaled ethylene oxide was absorbed into the bloodstream (Brugnone et al. 1985, 1986). Blood:air coefficients were 2.5–3.3 measured from venous blood samples collected 4 hours after the workshift.

Inhaled ethylene oxide was rapidly absorbed through the respiratory system of the rat (Filser and Bolt 1984; Koga et al. 1987; Matsuoka 1988; Nakashima et al. 1987; Tardif et al. 1987), mouse (Cumming et al. 1981; Ehrenberg et al. 1974; Tardif et al. 1987), and rabbit (Tardif et al. 1987). Absorption approached 100% among mice exposed to ethylene oxide for up to 2 hours at 2–55 mg/m^3 (1.1–30 ppm) (Ehrenberg et al. 1974).

No human or animal data were located regarding absorption of ethylene oxide after oral exposure. Available information regarding dermal absorption of ethylene oxide is limited to a reported permeation rate of 0.125 mg/(cm-hour) for absorption through excised human skin exposed to a 1% ethylene oxide solution (IARC 2008).

3.1.2 Distribution

No studies were located regarding the distribution of ethylene oxide in humans. However, studies in experimental animals demonstrate that absorbed ethylene oxide is readily distributed to a wide variety of tissues.

Brown et al. (1996) exposed rats and mice to ethylene oxide by 4-hour inhalation at 100 or 300 ppm and studied its distribution and elimination from blood, muscle, and brain. Tissue concentrations of ethylene oxide were calculated from headspace ethylene oxide concentrations using the appropriate air:tissue partition coefficient (Krishnan et al. 1992). For the 100 ppm exposure concentration, peak tissue concentrations averaged 0.57–0.60 µg/g for male rats, 0.56–0.72 µg/g for female rats, 0.29–0.36 µg/g for male mice, and 0.35–0.41 µg/g for female mice. Half-times of elimination of ethylene oxide from blood were 13.8 and 10.8 minutes for male and female rats, respectively, and 3.12 and 2.4 minutes for male and

female mice, respectively. Peak ethylene oxide concentrations in the testes were approximately 20% lower in rats and 50% lower in mice compared to concentrations in blood, muscle, and brain. Saturation kinetics were observed in mice (but not rats) exposed to ethylene oxide at 300 ppm for 4 hours. Brown et al. (1998) reported a linear increase in ethylene oxide levels in blood with increasing exposure concentrations in mice exposed to 50–200 ppm ethylene oxide for 4 hours; steady state was achieved within the first 2 hours of exposure. At higher concentrations (300 and 400 ppm), blood ethylene oxide levels increased sublinearly and continued to increase during the 4-hour exposure. The sublinear increase in blood levels correlated with tissue glutathione depletion. Ehrenberg et al. (1974) reported that 75 minutes after exposing mice to radiolabeled ethylene-[1,2-^{3}H] oxide, the highest levels of radioactivity were observed in the lungs, liver, and kidneys. Lesser amounts were found in the spleen, brain, and testes.

Krishnan et al. (1992) demonstrated relatively similar ethylene oxide tissue distribution for male Fischer rats based on calculated *in vitro* tissue:air partition coefficients for fat (44.1), brain (48.3), lung (60.9), liver (61.6), blood (64.1), and testes (83). Tyler and McKelvey (1982) reported the highest concentrations of ^{14}C-activity in urinary bladder, liver, packed blood cells, and adrenal glands, and the lowest concentration in fat from rats exposed to ^{14}C-ethylene oxide.

No studies were located regarding distribution of ethylene oxide after oral or dermal exposure.

