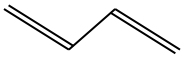
1,3-Butadiene Reference Exposure Levels

(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)

CAS 106-99-0



1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD), adopted in December 2008,that describes acute, 8-hour and chronic RELs. The TSD presents methodology that explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 et seq.). These guidelines have been used to develop the following RELs for butadiene; this document will be added to Appendix D of the TSD.

Butadiene is a major commodity product of the petrochemical industry. Workers acutely exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung. Some workers also experienced coughing, fatigue, and drowsiness. Inhalation of butadiene is mildly narcotic at low concentrations. Exposure to very high concentrations can result in narcosis, respiratory paralysis, and even death. Repeated exposure can damage human sperm cells and increase ovarian atrophy in mice. The studies reviewed for this document include those published through August 2012.

Although butadiene is a carcinogen (IARC Group 1, IARC, 2012), this document does not discuss issues related to the cancer potency factor. That has been previously derived and is available at www.oehha.ca.gov/air/hot_spots/index.html.

1.1 Butadiene Acute REL

Inhalation reference exposure level	**660 µg/m^3 (297 ppb)**
Critical effect(s)	Lowered male fetal weight
Hazard Index target(s)	Development

1.2 Butadiene 8-Hour REL

Inhalation reference exposure level	**9 µg/m^3 (4 ppb)**
Critical effect(s)	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

1.3 Butadiene Chronic REL

Inhalation reference exposure level	**2 µg/m^3 (1 ppb)**
Critical effect	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

2 Physical & Chemical Properties

Description	Colorless gas
Molecular formula	C_4H_6
Molecular weight	54.09 g/mol
Density	0.6211 g/ml at 20 ºC
Boiling point	-4.4 ºC
Melting point	-108.9 ºC
Vapor pressure	2100 mm Hg at 25 ºC
Flashpoint	- 76 ºC
Flammability limits	Extremely flammable
Solubility	Very slightly soluble in water ; soluble in ethanol, ether, acetone, benzene and organic solvents
Odor threshold	0.0014 mg/l; mildly aromatic with slight petroleum odor
Octanol-water partition coefficient	Log Kow = 1.99
Henry's Law constant	0.074 atm m^3/mole (HSDB, 2011)
Conversion factor	1 ppm = 2.21 mg/m^3 at 20 ºC, 1 bar 1 mg/m^3 = 0.445 ppm at 20 ºC, 1 bar

3 Occurrence and Exposure

1,3-Butadiene (butadiene, BD) is a major commodity product of the petrochemical industry. The largest quantities are used in the production of styrene-butadiene rubber copolymers for car and truck tire manufacturing. Butadiene polymers are also used in a variety of other industrial applications, including the production and synthesis of certain latex products, resins, and nitrile rubbers. Most environmental releases of butadiene (78.8%) are associated with non-point or mobile sources such as motor vehicles. About 1.6% of butadiene releases are fugitive or accidental emissions during manufacture, use, transport, storage, or disposal. The remainder comes from other sources including direct volatilization from gasoline, stack emissions from incinerators, cigarette smoke, and the burning of plastics (U.S. EPA, 2002; HSDB, 2004). Butadiene is a component of gasoline (Kane and Newton, 2010) as well as a product of the combustion of minor gasoline components, olefins and cyclohexane (Zhang et al., 2008). Approximately 26 percent of butadiene emissions in California can be attributed to on-road motor vehicles,

with an additional 27 percent attributed to other mobile sources, such as recreational boats, off-road recreational vehicles, and aircraft. Area-wide combustion sources contribute approximately 21 percent and include agricultural waste burning, open burning associated with forest management, and woodstoves and fireplaces. Stationary sources contribute less than one percent of the statewide 1,3-butadiene emissions. The primary stationary sources with reported 1,3-butadiene emissions are petroleum refining, manufacturing of man-made materials, and oil and gas extraction. The primary natural sources of 1,3-butadiene emissions are wildfires. The total statewide emission inventory for butadiene was estimated at 3,754 tons for 2008 (CARB, 2009).

Butadiene is subject to rapid destruction by reactions in the atmosphere in the presence of O_3, OH radical, and NO_x from which a number of reactive electrophiles are formed, including acrolein, formaldehyde, epoxybutene, and other aldehydes (Tuazon et al., 1999; Baker et al., 2005; Doyle et al., 2007). Baker et al. (2005) investigated the formation of butadiene and hydroxyl radical-initiated reaction products using atmospheric pressure ionization mass spectrometry (API-MS) and solid phase extraction followed by gas chromatography. The products from butadiene were identified as 4-hydroxy-2-butenal ($HOCH_2CH=CHCHO$) and its nitro derivative ($HOCH_2CH=CHCH_2ONO_2$) and isomers. The rate constant for the reaction of OH radicals (OH) with 1,3-butadiene was 6.66×10^{-11} $cm^3molecule^{-1}s^{-1}$ and with 4-hydroxy-2-butenal was $(5.7\pm1.4) \times 10^{-11}$ $cm^3molecule^{-1}s^{-1}$ both at 298±2K. The formation yields of acrolein and 4-hydroxy-2-butenal were 58±10% and 25+15-10%, respectively. Hydroxynitrates from the reaction of hydroxyperoxy radicals with NO account for an additional 7-10% of products from butadiene. Acrolein also reacts with OH radicals to form glycolaldehyde ($HOCH_2CHO$) with yields of 25 to 32 percent. For 1,3-butadiene the reaction with OH radicals is the dominant chemical loss process during daytime, with butadiene lifetimes of 1-2 hr at OH concentrations of 2×10^6 molecules/cm^3.

Sexton et al. (2007) investigated the photochemistry of 1,3-butadiene in an outdoor smog chamber. The butadiene atmospheric lifetime and transformation rates were dependent on concentrations of NO, NOx, other organic compounds, sunlight, temperature, and humidity. The results were compared with computer-based simulations of butadiene transformations (Morphecule Mechanism model). The major observed products with daytime BD/NOx were acrolein, formaldehyde, furan, 1,3-butadiene monoxide and an organic nitrate probably peroxyacylnitrate . Other carbonyls found were glyoxal, glycolaldehyde, glycidaldehyde, 3-hydroxy propanaldehyde, hydroxyl acetone, butenedial, and malonaldehyde. The model simulations were representative of the observed results.

Doyle et al. (2004) studied the photochemical degradation of 200 ppb 1,3-butadiene in presence of 50 ppb NO. The initial and major photochemical products were acrolein, acetaldehyde and formaldehyde. They observed that the photoproducts were more toxic to A549 human lung cells in vitro than butadiene. In a similar study Doyle et al. (2007) showed that BD + NOx + 5 hours light was more toxic to A549 cells than a synthetic mixture of acrolein, formaldehyde and ozone photoproducts or ozone alone. The photoproducts of butadiene were primarily acrolein, acetaldehyde, formaldehyde, furan and ozone. Toxicity was assessed by increased lactate dehydrogenase (LDH) release and increased interleukin-8 (IL-8) and interleukin-6 (IL-6) protein release.

3.1 Ambient Exposures

Despite its rapid removal, butadiene is almost always present at very low concentrations in US cities and large suburban areas.. Elevated concentrations have been measured in the vicinity of heavily trafficked areas, refineries, chemical manufacturing plants, and plastic and rubber factories (ATSDR 1992). Statewide levels throughout California have consistently dropped from an annual average high of 0.41 ppb in 1990 to approximately 0.1 ppb in 2007. Sampling of 18 communities in the San Francisco Bay area showed a maximum butadiene concentration of 0.26 ppb (San Jose). The majority of all samples were below the minimum detection limit (MDL) of 0.05 parts per billion by volume (ppbv) (BAAQMD, 2008). Similar sampling of 10 communities in the Los Angeles air basin showed a maximum butadiene concentration of 0.78 ppb (Compton). The majority of all samples were below the MDL of 0.2 ppbv (SCAQMD, 2008). In general, mean values in southern California are about twice those in the San Francisco Bay area e.g., San Jose, 0.069 ppb vs. Compton, 0.14 ppb.