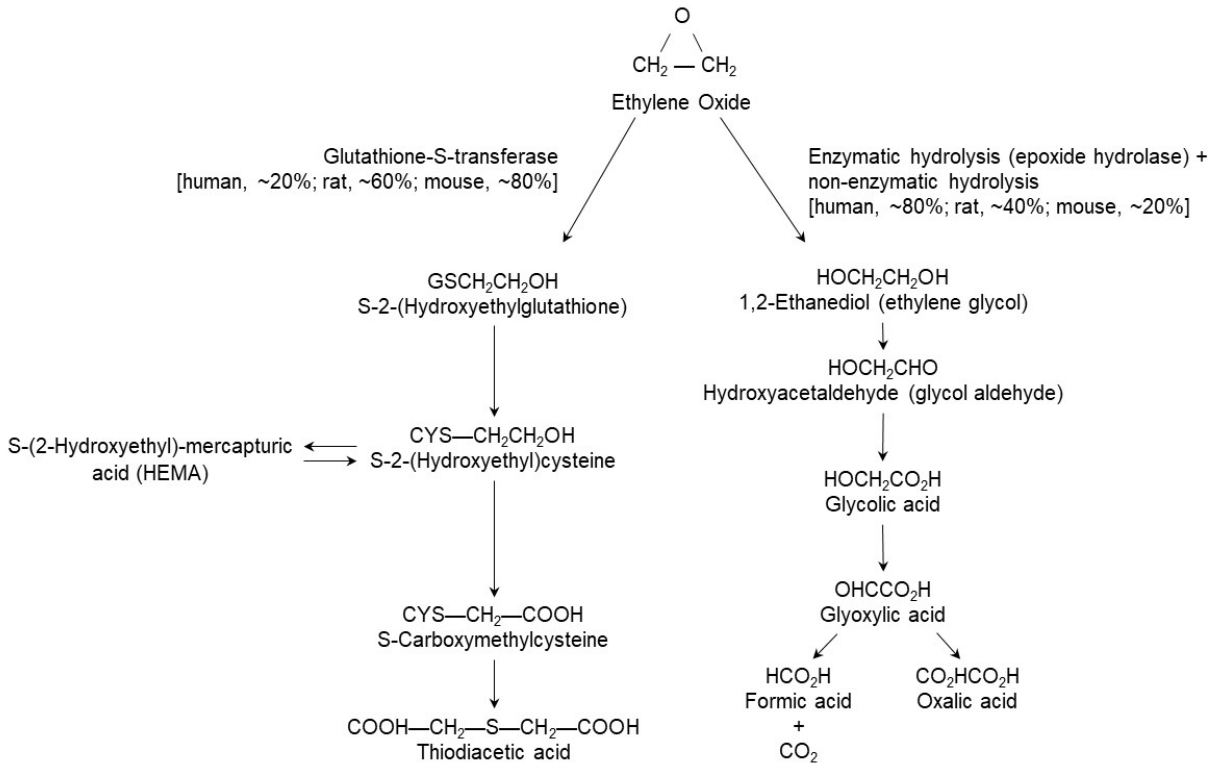
3.1.3 Metabolism

Ethylene oxide metabolism has been evaluated in a variety of experimental mammal systems *in vivo* and *in vitro*, and has resulted in the proposed metabolic scheme shown in Figure 3-1 (IARC 2008; Popp et al. 1994; Scheick et al. 1997). Metabolism is initiated via two separate pathways. One pathway involves enzymatic and nonenzymatic hydrolysis to ethylene glycol and subsequent conversion to oxalic acid, formic acid, and carbon dioxide. The other pathway involves glutathione conjugation to form mercapturic acid and meththio metabolites, some of which are converted to thiodiacetic acid. Metabolites recovered from the urine of ethylene oxide-exposed rats include ethylene glycol, 2-hydroxyethyl-mercapturic acid, and thiodiacetic acid (Scheick et al. 1997).

The relative contribution of each major pathway is species dependent. Results from a physiologically based pharmacokinetic (PBPK) model (Fennell and Brown 2001) designed to simulate the uptake and metabolism of ethylene oxide in rats, mice, and humans suggest that approximately 80% (rats), 60%

(mice), and 20% (humans) of metabolized ethylene oxide occurs via glutathione conjugation and 20% (rats), 40% (mice), and 80% (humans) occurs via the hydrolytic pathway. Although PBPK model simulations produced these species-specific differences in the relative contribution of each metabolic pathway, after accounting for species differences in uptake and metabolism, simulated peak blood concentrations were relatively similar for rats, mice, and humans (Fennell and Brown 2001). The order of area under the curve (AUC) values was humans>rats>mice.

Figure 3-1. Metabolism of Ethylene Oxide



Source: IARC 2008

3.1.4 Excretion

No studies were located regarding excretion in humans following inhalation, oral, or dermal exposure to ethylene oxide. No animal studies were located regarding excretion of ethylene oxide and/or its metabolites following oral or dermal exposure.

In a study of rats exposed to ^{14}C-ethylene oxide by inhalation, 59% of the ^{14}C-activity was recovered in the urine, 12% expired as $^{14}CO_2$, 4.5% in feces, and 1% expired unchanged (Tyler and McKelvey 1982). Ethylene oxide and its metabolites are rapidly excreted in urine. In a study of mice exposed to radiolabeled ethylene oxide for 60–75 minutes, an average of 78% of the absorbed radioactivity was eliminated in the urine within 48 hours (Ehrenberg et al. 1974). Filser and Bolt (1984) found that ethylene oxide administered in a closed-system inhalation chamber exhibited first-order elimination kinetics. Metabolites recovered in urine from rats exposed to airborne ethylene oxide include ethylene glycol, 2-hydroxyethylmercapturic acid, and thiodiacetic acid (Scheick et al. 1997).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

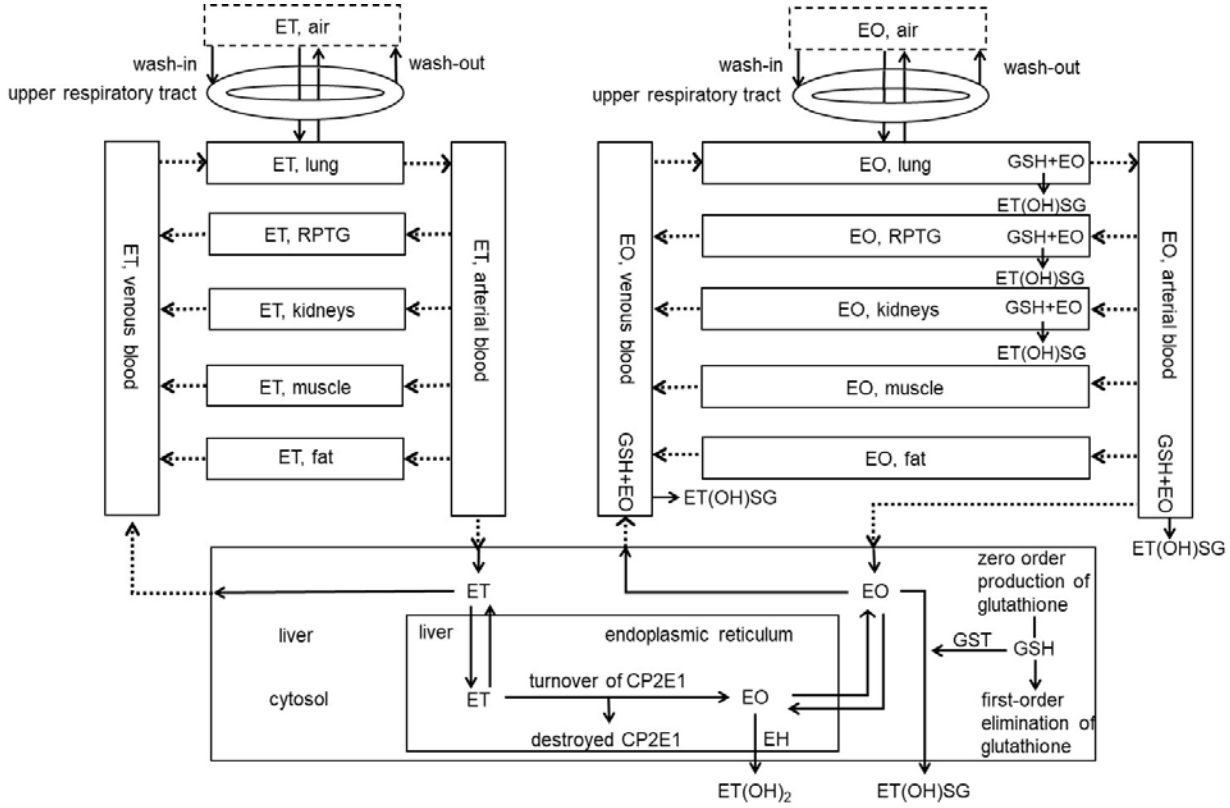
PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Several PBPK models of ethylene oxide have been reported. These include models of humans, mice, and rats (Csanady et al. 2000; Fennell and Brown 2001; Filser and Klein 2018a, 2018b; Krishnan et al. 1992; NIOSH 1987). The models simulate species differences in metabolism of ethylene oxide thought to contribute to interspecies differences in dose-response relationships for carcinogenicity. The model reported by Filser and Klein (2018a, 2018b) is an extension and enhancement of a model reported by Csanady et al. (2000) and includes simulations of adduct formation, thought to be important in the mechanism of carcinogenicity of ethylene oxide. The model reported by Fennell and Brown (2001) is an extension and enhancement of a model reported by Krishnan et al. (1992).

Filser and Klein (2018a, 2018b) Models of Human, Mouse, and Rat. Filser and Klein (2018a, 2018b) developed a model for simulating the kinetics of inhaled ethylene and ethylene oxide in humans, mice, and rats. The model is an extension and enhancement of a model reported by Csanady et al. (2000). The major enhancements made to the Csanady et al. (2000) model were as follows: (1) including glutathione transferase activity to extra-hepatic tissues; (2) including suicide substrate (ethylene) inhibition of hepatic

CYP2E1-mediated metabolism; (3) expanding metabolism pathways to include non-enzymatic hydrolysis and glutathione conjugation; and (4) updating some parameter values based on new data. The general structure of the model is depicted in Figure 3-2. Parameter values for the model are presented in Tables 3-1 and 3-2. Complete lists of parameters and parameter values and the basis for parameter values and evaluations of model predictions in comparison to observations are described in Filser and Klein (2018a, 2018b).

Figure 3-2. Physiologically Based Toxicokinetic Model for Inhaled Ethylene and Inhaled or Metabolically Formed Ethylene Oxide*



*Compartments in solid lines are characterized by defined volumes; the air compartment (dotted lines) can have a defined volume or can be infinitely large, depending on the exposure condition. Dashed arrows indicate transport in the blood; solid arrows indicate uptake or elimination

CYP2E1 = cytochrome P450 2E1; EH = microsomal epoxide hydrolase; EO = ethylene oxide; ET = ethylene; $ET(OH)_2$ = ethylene glycol; ET(OH)SG = S-(2-hydroxyethyl)glutathione; GSH = glutathione; GST = cytosolic glutathione S-transferase; RPTG = richly perfused tissue group

Source: Filser and Klein 2018a, 2018b (permission: Creative Commons CC BY 4.0, https://creativecommons.org/licenses/by/4.0/; minor revisions)