One recent study measured butadiene concentrations prior to and after the Ireland Public Health Tobacco Act of 2002 ban on smoking in pubs (McNabola et al., 2006). The average concentration of butadiene measured inside pubs prior to the ban was 4.15 $\mu g/m^3$ ($\pm$ 1.61 $\mu g/m^3$). This is compared to average ambient outside measurement of 0.12 $\mu g/m^3$ (0.05 ppm) and an average indoor measurement of 0.22 $\mu g/m^3$ (0.1 ppm) following the smoking ban. Similar results were obtained in a survey of 10 Finnish restaurants with 4.3 ± 3.2 SD $\mu g/m^3$ (1.9 ppm) butadiene (geometric mean = 2.7, range = 0.26-10.1, N = 20 measurements) in smoking areas compared with 1.1 ± 1.3 SD (0.5 ppm) butadiene (geometric mean = 0.52, range = 0.11-3.9, N =20) in non-smoking areas (Vainiotalo et al., 2008).

The TEACH (Toxic Exposure Assessment, Columbia/Harvard) study characterized personal exposures to urban air toxics among high school students living in inner city neighborhoods of New York City (Kinney et al., 2002). Exposure to air toxics was analyzed in 46 high school students using 48-hr personal monitoring, outdoor ambient monitoring, and in-home ambient monitoring. The students were mainly African American and Hispanic, and were required to be non-smokers from non-smoking families. The subjects ranged in age from 12.5 to 19.6 years old, with 42% male and 58% female. Investigators measured levels of particulates and concentrations of 17 volatile organic compounds (VOCs). Butadiene levels tended to be higher in the home and from personal exposures than what was measured in ambient outdoor air. The mean outdoor concentrations of butadiene were 0.13 $\mu g/m^3$ (winter) and 0.14 $\mu g/m^3$ (summer), versus in-home ambient concentrations of 1.18 $\mu g/m^3$ in winter and 1.01 $\mu g/m^3$ in summer. Mean concentrations obtained from personal air monitors were 0.87 $\mu g/m^3$ (0.39 ppm) in winter and 1.16 $\mu g/m^3$ (0.52 ppm) in summer (Kinney et al., 2002).

Marshall et al. (2006) estimated the intake of butadiene from ambient air pollution in California's South Coast Air Basin. Butadiene concentrations were based on air dispersion modeling for the period April, 1998 to March, 1999. Surveys from 25,064 respondents were used in the study. The 28,746 person-days in the data set are from 11,749 households. Travel survey data were collected for 15-24 week periods in 2000-

2002. The method used four main inputs: temporal information about people's position (latitude and longitude); microenvironment, and activity level; temporally and spatially explicit model estimates of ambient butadiene concentrations; and stochastically determined microenvironmental adjustment factors relating the exposure concentration to the ambient concentration, age-, gender-, and activity-specific breathing rates. The calculated average breathing rate (m^3/day-person) for the study was 13.1. The mean intake rate for 1,3-butadiene was 7.3 µg/day ±7.6 SD. This corresponds to an average concentration of 0.56 µg/m^3 (0.24 ppb)(7.3/13.1) or approximately 8% of the chronic REL for butadiene derived in this document.

A qualitatively similar difference between personal/indoor air and outdoor air concentrations was observed in metropolitan Mexico City (Serrano-Trespalacios et al., 2004). Personal exposures to 34 VOCs were measured for adolescents and their families living close to one of five central monitoring stations over the period of one year. Simultaneous 24-hr indoor, outdoor and central site measurements were also taken. Indoor butadiene concentrations (mean = 2.5 µg/m^3, 1.1 ppb) were 2 to 6 times higher than outdoors measurements (mean 0.9 µg/m^3, 0.40 ppb). Personal exposures for all participants including adolescents (mean = 2.9 µg/m^3, 1.3 ppb) were also significantly higher than ambient outdoor concentrations (Serrano-Trespalacios et al., 2004). Logue et al. (2011) identified 1,3-butadiene as one of nine indoor air pollutants categorized as priority hazards based on available acute and chronic health criteria. Two hundred and sixty-seven chemicals were evaluated with health criteria for 97. Butadiene was categorized based on its cancer risk, however the upper bound estimate of exposure (their Fig.2) is close to the U.S. EPA RfC of 0.9 ppb and the chronic REL derived in this document (1.0 ppb).

Gustafson et al. (2007) measured personal exposures and indoor air concentrations of butadiene, benzene, formaldehyde, and acetaldehyde due to wood burning in a small Swedish town. Ambient air concentrations of butadiene for 1 day sampling (N = 9) had a mean of 0.12µg/m^3(0.05 ppb) (95%CI, 0.04-0.16). Indoor personal monitoring in the wood burning group (N = 14) gave mean butadiene concentrations of 0.31 to 0.38 µg/m^3 (0.14 to 0.17 ppb) for 1 to 7 days sampling. The referent group (N=10) had mean values of 0.11 to 0.14 µg/m^3 (0.049 to 0.06 ppb) ($P < 0.015$-0.03). The authors concluded that domestic wood burning increased personal exposure to ambient butadiene as well as indoor exposures to butadiene.

Additional exposure analyses were conducted by Nazaroff and Singer (2004), who studied hazardous air pollutants including butadiene within US residences. The authors found that the population inhalation intake of butadiene was dominated by residential environmental tobacco smoke (ETS). Mean individual intake of butadiene was also dominated by residential ETS. Data analyses indicated that some 16 million U.S. juveniles (2 months to 16 years old) are exposed to ETS in the home. Assuming between 14-20 cigarettes smoked per day in each residence, with an average of 515 µg butadiene/cigarette, the resulting daily intake of butadiene for juveniles was 16 – 37 µg. The total mean individual inhalation intake for juveniles was estimated to be approximately 10 mg butadiene/year from residential ETS, far exceeding the exposure

to butadiene from ambient sources, estimated at approximately 0.3 mg/year (Nazaroff and Singer 2004).

3.2 Worker Exposures

Investigators in the US, Europe, and China report similar average exposures to butadiene in the workplace. However, approaches to exposure assessment vary. Because butadiene monomer production and extraction are generally operated currently as closed processes, exposures tend to be low. With certain tasks, or with accidents and engineering failures, there can be transiently high exposures that are brief and intense. Air measurement may not accurately reflect these peaks. Historical levels of butadiene exposure in the workplace are thought to have been much higher than present values (Lynch 2001). Few data are available for butadiene concentrations prior to the 1970s. Exposure modeling suggests that levels in the 1940s – 1950s were considerably higher, and estimated to be approximately 20 ppm (Lynch et al., 2001). There has been an annual trend of an approximate 6% drop in butadiene concentrations over time, with large decreases in the late 1950s and the early 1980s (Lynch 2001). More current measurements of butadiene in the workplace were provided by Chan et al. (2006), who measured worker exposure to air toxics in an 11-plant petrochemical complex in Taiwan. For the years 1997-1999, only 15.2% of the measurements were above the limit of detection (LOD), with a mean low concentration of 7.7 ppb, a mean high concentration of 10.5 ppb, and a maximum concentration of 3080 ppb butadiene. Specific LODs were not given for the 39 chemicals studied but the mean low concentrations were calculated using 0 for values below the LOD and the mean high concentrations were estimated using LOD/$\sqrt{2}$ for values below the LOD.

Sapkota and colleagues evaluated butadiene exposure of tollbooth workers in the Baltimore Harbor Tunnel during the summer of 2001. Mean ambient butadiene concentrations outside the tollbooths varied by shift, with the morning levels (19.8 $\mu g/m^3$) exceeding afternoon levels (14.9 $\mu g/m^3$). The lowest concentrations were measured at night, and averaged 4.9 $\mu g/m^3$. Considerable protection was offered by the tollbooth itself, within which ambient concentrations even in the height of traffic only measured 6.7 $\mu g/m^3$ (3 ppb) butadiene (Sapkota et al., 2005).

Ammenheuser et al. (2002) evaluated BD exposure in 49 workers in a styrene-butadiene rubber plant. The concentration of the butadiene metabolite 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (M1) in urine was used as a biomarker of butadiene exposure and the frequency of hypoxanthine-guanine phosphoribosyl transferase (*HPRT)* mutations in peripheral blood lymphocytes was used as a biomarker of effect. The workers were divided into high and low exposure groups according to job description and work area. The average butadiene exposure concentrations (by personal organic vapor badge dosimeters) for the two groups were high, 1.48 ppm and low, 0.15 ppm ($P < 0.002$). Both mean M1 and *HPRT* mutant frequencies were more than three-fold higher in the high group than in the low group ($P < 0.0005$). For the high exposure group (N = 19-22) urine M1 mean was 2,046 ± 348 SE ng/mg creatinine and the *HPRT* frequency was 6.8±1.2 SE x 10^{-6}. The values for the low exposure group (N = 20-24) were 585±98 ng/mg creatinine and 1.8±0.2 x 10^{-6}, respectively. The study

demonstrated correlation between the three measures of butadiene exposure. All workers, even those not exposed to butadiene, excreted some M1 suggesting other exogenous and/or endogenous sources. It is uncertain whether the observed increase in mutant frequency represents an adverse effect.

4 Metabolism

Common routes of exposure to butadiene include inhalation and dermal exposure.The inhalation absorption of butadiene is dependent on its partition coefficient (log Kow = 1.99) and Henry's Law constant (0.074 atm m^3/mole). Butadiene is absorbed very quickly into the lungs and blood. Physiological modeling uses blood/air partition coefficients of about 1 for butadiene (e.g., lung/air = 0.65 in humans, Pery & Bois, 2009; blood/air = 1.22 in humans, Brochot et al., 2007). The dosimetry of butadiene in rodents has been assessed by measuring steady-state concentrations of butadiene epoxides in blood and deposition in tissues following butadiene exposure. The steady-state in vivo concentrations of epoxybutene in the blood of mice exposed to 62.5, 625, and 1250 ppm butadiene for 4 to 6 hours was 4-8 times higher than those measured in rats at these BD concentrations and exposure periods, i.e. 0.4 to 8.6 µM in mice vs. 0.01 to 2.5µM in rats (Bond and Medinsky, 2001). Average tissue epoxides in mice for exposures of 625 ppm or 1250 ppm butadiene for 6 hours ranged from 2 to 3.5 mmol/g for lung epoxybutene, 0.5 to 0.75 mmol/g for liver epoxybutene, and 0.6 to 1.5 mmol/g for lung diepoxybutane, respectively. In rats at exposures of 625, 1250, or 8000 ppm butadiene, lung epoxybutene ranged from 0.2 to 1.1 mmol/g and liver epoxybutene ranged from 0.1 to 1.2 mmol/g (Bond et al., 1996).

Butadiene metabolites play a role in the toxicity of butadiene. It is not always clear which metabolites are responsible for various observed toxicities (e.g., cardiovascular). In some cases, strong evidence implicates specific toxicological endpoints are associated with particular metabolites.

In general, studies using human tissues showed that humans metabolized butadiene more like rats than mice, with lower rates of diepoxybutane formation (Bond et al., 1996). Perez et al. (1997) report the formation of N-(2,3,4-trihydroxybutyl)valine hemoglobin adducts (THBV) from the butadiene metabolite epoxybutanediol in rats and occupationally exposed humans. The adduct levels in rats exposed to 50, 200, or 500 ppm butadiene were 13.4, 5.9, and 1.15 pmol THBV/g globin/ppm butadiene, respectively. In humans exposed to 1 ppm butadiene, hemoglobin adducts were 8.2 and 10.7 pmol THBV/g globin/ppm. The results indicate that butadiene is absorbed into human blood at relatively low concentrations and forms hemoglobin adducts at levels comparable to those seen in rats.

Once in the body, butadiene is activated, creating the toxic metabolite(s) associated with adverse effects. Activation of butadiene typically occurs in liver microsomes via P450-mediated oxidative metabolism in mammals (Bond and Medinsky, 2001). Epoxybutene is the major oxidative metabolite, which is further oxidized to diepoxybutane and hydrolyzed to epoxybutane diol by microsomal epoxide hydrolase (Bond and Medinsky, 2001; Elfarra et al., 2001) (Figure 1).

Available data indicate that metabolism is qualitatively similar among all species studied, although the rates of metabolite formation differ. Mice form the initial oxidative metabolite, epoxybutene, approximately 6 to 8 times faster than rats or humans (Csanady et al., 1992) and produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995; Csanady et al., 2011).

This greater formation of epoxy metabolites may be related to differences in the amino acid composition of the binding pocket of CYP2E1 in rodents that facilitate hydrogen bonding and increase the probability of a second epoxidation. Compared to the rat, mouse CYP2E1 has an additional methylene group in the active site that binds epoxybutene more tightly, thus further enhancing formation of the second epoxide (Lewis et al., 1997). This observed difference in the quantity of epoxides formed correlates with the observed sensitivity and higher overall toxic response in mice versus rats. In vitro data from adult liver samples indicate that humans form epoxides of butadiene at rates (Vmax/Km, where Vmax = maximum reaction rate e.g. µmoles/hr and Km = reactant or substrate concentration (S) in µmol/L giving half the Vmax, where the overall reaction rate is more precisely defined by the Michaelis-Menten relation v = Vmax x S/(Km + S)) similar to mice but less than rats (Duescher and Elfarra 1994; Krause and Elfarra 1997), although it is unclear how this relates to in vivo toxicity in humans. Other authors, notably Csanady et al. (1992), reported that the Vmax/Km ratio obtained with mouse liver microsomes was 5.6- and 8.2-fold higher than the ratios obtained with human or rat liver microsomes, respectively. The observed differences in human butadiene oxidation rates between the studies may be attributed to variability in the expression of P450 2E1, 2A6 or other P450 enzymes in the human liver samples (Duescher and Elfarra, 1994). Detoxification of butadiene epoxides also varies across species, with human tissues preferentially detoxifying the derived epoxides through microsomal epoxide hydrolase to form butane diol followed by conjugation with GSH, whereas detoxification in mice occurs through glutathione-*S*-transferase (Boogaard and Bond 1996; Kemper et al., 2001). As a result, mice predominantly excrete 1-hydroxy-2-(N-acetylcysteinyl)-3-butene (MII), while adult humans excrete mainly 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (MI) (Figure 1). Boogard et al. (2001) reported urinary metabolites resulting from 8 hours occupational exposure to 1 ppm butadiene: MI, 2213 µg/L and MII, 39 µg/L.

The reactive metabolites of butadiene, including 1,2-epoxy-3-butene (EB), 3,4-epoxy-1,2-butane diol (EB diol), diepoxybutane (DEB), and hydroxymethylvinyl ketone (HMVK) have been shown to covalently bind to biological macromolecules, including DNA in the testes, lung, and liver, and certain proteins, including hemoglobin (Koivisto et al., 1998; Hayes et al., 2000; Begemann et al., 2001; Boogaard et al., 2004). In vivo experiments have demonstrated species differences in adduct formation. For example, following a nose-only 6 hr inhalation exposure of 200 ppm ^{14}C-1,3-butadiene, measured uptake in mice was almost twice that for rats; the formation of specific DNA adducts was nearly 10 times higher in mice than rats (Boogaard et al., 2004). Quantitatively similar profiles of adducts were observed in an inhalation study using 20 ppm, although the overall level of DNA adducts formed in rats and mice was very low (Booth et al., 2004).