Table 3-1. Physiological and Physicochemical Parameters Used in the Physiologically Based Toxicokinetic Model

Parameter	Abbreviation	Mouse	Rat	Human
Body weight (kg)	BW	0.025	0.25	70
Alveolar ventilation at rest (L/hour)	Qalv	1.2	7.02	300
Cardiac output at rest (L/hour)	Qcard	1.02	5.04	372
Blood flow (L/hour)				
RPTG	Qr	0.355 x Qcard	0.355 x Qcard	0.285 x Qcard
Kidneys	Qk	0.155 x Qcard	0.155 x Qcard	0.155 x Qcard
Muscle	Qm	0.223 x Qcard	0.223 x Qcard	0.250 x Qcard
Fat	Qa	0.017 x Qcard	0.017 x Qcard	0.050 x Qcard
Liver	QL	0.250 x Qcard	0.250 x Qcard	0.260 x Qcard
Compartment volumes (L)				
Arterial blood	Vart	0.0159 x BW	0.0241 x BW	0.0257 x BW
Venous blood	Vvnb	0.0331 x BW	0.050 x BW	0.0533 x BW
RPTG	Vr	0.026 x BW	0.0377 x BW	0.038 x BW
Kidneys	Vk	0.0167 x BW	0.0073 x BW	0.0044 x BW
Lung	Vp	0.0073 x BW	0.005 x BW	0.0076 x BW
Muscle	Vm	0.66 x BW	0.676 x BW	0.541 x BW
Fat	Va	0.10 x BW	0.07 x BW	0.19 x BW
Liver	VL	0.055 x BW	0.04 x BW	0.026 x BW
Water content as fraction of compartment volume				
Blood	wfb	0.842	0.842	0.830
RPTG	wfr	0.783	0.783	0.76
Kidneys	wfk	0.771	0.771	0.827
Lung	wfp	0.842	0.842	0.790
Muscle	wfm	0.756	0.756	0.756
Fat	wfa	0.183	0.183	0.100
Liver	wfL	0.705	0.705	0.683
Partition coefficients of ET				
Blood:air	Pbair	0.48	0.48	0.22
RPTG:blood	Prb	1.04	1.04	2.18
Kidney:blood	Pkb	1.04	1.04	2.18
Lung:blood	Ppb	1.04	1.04	2.18
Muscle:blood	Pmb	1.31	1.31	2.95
Fat:blood	Pab	4.29	4.29	8.73
Liver:blood	PLb	1.19	1.19	2.05
Partition coefficients of EO				
Blood:air	PEObair	61	61	61
RPTG:blood	PEOrb	1.03	1.03	1.03
Kidney:blood	PEOkb	1.03	1.03	1.03
Lung:blood	PEOpb	1.03	1.03	1.03

Table 3-1. Physiological and Physicochemical Parameters Used in the Physiologically Based Toxicokinetic Model

Parameter	Abbreviation	Mouse	Rat	Human
Muscle:blood	PEOmb	1.08	1.08	1.08
Fat:blood	PEOab	0.70	0.70	0.70
Liver:blood	PEOLb	0.89	0.89	0.89
Wash-in–wash-out effect				
Fraction of inhaled ET reaching the alveoli	fET	0.6	0.6	1.0
Fraction of inhaled EO reaching the alveoli	fEO	0.6	0.6; 0.3	0.8

EO = ethylene oxide; ET = ethylene; RPTG = richly perfused tissue group

Source: Filser and Klein 2018a, 2018b (permission: Creative Commons CC BY 4.0, https://creativecommons.org/licenses/by/4.0/; minor revisions)

Table 3-2. Biochemical Parameters Used in the Physiologically Based Toxicokinetic Model

Parameter	Abbreviation	Mouse	Rat	Human
Metabolism of ET in the liver				
Rate constant of CYP2E1 catalyzed formation of EO from ET ($hour^{-1}$)	k3	260	420	50
Rate constant of suicide inhibition of CYP2E1 by ET ($hour^{-1}$)	k4	1.9	0.8	1.1
Apparent Michaelis constant of ET oxidation in venous liver blood (mmol/L)	Kmmo	0.001	0.003	0.003
Rate constant of physiological degradation of CYP2E1 ($hour^{-1}$)	ke	0.187	0.120	0.0139
Initial concentration of CYP2E1 (mmol/kg liver)	CYPo	0.00288	0.001074	0.002115
Metabolism of EO				
EH in the liver				
Maximum rate of hydrolysis of EO catalyzed by EH (mmol/hour/kg liver)	VmaxEOEH	2.70	3.60	25.83
Apparent Michaelis constant of EO hydrolysis in the liver (mmol/L)	KmapEO	0.2	0.2	0.46
Intrinsic Michaelis constant of EO hydrolysis in the endoplasmic reticulum (mmol/L)	KmihEO	0.1999	0.1999	0.31
GST in the liver				
Maximum metabolic elimination rate of EO catalyzed by GST in the liver (mmol/hour/kg liver)	VmaxgstL	1,431	272	58.4
Apparent Michaelis-constant of EO with GST in the liver (mmol/L)	KmEOL	9	9	9
Apparent Michaelis constant of GSH with GST in the liver (mmol/L)	KmgshL	0.1	0.1	0.1