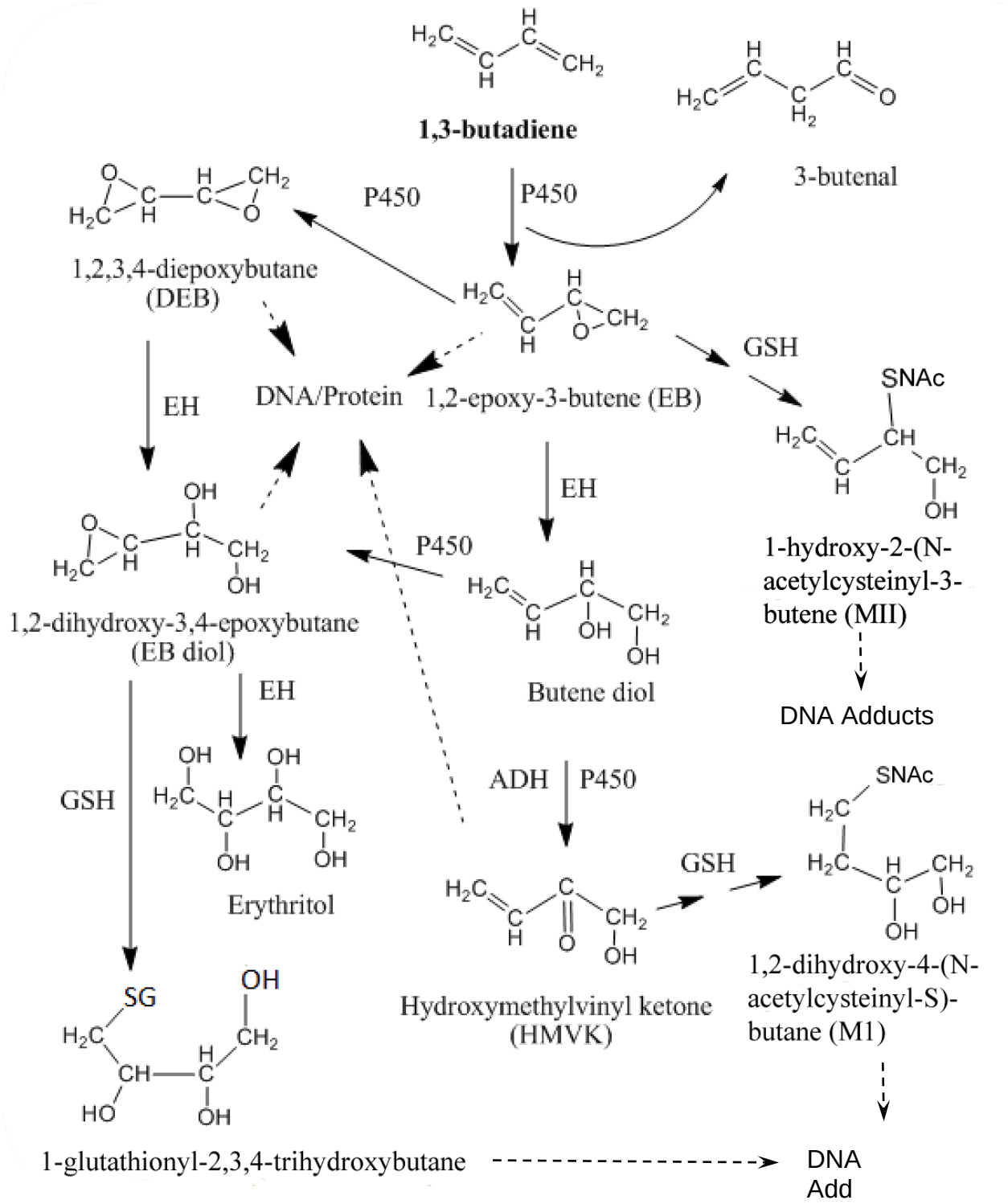


Figure 1 Metabolic scheme for 1,3-butadiene

This scheme presents the major identified reactive metabolites. Dashed arrows emanate from butadiene metabolites reported to form adducts with DNA or proteins. Abbreviations: EH, epoxy hydrolase; GSH, glutathione; ADH, alcohol dehydrogenase; P450, Cytochrome P450; NAc, N acetylcysteinyl; G, glutathionyl.

Cho and Guengerich (2012b) reported the mutagenicity in the *Escherichia* coli *rpoB* gene of the DEB-GSH conjugate *S*-(2-hydroxy-3,4-epoxybutyl)glutathione. Three DNA adducts were identified in the *E.coli* DEB/GST/GSH system: *S*-[4-(N(7)-guanyl)-2,3-dihydroxybutyl]GSH; *S*-[4-(N(3)-adenyl)-2,3-dihydroxybutyl]GSH; and *S*-[4-(N(6)-deoxyadenosinyl)-2,3-dihydroxybutyl]GSH.

Butadiene reactive metabolites also appear to form stable hemoglobin adducts in humans and experimental animals. In one study, Begemann et al. (2001) found that workers in a butadiene monomer production plant exposed to a median concentration of 440 $\mu g/m^3$ (0.2 ppm) had significantly increased levels of epoxybutene hemoglobin adducts versus unexposed controls. Results from another study indicated that levels of hemoglobin adducts of epoxybutane diol were substantially higher than those of epoxybutene in both inhalation-exposed rats and occupationally exposed humans (Perez et al., 1997). Forty-one butadiene polymer production workers in China were exposed to median butadiene concentrations of 2 ppm (6 hr time-weighted average) and had greater levels of hemoglobin adducts when compared with unexposed controls ($p < 0.0001$) (Hayes et al., 2000). These adduct levels were significantly correlated with butadiene concentrations ($p < 0.03$). The exposed workers did not differ from unexposed workers with respect to frequency of diepoxybutane-induced sister chromatid exchanges or aneuploidy, leading the authors to suggest that there were no correlations of biomarkers of butadiene exposure with these specific genotoxic effects (Hayes et al., 2000).

Sangaraju et al. (2012) used a more sensitive liquid chromatography-electrospray ionization tandem mass spectrometry method of measuring DEB-specific adducts in mouse liver DNA (bis-N7G-BD) following 2 weeks exposure (6hr/d, 5d/week) to various concentrations of 1,3-butadiene from 0.5 to 625 ppm. At low concentrations of 0.5, 1.0 and 1.5 ppm butadiene they found bis-N7G-BD (1,4-bis-(guan-7-yl)-2,3-butanediol) adducts at 0.17, 0.14, and $0.14/10^7$ nucleotides/ppm BD, respectively. At higher concentrations of 6.25, 62.5, 200 and 625 ppm BD the adduct levels were significantly lower at 0.051, 0.013, 0.009, and $0.006/10^7$ nucleotides/ppm BD, respectively. A similar pattern of higher efficiency of adduct formation at lower exposures was also seen with DEB-specific hemoglobin adducts by Georgieva et al. (2010). At the same low concentration butadiene exposures (0.5, 1.0 and 1.5 ppm), pyr-Val (N,N-(2,3-dihydroxy-1,4-butadiyl)valine) Hb adducts were formed at 21.0, 20.0 and 26.0 pmol pyr-Val-Hb/g globin/ppm BD. At 6.25, 62.5, 200 and 625 ppm butadiene concentrations, adduct formation was 12.0, 6.7, 3.7, and 2.5 pmol pyr-Val Hb/g globin/ppm BD, respectively.

Overall the results indicate that the efficiency of DEB reaction with cellular macromolecules is higher at lower exposure levels in mice. It is unknown whether a similar increased efficiency at low exposure levels occurs in humans. Boysen et al. (2012) have observed low but clearly detectable levels of pyr-Val-Hb in workers with 8-hr time weighted average butadiene exposures of 0.01 ppm (N = 23, controls), 0.24 ppm (N = 24, monomer workers), or 0.81 ppm (N = 34, polymerization workers). The levels of pyr-Val-Hb adducts found were 0.13, 0.16, and 0.29 ($P<0.05$ vs controls and monomer workers) pmol/g globin, respectively. The authors concluded that humans were less efficient in the formation of DEB than mice or rats at similar butadiene

exposures. HB-Val, indicative of epoxybutene concentrations, was found at 0.22, 0.47, and 2.23 pmol/g globin and THB-Val, indicative of epoxybutane diol concentrations, was found at 95, 179, and 716 pmol/g globin, respectively. The formation of DEB relative to other epoxides was significantly different in the highest exposed workers compared to controls and lower exposures. The pyr-Val concentrations in individual workers were significantly correlated with their HB-Val concentrations when analyzed combined or separately ($P < 0.01$, $P < 0.05$, respectively). In contrast the pyr-Val concentrations were significantly correlated with the THB-Val concentrations when analyzed combined or separately only for the high exposure group ($P < 0.01$).

5 Acute Toxicity of 1,3-Butadiene

5.1 Acute Toxicity to Adult Humans

There is a paucity of data on acute human exposure to butadiene. For eyes and skin, direct contact with liquid butadiene can cause burns similar to frostbite, while vapors or fumes may be irritating to the eyes. Some early occupational health studies indicated that workers exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung (Wilson 1944). Some workers also experienced coughing, fatigue and drowsiness, although the symptoms diminished after the exposure ceased. Inhalation of butadiene is mildly narcotic at low concentrations, and may result in a feeling of lethargy and drowsiness (Sandmeyer, 1981). Exposure to very high concentrations of butadiene can result in narcosis, respiratory paralysis, and even death. The first signs of acute exposure to high concentrations are blurred vision, nausea, paresthesia, and dryness of the mouth, throat, and nose. This may be followed by fatigue, headache, vertigo, hypotension, slowed pulse rate, and unconsciousness (ATSDR, 1992).

In more recent occupational health studies, while there were significant differences in short-term inhaled concentrations of butadiene between exposed workers (mean 1.5 $\mu g/m^3$, range 0.2 – 69.0 $\mu g/m^3$) and unexposed workers (mean 0.4 $\mu g/m^3$, range < 0.1 – 3.8 $\mu g/m^3$), biomarkers of effect, such as sister chromatid exchanges, were not different between groups of workers (Lovreglio et al., 2005). A study of 437 full-shift and 14 short-term samples indicated that exposure to butadiene in the polymer industry ranged from < 0.005 – 43.2 ppm for 8 hr samples, and 0.088 – 210 ppm for acute (15 – 120 min) exposures (Fajen et al., 1990). Data on potential adverse effects from acute exposure were not reported. Since the workers in many of these studies were exposed to mixtures of chemicals, the relationship of acute butadiene exposure and adverse effects remains unclear.

In an acute study of human physiological factors, 133 adult subjects (both male and female) were exposed to 2 ppm (4.42 mg/m^3) butadiene for 20 minutes (Lin et al., 2001) followed by purified air for 40 minutes. Five exhaled breath samples collected during exposure were used to measure the respiratory uptake of butadiene. The absorbed dose was defined as µg butadiene uptake/kg body weight. Although the subjects were given identical administered doses, there was a wide range of uptakes, 0.6-4.9 µg/kg.. The total butadiene concentration inhaled was significantly higher in males than in

females; however there was no significant difference in the respiratory uptake fraction of the total concentration inhaled, which ranged from 18-74% for all participants. Females had larger body burdens per kilogram of body weight than males. Additionally, both age and smoking were negatively associated with butadiene uptake. The authors suggested that the wide range of butadiene uptake values indicates that administered dose is a poor estimator of absorbed dose or body burden (Lin et al., 2001).

Khalil et al. (2007) evaluated neurological parameters for a random cohort of 310 subjects who had been exposed to 1,3-butadiene due to accidental leakage and release of BD in an industrial area and 50 matched unexposed controls. A set of clinical neurological tests was utilized including electroencephalograms (EEGs), somatosensory evoked potentials (SSEPs), brainstem auditory evoked potentials (BAEPs), visual evoked potentials (VEPs), and electromyography/nerve conduction tests (EMG/NCT). Exposed individuals were divided into mild (N = 212) and severe (N = 98) subgroups based on preliminary testing. Among the mild subgroup the most common finding was encephalopathy characterized by headaches and light headedness that resolved within hours to six months. The electroneurodiagnostic tests were normal except for abnormal BAEPs in 21/212 (9.91%) subjects. In the severe subgroup encephalopathy was characterized by confusion, irritability, impaired judgment, altered levels of consciousness, disequilibrium, numbness, paresthesia, ataxia, weakness, nausea, vomiting, and memory deficits. The EEGs were mildly abnormal (12/98, 12%) and BAEPs and VEPs strongly so (63/98, 49/98, respectively). Within the 50 controls, one had an abnormal EEG on heavy medication and 2 (4%) had polyneuropathy with diabetes and alcohol abuse, and two had abnormal BAEPs due to deafness or hearing abnormalities. All controls had normal VEPs. The authors concluded that butadiene met the definition of a neurotoxicant. The butadiene-induced neurotoxic effects of most of the subjects were reversible except for 18 (6%) subjects that demonstrated irreversible neurotoxic effects.

5.2 Acute Toxicity to Infants and Children

The exposures most infants and children have to butadiene tend to be chronic and low level. However, by correlating changes in health effects with short-term changes in ongoing measurements of butadiene and other VOC's, it may be possible to discern the potential toxicity associated with acute exposures. This is the approach taken below to determine the effects of air pollutants on asthma exacerbation in children.

Delfino and colleagues studied the relationship between asthma symptoms in children and concentrations of ambient VOCs and criteria air pollutants, including butadiene (Delfino et al., 2003). To be eligible to participate, children were required to have a minimum 1-year history of physician diagnosed asthma and at least two symptomatic days per week that required use of a β-agonist inhaler. Twenty-two Hispanic children, aged 10 – 16, who were living within the Los Angeles air basin and who were nonsmokers from nonsmoking households, were enrolled in the study. The children kept daily symptom diaries from November 1999 through January 2000 and the severity of their symptoms was analyzed for association with ambient concentrations of particulates, ozone, and VOCs. Regression analysis examined air pollution

concentrations on the same day or up to 4 days prior to the reported asthma symptoms, thereby allowing the analysis of the lag between the measured concentrations and the onset of health effects.

Adjusted odds ratios (OR's) for more severe asthma symptoms were as follows: for 1.4 ppb NO_2, 1.27 (95% CI, 1.05-1.54); 1.00 ppb benzene, 1.23 (95% CI, 1.02-1.48); 3.16 ppb formaldehyde, 1.37 (95% CI, 1.04-1.80); 37 µg/m^3 PM_{10}, 1.45 (95% CI, 1.11-1.90); 2.91 µg/m^3 elemental carbon, 1.85 (95% CI, 1.11-3.08); and 4.64 µg/m^3 organic carbon, 1.88 (95% CI, 1.12-3.17).

Butadiene was detected in 74 daily air pollution measurements with a mean concentration of 0.51 ppb (range 0.05 – 1.50 ppb). This study presented an OR of 1.32 (95% CI = 0.97-1.80) for the association between asthma symptoms and ambient butadiene concentrations on the previous day, and an OR of 1.16 (95% CI = 0.90-1.49) for the association between asthma symptoms and same-day butadiene levels. While these estimates are elevated, the confidence intervals included no effect. Thus, the authors interpreted these results as suggestive of an increased risk of asthma symptoms in children with butadiene exposure. However, the study was limited by small numbers, as only seven subjects reported symptom scores >2 and only 16 with scores >1. Also, associations for scores >2 were strongly influenced by one subject with moderate persistent asthma (Delfino et al., 2003). Since many of these pollutant chemicals occur together it will always be difficult to tease out effects due to specific pollutants. However, such studies may be of use in comparison with studies of accidental releases of butadiene where the problem of concurrent exposures is much less.

5.3 Acute Toxicity to Experimental Animals and In Vitro Toxicity

Few studies have reported the acute or short-term effects of butadiene. The lethal concentration resulting in 50% mortality in a population (LC_{50}) was measured in an acute inhalation study using 2 hr exposures for mice and 4 hr exposures for rats (Shugaev 1969). The mouse LC_{50} was determined to be 122,000 ppm, and the rat LC_{50} was 129,000 ppm; no other effects were reported. In a National Toxicology Program study, male and female B6C3F1 mice were exposed to 0, 625, 1,250, 2,500, 5,000, or 8000 ppm butadiene in air for 15 days (NTP, 1984). Survival rates were unaffected by dosing, and no respiratory effects, cardiovascular lesions, or hepatic/gastrointestinal histopathology were noted even at the highest concentration. There was, however, a non-significant decrease in mean body weight at 8000 ppm butadiene when compared with controls (NTP 1984). No severe immunological changes were noted in humoral or cell-mediated immunity in mice exposed to 1250 ppm butadiene for 6 weeks (Thurmond et al., 1986).

The effects of butadiene inhalation (12.5 and 1250 ppm) on birth outcomes following a single acute 6-hr exposure of male mice were reported by Anderson et al. (1996). The mean number of implants per female was reduced at both concentrations compared to controls, but statistically significant only at the higher level ($p < 0.05$). A dominant lethal

effect (see Section 7.2) was not evident with the acute exposure, and there was no significant increase in post-implantation losses or fetal abnormalities.