Table 3-2. Biochemical Parameters Used in the Physiologically Based Toxicokinetic Model

Parameter	Abbreviation	Mouse	Rat	Human
Elimination rate constant for GSH turnover in the liver (hour^{-1})	kdgshL	0.2	0.2	0.2
Initial concentration of cytosolic GSH in the liver (mmol/L)	CgshoL	8.62	6.4	5.9
GST in the lung				
Maximum metabolic elimination rate of EO catalyzed by GST in the lung (mmol/hour/kg lung)	Vmaxgstp	97.6	27.5	5.84
Apparent Michaelis-constant of EO with GST in the lung (mmol/L)	KmEOp	9	9	9
Apparent Michaelis constant of GSH with GST in the lung (mmol/L)	Kmgshp	0.1	0.1	0.1
Elimination rate constant for GSH turnover in the lung (hour^{-1})	kdgshp	1.3	1.8	2.0
Initial concentration of cytosolic GSH in the lung (mmol/L)	Cgshop	1.86	1.7	1.95
GST in the kidneys				
Maximum metabolic elimination rate of EO catalyzed by GST in the kidneys (mmol/hour/kg kidney)	Vmaxgstk	264	172	5.0
Apparent Michaelis-constant of EO with GST in the kidneys (mmol/L)	KmEOk	9	9	9
Apparent Michaelis constant of GSH with GST in the kidneys (mmol/L)	Kmgshk	0.1	0.1	0.1
Elimination rate constant for GSH turnover in the kidneys (hour^{-1})	kdgshk	0.2	0.2	0.2
Initial concentration of cytosolic GSH in the kidneys (mmol/L)	Cgshok	3.06	2.6	0.5
GST in the RPTG				
Maximum metabolic elimination rate of EO catalyzed by GST in the RPTG (mmol/hour/kg RPTG)	Vmaxgstr	43	8.16	1.75
Apparent Michaelis-constant of EO with GST in the RPTG (mmol/L)	KmEOr	9	9	9
Apparent Michaelis constant of GSH with GST in the RPTG (mmol/L)	Kmgshr	0.1	0.1	0.1
Elimination rate constant for GSH turnover in the RPTG (hour^{-1})	kdgshr	0.2	0.2	0.2
Initial concentration of cytosolic GSH in the RPTG (mmol/L)	Cgshor	2.29	2.85	1.20
GST in blood				
Maximum metabolic elimination rate of EO catalyzed by GST in the blood (mmol/hour/L blood)	Vmaxgstb	57.24	10.88	2.33
Apparent Michaelis-constant of EO with GST in the blood (mmol/L)	KmEOb	9	9	9

Table 3-2. Biochemical Parameters Used in the Physiologically Based Toxicokinetic Model

Parameter	Abbreviation	Mouse	Rat	Human
Apparent Michaelis constant of GSH with GST in the blood (mmol/L)	Kmgshb	0.1	0.1	0.1
Elimination rate constant for GSH turnover in the blood ($hour^{-1}$)	kdgshb	0.2	0.2	0.2
Initial concentration of cytosolic GSH in the blood (mmol/L)	Cgshob	1.255	0.945	0.766
Non-enzymatic GSH conjugation of EO				
Rate constant of the conjugation reaction (L/(mmol GSHxhour))	kEOG	0.01248	0.01248	0.01248
Non-enzymatic hydrolysis of EO				
Rate constant of EO hydrolysis ($hour^{-1}$)	kEOh	0.06	0.06	0.06

CYP2E1 = cytochrome P450 2E1; EH = microsomal epoxide hydrolase; EO = ethylene oxide; ET = ethylene; SH = glutathione; GST = cytosolic glutathione S-transferase; RPTG = richly perfused tissue group

Source: Filser and Klein 2018a, 2018b (permission: Creative Commons CC BY 4.0, https://creativecommons.org/licenses/by/4.0/; minor revisions)

The ethylene oxide model includes compartments representing venous and arterial blood, lung, kidney, liver, muscle, fat, and a lumped compartment representing all other richly perfused tissues. Absorption from the respiratory tract is simulated as a flow-limited transfer from the upper respiratory tract governed by the fraction of inhaled ethylene oxide reaching the alveolar region of the lung, the alveolar-blood concentration difference, alveolar ventilation rate, cardiac output, and the blood:air partition coefficient. Reported blood:air partition coefficients are 61 for humans (Csanady et al. 2000) and 64.1 for rats (Krishnan et al. 1992). Exchanges between ethylene oxide in tissues and blood are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:blood partition coefficient. Elimination of ethylene oxide is simulated as exhalation and metabolism. Four metabolism pathways are simulated: (1) conversion to ethylene glycol mediated by liver microsomal epoxide hydrolase in liver; (2) conversion to S-(2-hydroxyethyl)glutathione mediated by cytosolic glutathione S-transferase in lung, kidney, liver, and richly perfused tissue; (3) non-enzymatic conversion to ethylene glycol in all tissues; and (4) non-enzymatic conjugation with glutathione in all tissues.

The epoxide-hydrolase pathway is simulated as a capacity-limited process governed by a maximum rate (Vmax, mmol/hour/kg liver) and a Michaelis half-saturation constant (Km, mmol/L). The non-enzymatic hydrolysis pathway is unlimited and governed by a first-order rate constant ($hour^{-1}$). The glutathione transferase pathway is simulated as a capacity-limited process governed by a maximum (Vmax, mmol/hour/kg liver) and Michaelis constants (Km, mmol/L) for ethylene oxide and reduced glutathione

(GSH). Conjugation with glutathione is limited by availability of GSH, which is consumed by conjugation with ethylene oxide as well as physiologic GSH turnover unrelated to conjugation. Physiologic turnover of GSH is governed by a first-order elimination rate constant ($hour^{-1}$). The non-enzymatic glutathione conjugation pathway is unlimited and governed by a clearance constant (L/mmol GSH/hour). The Filser and Klein (2018a, 2018b) ethylene oxide model is connected to a similar model for ethylene. The ethylene model simulates production of ethylene oxide in the liver mediated by the microsomal CYP2E1 system and includes suicide inhibition of CYP2E1 by ethylene. Production of ethylene oxide is governed by a first-order rate constant ($hour^{-1}$) for the reaction with ethylene, a first-order rate constant for suicide inhibition of CYP2E1 by ethylene ($hour^{-1}$), and a first-order rate constant for physiologic turnover of CYP2E1 ($hour^{-1}$). The model simulates the formation of adducts between ethylene oxide and HEV and DNA guanine (N7-(2-hydroxyethyl)guanine; HEG). Adduct formation is governed by clearance terms (L/hour/g Hb or DNA).

Partition coefficients were estimated from *in vitro* head-space studies (Csanady et al. 2000). Parameters (Vmax, Km) for liver epoxide hydrolase and tissue-specific glutathione transferase activity were derived from *in vitro* studies, with Vmax scaled to whole tissue from estimates of rates per unit mass of protein (Filser and Klein 2018a, 2018b). Parameters for liver epoxide hydrase were subsequently calibrated to fit data on blood ethylene oxide concentration in mice, rats, or humans exposed to ethylene or ethylene oxide (Brugnone et al. 1986; Fennell et al. 2004; Filser et al. 2013). Calibration was achieved by visual inspection of plots of observed and predicted values. The rate of non-enzymatic conjugation with GSH was estimated from *in vitro* studies (Fennell and Brown 2001). The rate of non-enzymatic hydrolysis was estimated by model calibration to blood ethylene oxide concentrations measured in workers exposed to ethylene oxide (Fennell and Brown 2001). Adduct formation rates were derived from *in vitro* studies of adduct formation (Segerbäck 1990; Walker et al. 1992b).

The calibrated model was evaluated against observations of blood ethylene oxide concentrations and rates of uptake from closed space air obtained from studies in which mice, rats, or humans were exposed to ethylene or ethylene oxide (Brown et al. 1998; Brugnone et al. 1986; Filser and Bolt 1984; Filser et al. 1992, 2013, 2015). Exposures to ethylene oxide were 9–2,500 ppm in mice, 140–1,450 ppm in rats, and 2–10 ppm in humans. Goodness-of-fit was assessed by the sum of squared errors from observations and comparison of plotted predictions and observations.

The model predicts that, over a range of exposures (0.5–500 ppm for 6 hours), the epoxide hydrolase pathway will dominate metabolism in the human relative to the glutathione transferase pathway (EH/GST

ratio: 85/15). In mice and rats, the glutathione transferase pathway is predicted to dominate; however, depletion of the glutathione will limit the capacity for conjugation at concentrations exceeding 100 ppm in the mouse and 200 ppm in the rat. The model predicts that clearance of ethylene oxide in the mouse is capacity limited. At exposure concentrations below 200 ppm, rates of uptake of ethylene oxide from closed chambers (an indicator of systemic clearance) are predicted to be similar in mice and rats. However, at exposure concentrations exceeding 200 ppm, uptake rates in mice are slower than those predicted for rats, indicating slower clearance by mice. The model also predicts that exposure concentrations exceeding 200 ppm in mice would result in a super-linear increase in blood ethylene oxide concentrations (i.e., an increase in blood concentration larger than the proportional increase in exposure concentration). The predicted differences in the exposure-clearance and exposure-blood concentration relationship in mice agree with observations (Filser and Klein 2018a, 2018b). These differences in metabolic pathways and systemic clearance could contribute to species differences in dose-response relationships for toxicity endpoints that derive from metabolites of ethylene oxide. The model was applied to estimating exposure concentrations of ethylene and ethylene oxide that would result in the same HEV or HEG levels. The model predicts that an inhalation exposure of humans to 0.3 ppm ethylene oxide (8 hours/day, 5 days/week) would produce the same levels of HEV or HED as a similar exposure to 200 ppm ethylene.