In contrast to the results of the studies of the parent compound cited above, short-term studies of butadiene metabolites have shown acute effects, some of which were severe. Henderson and colleagues investigated the acute and sub-acute toxicity of the oxidative metabolite butadiene diepoxide in female B6C3F1 mice and in Sprague Dawley rats. Examination of the organs, including the liver, kidney, marrow, heart, and ovaries revealed that a single 6 hr inhalation exposure to 18 ppm butadiene diepoxide (3 to 4/group) induced no clinical signs of toxicity. However, the mice showed a slight increase in the number of alveolar macrophages and focal necrosis of the olfactory epithelium that started to reverse 7 days after exposure (Henderson et al., 1999). In the sub-acute portion of the study, mice (15/group) were exposed to either 2.5 or 5 ppm butadiene diepoxide for 6 hr/day for 5 days. The animals showed no body weight changes and no histopathology of the nose, larynx, or lung. However, in an earlier part of the study there was significant toxicity when mice and rats (56/group) were exposed to either 10 or 20 ppm butadiene diepoxide for 6 hr/day for 7 days. Both mice and rats experienced significant weight loss, corneal opacity, labored breathing, ruffled coats and hunched posture, as well as nasal mucosa degeneration, necrosis, inflammation, and ulceration. Additionally there were individual instances of focal hepatic necrosis and bone marrow atrophy in the mice. All but one of 56 mice from the high dose group were dead by the end of 12 days (98.2 %); by comparison, mortality in the low dose group was only 29% at 10 days of the 21 day study duration.

In a study of the butadiene metabolite 3-butene-1,2-diol, male B6C3F1 mice (4 to 5/group) were given a one-time intraperitoneal (i.p.) dose of 250 mg/kg and male Sprague Dawley rats (4 to 5/group) were given either 125, 200, or 250 mg/kg 3-butene-1,2-diol i.p. (Sprague et al., 2004). Two of the four rats dosed with 250 mg/kg died within 24 hours. Prior to death, the rats were lethargic and experienced seizures. At necropsy, it was evident that rats dosed at the highest level experienced severe hepatic necrosis and hemorrhage. The rats dosed with 200 mg/kg also showed indications of hepatic necrosis, inflammation, and hemorrhage. No other rat organ examined showed consistent dose-related lesions, although dosed rats exhibited significant hematological differences and significantly lower hepatic levels of glutathione when compared with controls (Sprague et al., 2004). Mice necropsied 4 hrs after treatment with 250 mg/kg 3-butene-1,2-diol did not exhibit any lesions, or any significant hematological changes. However, the mice did have significantly lower levels of hepatic glutathione 1 hr after treatment when compared to controls (Sprague et al., 2004). Half-lives for 3-butene-1,2-diol were calculated to be 2.12 hr in the rat and 0.44 hr in the mouse, which may partially account for the observed differences in acute toxicity between the two species (Sprague et al., 2004).

5.3.1 Toxicity In Vitro

Results from a human embryonic lung fibroblast study suggest that in vitro exposure to the butadiene metabolite 1,2:3,4-diepoxybutane (100 μM for 1 hr) is associated with substantial inhibition of cell proliferation and cell cycle arrest at G_1 (Schmiederer et al.,

2005). The authors suggested that cell proliferation was inhibited by a diepoxybutane-mediated alteration to cell division (Schmiederer et al., 2005). Another in vitro study found that products from the incomplete combustion of butadiene were differentially taken up and retained by human bronchial epithelial cells (Penn et al., 2005). Investigators found that the combustion-generated "ultrafine" particles (defined by the authors as <2.5 µm aerodynamic diameter) migrated from culture medium to the cell membrane, but not into the cell interior. The organic chemicals bound to the particles, however, were found to migrate from the particle surface, through the cell membrane into the cytosol, and into cellular vesicles. The authors suggested that toxicants, including butadiene, may transfer into cells directly without the cellular uptake of the carrier particles i.e., direct uptake from the vapor phase (Penn et al., 2005).

6 Developmental and Reproductive Toxicity

6.1 Developmental Effects

Many investigators have studied the potential for butadiene and its metabolites to cause reproductive or developmental toxicity. Earlier studies focused on overall reproductive fitness following short-term exposures, while more recent studies have focused on specific effects and mechanisms of toxicity.

Some of the first studies on the reproductive and developmental toxicity of butadiene were nested within chronic studies (Owen et al., 1987; Melnick et al., 1990). The National Toxicology Program developed the first series of short-term studies to evaluate developmental and reproductive toxicity of inhaled butadiene in mice and rats (Morrissey et al. 1990). In a three-part study, Sprague Dawley rats (24-28/group), and B6C3F1 and Swiss (CD-1) mice (18-22/group) were exposed to 0, 40, 200, or 1000 ppm butadiene on gestation days 6-15 for 6 hr/day and examined for teratological effects. A separate set of male mice was exposed to 0, 200, 1000, or 5000 ppm for 6 hr/day for 5 days for a sperm morphology study. Pregnant rats exhibited toxicity at 1000 ppm in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain; there were no other differences in fertility or developmental parameters between exposed and control groups. In mice, however, there were increased numbers of fetal variations (supernumerary ribs, reduced ossification) in litters from dams exposed to 200 and 1000 ppm butadiene. These teratogenic effects occurred in the presence of maternal toxicity and fetal weight gain reductions. Male mice treated with 1000 or 5000 ppm butadiene showed significant increases in abnormal sperm 5 wks after exposure, consistent with an effect of butadiene on mature spermatozoa and spermatids (Morrissey et al., 1990).

Teratological effects at maternal butadiene exposures of 200 ppm and higher were also observed by Hackett et al. (1987a), along with signs of maternal toxicity. However, in their study of reproductive performance, and maternal and fetal toxicity, Hackett et al. included a lower dose of 40 ppm. For this study, 78 pregnant female CD-1 mice received whole-body exposure to 0, 40, 200, or 1000 ppm butadiene for 6 hr/day on gestation days (gd) 6-15, with necropsy on gd 18. Mice were weighed prior to mating and repeatedly during gestation. They were observed for signs of toxicity during

exposure and examined for gross tissue abnormalities at necropsy. Reproductive parameters included numbers of implantations sites, resorptions, and live and dead fetuses. Live fetuses were examined for signs of morphological anomalies and growth retardation. The incidences of fetal variations (supernumerary ribs and reduced ossification of the sternebrae) were significantly elevated in litters from mice exposed to 200 and 1000 ppm ($P < 0.05$). At these exposure levels, there was also evidence of maternal toxicity as shown by significantly lower maternal weight gain ($P \leq 0.05$). There was a significant dose-dependent reduction of fetal body and placental weights at the two higher doses for female fetuses, and at all doses in males ($P \leq 0.05$) (Table 1). The observation that males fetuses appeared to be susceptible to butadiene at levels that were not maternally toxic is the basis of the acute REL.

Green (2003) reanalyzed the data of Hackett et al. (1987) and found inconsistencies associated with the presentation and calculation of mean values for maternal and fetal body weights, sex ratio, and reproductive data. When the data were analyzed in the context of analysis of covariance (ANCOVA) with sex ratio and litter size as covariates, no statistically significant difference was found between fetal weights at the 40 ppm exposure level and the controls. The results of the Green (2003) re-analysis are shown in Table 2B. In addition to the covariance adjusted pairwise tests of means in Table 2, Green also evaluated the ratios of treatment means to control means and calculated a 95% confidence interval (CI) for this ratio using the variance-covariance matrix from the ANCOVA. For male fetal weights the ratios (and 95% CI) were: control, 100 (referent); 40 ppm, 95.7(100.1); 200 ppm, 82.6(87.2); and 1000 ppm, 77.0(81.5). Since the upper 95% CI for the 40 ppm exposure level includes 100 there is no statistically significant difference from the control at this level. The other data sets for combined sexes and female fetal weights gave similar results.

In a companion study, Hackett et al. (1987b) exposed female Sprague-Dawley rats to whole body inhalation of 1,3-butadiene for 6 hr/day at 0, 40, 200, or 1000 ppm. The female rats (24 to 28/dose group) that had been mated to unexposed males were exposed on gestation days 6 thru 15 and sacrificed on gd 20. There were no significant differences among treatment groups in maternal body weights or extragestational weights at 40 or 200 ppm, but at 1000 ppm depressed body weight gains were observed during the first 5 days of exposure and extragestational weight gains were lower than control values. Placental weights, fetal body weights and sex ratios were unaffected by butadiene treatment. There were no significant differences among groups for incidences of fetal malformations.

The dose-response analyses of the Hackett et al. (1987) and the Green (2003) re-analysis data sets are described and discussed in Section 8.1. Those analyses employ the benchmark dose software (BMDS v. 2.3.1) and do not depend on a specific no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL). Rather, the benchmark model uses the entire dose-response data set. The key value for a non-cancer dose response is usually the benchmark concentration level $BMCL_{05}$, or the 95% lower confidence limit on the concentration causing a 5% response. Considerable experience has shown that the $BMCL_{05}$ is a better choice for a point of departure than a single dose NOAEL.

Table 1. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Hackett et al., 1987a)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Maternal weight gain (g)[a] (11-16 gd)	13.3 ± 0.6[b]	12.7 ± 0.4[b,c]	11.4 ± 0.5[c,d]	10.6 ± 0.4[d]
Pup body weight (g)[a]				
Females	1.30 ± 0.02[b]	1.25 ± 0.01[b]	1.10 ± 0.02[c]	1.02 ± 0.03[d]
Males	1.38 ± 0.03[b]	1.31 ± 0.02[c]	1.13 ± 0.02[d]	1.06 ± 0.02[e]
Sex ratio (% male)	51.6 ± 3.91	49.8 ± 3.06	51.5 ± 3.68	51.8 ± 3.29
Placental weight (mg)[a]				
Females	83.1 ± 3.03[b]	80.9 ± 2.46[b]	74.7 ± 3.52[b,c]	70.1 ± 2.33[c]
Males	89.3 ± 3.05[b]	89.5 ± 2.27[b]	80.1 ± 2.35[c]	74.5 ± 1.81[c]

[a]Mean ± standard error; [b-e] Values that do not share a common superscript letter are significantly different ($p \leq 0.05$) from one another.

Table 2. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Green, 2003 Reanalysis of Hackett et al., 1987a data)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Pup body weight (g)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Combined	1.350 ± 0.028	1.283 ± 0.013	1.126 ± 0.021*	1.038 ± 0.025*
Females	1.309 ± 0.021	1.253 ± 0.012	1.100 ± 0.022*	1.015 ± 0.026*
Males	1.382 ± 0.033	1.307 ± 0.016	1.132 ± 0.016*	1.060 ± 0.024*
Sex ratio (% male)	51.55 ± 3.866	49.66 ± 2.947	51.44 ± 3.677	51.80 ± 3.310
Placental weight (mg)				
Combined	86.99 ± 2.960	85.30 ± 2.283	78.65 ± 3.243	73.06 ± 1.826*
Females	83.15 ± 3.023	80.89 ± 2.474	74.33 ± 3.540	70.84 ± 2.284*
Males	89.58 ± 2.995	89.71 ± 2.263	80.27 ± 2.324	74.64 ± 1.785*

Note: * significantly different from controls ($P < 0.05$).

6.2 Dominant Lethal Effects

The primary purpose of the dominant lethal assay is to identify toxicants that have the ability to enter the gonad and cause a genetic lesion in the gamete, which results in cytogenetic damage in the fertilized zygote without recognized changes in fertilizability. Generally, male animals are either given 1 – 5 doses of the test agent, or are dosed over the entire cycle of spermatogenesis, then mated at weekly intervals with untreated females. The females are necropsied prior to giving birth and examined for uterine contents. The total number of implanted embryos is compared over dose groups and with controls. Dominant lethal assays use conceptus failure as an endpoint for potential male-mediated developmental toxicity.

Nested within the larger National Toxicology Program study discussed above, dominant lethality was studied in litters sired from male mice exposed to 70, 200, 1000, or 5000 ppm butadiene for 6 hr/day for 5 days (Morrissey et al., 1990). There was no mortality noted in the male mice even at the highest butadiene concentrations, and body weights were unaffected. One week following exposure to 1000 ppm, investigators observed a statistically significant increase in dead implants as a percentage of total implants ($p \leq 0.05$). There were smaller non-statistically significant increases in dead implants at 200 and 5000 ppm (p values not given). During the second week following exposure, there were significant increases in dead implants in both the 200 and 1000 ppm groups ($p \leq 0.05$). The investigators noted that the dose-dependency of the effects weakens at higher dose levels, but the effect in week 1 and 2 is consistent with an effect of butadiene on mature spermatids and spermatozoa. The authors suggested that because of the effects noted in sperm morphology and dominant lethality, butadiene might act as a germ cell mutagen in mice (Morrisey et al., 1990).

In 1993, Anderson and colleagues considered dominant lethality in a more focused study of male-mediated reproductive toxicity following acute and short-term butadiene exposure. In the acute study, male CD-1 mice were exposed to 0 (n = 25), 1250 (n = 25), or 6250 (n = 50) ppm butadiene one time for 6 hr, then mated with untreated females. In the short-term study, male CD-1 mice were also exposed to 0, 12.5, or 1250 ppm butadiene for 6 hr/day 5 days/wk for 10 weeks prior to mating, then mated with untreated females. The necropsied dams were examined for the number of live fetuses, the number of early and late post-implantation deaths, and the number and type of fetal gross malformations. A single 6 hr exposure did not elicit a dominant lethal effect, although there was a non-significant reduction in the mean number of implants in the 1250 and 6250 ppm dose groups. Such findings may indicate germ cell toxicity or pre-implantation losses, which are generally not associated with heritable changes (Anderson et al., 1993). Subchronic exposures to 12.5 ppm butadiene were associated with increased congenital malformations and late fetal deaths. In addition, there was a significant incidence of dominant lethality at 1250 ppm butadiene. The authors concluded that butadiene is mutagenic and teratogenic following subchronic exposures (Anderson et al., 1993).

To further elucidate the dominant lethality of butadiene, Adler and Anderson (1994) exposed CD-1 male mice to 1250 ppm for 6 hr/day, 5 day/week for 10 weeks prior to

mating, and then mated the exposed males with unexposed females for 4 successive weeks. There was a total (summed) incidence of 23.1% dominant lethality over 3 weeks of mating. The highest weekly incidence of dominant lethality was 12.4% after week 2 of mating. No other fertility differences were noted between exposed and control animals. From the timing of the dominant lethal effect, the authors suggested that spermatozoa and late spermatids were the most sensitive germ cell types to butadiene inhalation (Adler and Anderson, 1994). Most of these results were duplicated in another report by Anderson and colleagues (Anderson et al., 1996) and are consistent with dominant lethal findings by Morrisey et al. (1990).

In 1994, Adler and colleagues designed a cross-bred (102/E1 x C3H/E1) mouse study to consider not only dominant lethality but teratogenicity in pregnancies allowed to come to term. Male mice (20 per group) were exposed to 0 or 1300 ppm butadiene for 6 hr/day for 5 days, and then mated with unexposed females for 4 consecutive weeks. Groups of pregnant dams were sacrificed at gestation day 14 following successive weeks of mating. The percentage of dead implants and dominant lethals was elevated in litters that were sired during weeks 1, 2, and 3 following butadiene exposure in the males. The effect, however, was highly significant ($p \leq 0.01$) in week 2. All other fertility parameters were similar to controls (Adler et al., 1994). From the timing of effects, the authors concluded that dominant lethal mutations were induced in spermatozoa and late spermatids, suggesting that butadiene may induce heritable translocations in these germ cell stages (Adler et al., 1994), consistent with their previous work in CD-1 mice (Adler and Anderson 1994).

Two other studies have demonstrated a positive association between inhalation of low concentrations of butadiene and dominant lethality, with mice being more susceptible than rats (Anderson et al., 1998; Brinkworth et al., 1998). Brinkworth and colleagues exposed male CD-1 mice to 0, 12.5 or 125 ppm butadiene for 6 hrs/day for 5 days a week for 10 weeks followed by mating with untreated females. A significant increase in dominant lethality was noted, but only in the 125 ppm group. Anderson and colleagues exposed male CD-1 mice to 0, 12.5, 65, or 130 ppm butadiene for 6 hr/day, 5 days/week for four weeks and groups of male Sprague Dawley rats to 0, 65, 400, or 1250 ppm butadiene for 6 hr/day, 5 days/week for 10 weeks. There was a significant increase in dominant lethality in mice in the 65 and 130 ppm dose groups, although the effect was not dose-related. Male-specific effects in the rats were not observed at any exposure level (Anderson et al., 1998). Additionally, one study showed that the butadiene metabolite diepoxybutane is also associated with dominant lethality. Male mice were given either 0 or 54 mg/kg diepoxybutane (DEB), and mated for up to 16 days after treatment with untreated females. A single dose of 54 mg/kg DEB was toxic to mature spermatozoa; therefore, there was a low number of total implants in the first 8 days after treatment. However, there was an increase in dominant lethals throughout all mating intervals. The authors suggested that diepoxybutane is likely a late spermatid mutagen (Adler et al., 1995).