Fennell and Brown (2001) Models of Human, Mouse, and Rat. Fennell and Brown (2001) developed a model for simulating the kinetics of inhaled ethylene oxide in humans, mice, and rats. The model is an extension and enhancement of a model reported by Krishnan et al. (1992). The major enhancements made to the Krishnan et al. (1992) model were as follows: (1) including a kidney compartment; (2) simulating enzymatic hydrolysis (liver) and glutathione conjugation (liver, kidney, testes) as capacity limited (Vmax, Km); (4) simulating exchanges between blood and testes as diffusion-limited; and (4) updating some parameter values based on new data. Complete lists of parameters and parameter values and the basis for parameter values and evaluations of model predictions in comparison to observations are described in Fennell and Brown (2001).

The model includes compartments representing venous and arterial blood, lung, kidney, liver, brain, testes, fat, and lumped compartments representing all other richly perfused tissues or slowly perfused tissues. Absorption from the respiratory tract is simulated as a flow-limited transfer from a gas exchange compartment of the lung to a metabolic lung compartment, governed by an uptake fraction, concentration difference across the lung compartments, alveolar ventilation rate, cardiac output, and the blood:air partition coefficient. Exchanges between ethylene oxide in blood and all tissues except testes are

assumed to be flow-limited (governed by blood flow) with equilibrium determined by a tissue:blood partition coefficient. Exchange between blood and testes was simulated as a diffusion-limited process governed by a permeability coefficient ($hour^{-1}$). The diffusion-limited assumption was needed to accurately predict the observed testes concentrations of ethylene oxide. Elimination of ethylene oxide is simulated as exhalation and metabolism. Three metabolism pathways are simulated: (1) conversion to ethylene glycol mediated by liver microsomal epoxide hydrolase in liver; (2) conversion to S-(2-hydroxy-ethyl)glutathione mediated by cytosolic glutathione S-transferase in lung, kidney, and testes; and (3) non-enzymatic hydrolysis in all other tissues except fat. The epoxide hydrolase pathway is simulated as a capacity-limited process governed by a maximum rate (Vmax, mmol/hour) and a Michaelis half-saturation constant (Km, mmol/L). The non-enzymatic hydrolysis pathway is unlimited and governed by a first-order rate constant ($hour^{-1}$). The glutathione transferase pathway is simulated as a capacity-limited process governed by a maximum (Vmax, mmol/hour) and Michaelis half-saturation constants (Km, mmol/L) for ethylene oxide and GSH. Conjugation with glutathione is limited by availability of GSH, which is consumed by conjugation with ethylene oxide as well as physiologic GSH turnover unrelated to conjugation. Physiologic turnover of GSH is governed by first-order synthesis and elimination rates ($hour^{-1}$). The model simulates the formation of adducts between ethylene oxide and the protein and DNA in kidney, liver, and testes, governed by first-order binding rates ($hour^{-1}$).

Partition coefficients were estimated from *in vitro* head-space studies (Csanady et al. 2000; Filser et al. 1992; Krishnan et al. 1992). Parameters (Vmax, Km) for liver epoxide hydrolase and tissue-specific glutathione transferase activity were derived from *in vitro* studies, with Vmax scaled to whole tissue from estimates of rates per unit mass of protein (Brown et al. 1996; Fennell and Brown 2001). Other parameters were calibrated to achieve better agreement with observations. These included the pulmonary uptake factor, the diffusion permeability coefficient for testes, partition coefficients, and various physiological parameters. The model was calibrated with observations made in mice and rats on kinetics of ethylene oxide concentrations in closed exposure chamber air, blood, and tissues; and blood GSH concentrations (Brown et al. 1996, 1998; Krishnan et al. 1992). The human model was calibrated with observations of blood ethylene oxide concentrations exposed to work room air (Brugnone et al. 1986).

The model predicts that, over a range of exposures (20–500 ppm for 4 hours), the hydrolysis pathway will dominate metabolism in the human relative to the glutathione transferase pathway (EH/GST ratio: 60/20). In mice and rats, the glutathione transferase pathway is predicted to dominate; however, depletion of the glutathione will limit the capacity for conjugation at concentrations exceeding 200 ppm in the mouse. The EH/GST ratio was relatively constant (30:60) in the rat. The model predicts that clearance of

ethylene oxide in the mouse is capacity limited. At exposure concentrations below 200 ppm, blood ethylene oxide concentrations are predicted to be similar in mice, rats, and humans. However, at exposure concentrations exceeding 200 ppm, blood ethylene oxide concentrations in mice exceed those predicted for rats and humans. The predicted differences in the exposure-blood concentration relationship in mice and rats agree with observations (Fennell and Brown 2001). These differences in metabolic pathways and clearance could contribute to species differences in dose-response relationships for toxicity endpoints that derive from metabolites of ethylene oxide. However, at exposure concentrations below 100 ppm, the models predict similar peak and blood ethylene oxide concentrations and areas under the curve (AUCs) in mice, rats, and humans (Fennell and Brown 2001).

3.1.6 Animal-to-Human Extrapolations

The disposition and metabolism of inhaled ethylene oxide is species dependent. Species differences exist regarding the relative contribution of the metabolic pathways discussed in Section 3.1.3 (the nonenzymatic hydrolysis pathway and the glutathione conjugation pathway) (Fennell and Brown 2001). Although ethylene oxide exposure concentrations <200 ppm are predicted to result in similar blood levels among rats, mice, and humans, higher exposure concentrations would result in higher blood levels in mice compared to rats and humans. The higher blood levels in mice are likely the result of glutathione depletion. Interspecies extrapolation would need to account for species differences in metabolic pathways, species-specific contribution of exposure concentration, and the identity of the toxicant or toxicants (ethylene oxide itself and/or its metabolite[s]) responsible for a particular toxic effect.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic

makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to ethylene oxide are discussed in Section 5.7, Populations with Potentially High Exposures.

No data were located regarding age-related differences in susceptibility to ethylene oxide toxicity or carcinogenicity. However, because detoxification of ethylene oxide occurs via hydrolytic and glutathione-S-transferase pathways, very young children with incomplete development of these detoxification pathways (McCarver and Hines 2002; Zhong et al. 2018) may exhibit increased susceptibility to ethylene oxide toxicity. Limited data do not suggest significant sex-related differences in ethylene oxide metabolism (Fennell and Brown 2001; Mertes et al. 1985). Individuals with genetic deficiencies in activities of detoxification enzymes would likely be at increased risk of ethylene oxide toxicity/carcinogenicity. For example, ethylene oxide hemoglobin adduct levels in occupationally-exposed workers were 2-fold greater among individuals expressing a null GSTT1 genotype than those expressing a nonnull GSTT1 genotype (Yong et al. 2001). Haufroid et al. (2007) reported increased urinary excretion of an ethylene oxide glutathione conjugate among nonnull GSTT1 genotype hospital workers, suggestive of increased ethylene oxide detoxification. People with DNA repair deficiencies might experience increased sensitivity to DNA-damaging effects of ethylene oxide.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to ethylene oxide are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/

exposurereport/). If available, biomonitoring data for ethylene oxide from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by ethylene oxide are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Ethylene oxide can be measured in blood and alveolar air (Brugnone et al. 1986). The urinary metabolite, HEMA (*S*-[2-hydroxyethyl]mercapturic acid), has been used as a biomarker of exposure to ethylene oxide (Alwis et al. 2012; Eckert et al. 2011; Popp et al. 1994), including occupational levels <1 ppm (Haufroid et al. 2007). However, HEMA is not specific to ethylene oxide; it is also a metabolite of acrylonitrile and vinyl chloride.

Ethylene oxide forms adducts with DNA and hemoglobin, which are considered markers of biological effects (Angerer et al. 1998; Boogaard 1999, 2002; Rusyn et al. 2005; Walker and Skopek 1993). However, these adducts also can implicate that exposure to ethylene oxide has occurred. Additional information is provided in Section 3.3.2 (Biomarkers of Effect).

3.3.2 Biomarkers of Effect

Ethylene oxide is a direct acting alkylating agent that can form adducts with macromolecules such as DNA and hemoglobin. The detection of these adducts can be used as a biomarker of effect, even in the absence of adverse effects. The primary DNA adduct formed is 7-HEG (EPA 2016; IARC 2012). Studies in rats and mice have found concentration- and duration-related increases in 7-HEG levels following inhalation exposure (Rusyn et al. 2005; Walker and Skopek 1993). EPA (2016) notes that DNA adducts are less reliable measures of exposure because they can be repaired or fixed as mutations. The ethylene oxide hemoglobin adduct, hydroxylated *N*-terminal valine (HOEtVal), has been widely used as a biomarker for ethylene oxide (see Angerer et al. 1998; Boogaard 2002; Boogaard et al. 1999). Occupational exposure studies have found a correlation between ambient air levels of ethylene oxide and HOEtVal levels (Angerer et al. 1998; Boogaard 2002). Studies in rats and mice have reported increases in HOEtVal levels following a single inhalation exposure (Walker et al. 1992a) or intraperitoneal injection (Tates et al. 1999; Walker et al. 1992a) or repeated inhalation (Tates et al. 1999; Walker and Skopek 1993; Walker et al. 1992a) or drinking water (Tates et al. 1999) exposures. A 4-week inhalation exposure to 3–33 ppm resulted in a linear increase in HOEtVal levels; at 100 ppm, the slope estimated from the 3–33 ppm exposure underpredicted the HOEtVal levels by 20 and 25% in rats and mice, respectively (Walker et al. 1992a). In humans, HOEtVal blood levels are influenced by endogenous production of ethylene oxide, genetic status of the polymorphic glutathione transferases *hGSST1* (Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999, 2001; Yong et al. 2001), and smoking status (Bono et al. 1999; Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999). *hGSTM1* genotypes did not influence HOEtVal blood levels (Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999).

3.4 INTERACTIONS WITH OTHER CHEMICALS

No information was located regarding toxicologically-relevant interactions between ethylene oxide and other substances.