Accumulated data appear to suggest that inhalation exposure of butadiene is associated with an increase in dominant lethal effects even at concentrations below the threshold for acute toxicity. There is evidence of species and strain differences in

susceptibility, with mice being more susceptible than rats, and outbred CD-1 mice appearing to show dominant lethality at lower butadiene concentrations than other strains of mice. Regardless of the length of pre-mating dosing (i.e., a single 6 hr, 5 day, or 4-10 week exposures), dominant lethal effects were associated with butadiene effects in the more mature male germ cells, specifically mature sperm and late spermatids.

6.3 Germ Cell Mutagenicity

Results from dominant lethal experiments suggest that butadiene exposure may be associated with germ cell mutagenicity. As such, several researchers investigated the potential for butadiene to induce heritable mutations. In one study, C3H/E1 male mice were exposed to 1300 ppm butadiene for 6 hr/day for 5 days and mated with untreated females. Half the offspring were examined for dominant lethality while the other half were allowed to mature in order to study heritable effects in subsequent generations (Adler et al., 1995). There was a non-significant increase in dominant lethal mutations in F_1 litters sired from butadiene-exposed males. However, when the F_1 males were allowed to mature and mate, the resulting F_2 litters were approximately half as large as litters sired by control F_1 mice. Additionally, there was a 2.7% translocation frequency in F_2 pups following F_0 paternal butadiene exposure compared with a 0.05% translocation frequency from historical controls (Adler et al., 1995).

In a follow-up study, mice exposed to 0, 130, or 500 ppm butadiene for 6 hr/day for 5 days were mated for 4 consecutive weeks with untreated females. A positive dominant lethal effect was observed for 130 and 500 ppm during week one of mating (a sperm effect) and for 500 ppm during week two of mating (a late spermatid effect). Among 434 F_1 offspring from butadiene-exposed F_0 sires, there was a 1.15% incidence of translocation when compared with 0.05% translocation in historical controls ($p < 0.001$) (Adler et al., 1998). The heritable mutation was identified as a reciprocal translocation with insertion of the central portion of chromosome 9 into chromosome 19 (Adler et al., 1998). The genetic risk from butadiene exposure was calculated using data from these two studies with a linear dose-response model [$Y = 0.05 + 6.9 \times 10^{-5} X$] where Y is the percentage of offspring with heritable translocations and X is butadiene exposure in ppm hr. Results from the calculations suggest that butadiene has the potential to cause heritable mutations in mouse sperm that can be transmitted to subsequent generations with a doubling dose of 725 ppm-h butadiene (Adler et al., 1998).

Pacchierotti et al. (1998) investigated the paternal transmission of butadiene-induced chromosomal aberrations to one cell embryos. Male mice (102/E1 x C3H/E1) were exposed to 0, 130, 500, or 1300 ppm butadiene for 6 hr/day for 5 days then mated 1:2 with untreated B6C3F1 females. The resulting zygotes were arrested at first cleavage and analyzed for chromosomal aberrations in each of the first three weeks of mating following paternal exposure. Zygotes conceived in weeks one and two of mating were produced from oocytes from untreated females fertilized with either mature sperm or late spermatids. Zygotes conceived in week three of mating were the product of oocyte-early spermatid or oocyte-late prophase spermatocyte unions. Investigators found a dose-dependent increase in structural chromosomal aberrations in first-cleavage

embryos conceived in week one of mating, with significance ($p < 0.005$) achieved at 500 ppm butadiene. Embryos conceived during week two also showed dose-dependent increases in chromosomal aberrations, with significance achieved at 1300 ppm butadiene ($p < 0.025$). The authors concluded that the male germ cells associated with the observed embryo mutations were likely the same types as those associated with male-mediated dominant lethality in other studies, and that sperm in the final stages of maturation were most susceptible to the mutagenic effects of butadiene.

Anderson et al. (1997) evaluated the effects of 1,2-epoxybutene (BMO) and three other chemicals (β-estradiol, daidzein, dibromochloropropane) on human sperm using the alkaline Comet assay for DNA damage in vitro. Fresh and frozen samples from two fertile donors each and frozen samples from two infertile donors were analyzed. All the chemicals induced significant effects in all exposed samples ($P < 0.01$). For BMO exposures of 0, 80, 160, and 320 µM, significant dose-response model fits were obtained only with frozen samples. Using the continuous Hill and polynomial models, $BMCL_{05}$ values ranged from 0.6 to 24.2 µM with an arithmetic mean of 12.3 µM and a geometric mean of 4.64 µM. The effects seen with BMO were comparable to those seen with dibromochloropropane, a known male reproductive toxicant, at 20 to 200 µM. Internal exposure estimates obtained with human PBPK models based on Kohn and Melnick (2001) indicate that BMO blood concentrations in the range of $BMCL_{05}$'s might be achieved by acute occupational exposures to butadiene (e.g., 200 ppm butadiene x 2 hr = 6.8 to 15.6 µM BMO Cmax in venous blood). Alternatively, the human PBPK model of Brochot et al. (2007) predicts lower blood and tissue concentrations of BMO for the same exposure scenario (i.e., 0.1 to 0.7 µM).

The majority of acute reproductive toxicity studies of butadiene have focused on male animals. No similar studies were found for female animals. However, one study by Tiveron and colleagues (1997) considered the acute female reproductive toxicity of the butadiene metabolite diepoxybutane. Female B6C3F1 mice received a single intraperitoneal injection of 26, 34, 43, or 52 mg/kg diepoxybutane, and then mated with unexposed males. The embryos were arrested at first-cleavage metaphase and examined for chromosomal aberrations. There were no consistent diepoxybutane-associated effects on mating, fertilization, or cell-cycle progression of the fertilized oocytes, although there was a decrease in the average number of zygotes harvested from each female. There was, however, a significant increase in frequency and number of chromosomal aberrations in exposed zygotes when compared with controls in all dose groups ($p < 0.05$-0.001).

When comparing the sensitivity of male and female gametes to pre-conception exposures, it appears that zygotes produced from exposed sperm-nonexposed oocytes are many times more likely to contain chromosomal aberrations (Adler et al., 1995) than zygotes conceived with exposed oocytes (Tiveron et al., 1997). While chromosomal damage may be transmitted via female germ cells (Adler et al., 1995), sperm appear to be at much higher risk of the mutagenic effects of butadiene and butadiene metabolites.

6.4 Ovarian Atrophy

Reproductive organs appear to be critical targets of chronic butadiene exposure, and ovotoxicity is the basis of the chronic and 8-hour RELs. Effects in the female reproductive tract were identified in an NTP chronic study, where B6C3F1 mice were exposed to 0, 625, or 1250 ppm butadiene for 6 hr/day, 5 days/wk for a planned period up to 103 weeks (NTP, 1984). Significant ovarian atrophy was observed in female mice in both dose groups (40 of 45 females at 625 ppm; 40 of 48 females at 1250 ppm). NTP investigators identified a chronic LOAEL of 625 ppm, based on the observed gonadal effects (NTP, 1984). In an effort to further elucidate the reproductive toxicity, NTP investigators conducted a study in B6C3F1 mice with lower butadiene concentrations (Melnick et al., 1990; NTP 1993). The animals were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm for 6 hr/day, 5 days/wk for up to 65 weeks. While there was significant mortality, a concentration-related increase in ovarian atrophy was also observed. At 40 weeks, ovarian atrophy was present in females exposed to 200 and 625 ppm butadiene. At 65 weeks, ovarian atrophy was present in all groups exposed to ≥ 20 ppm butadiene, and female mice exposed to the lowest concentrations of butadiene (6.25 ppm) exhibited atrophy at the end of the study at 105 weeks (Melnick et al., 1990; NTP, 1993). Based on these results, NTP investigators identified a chronic LOAEL of 6.25 ppm for reproductive toxicity (NTP, 1993). The 9-, 15- and 24-month ovarian atrophy data from NTP (1993) were subjected to benchmark dose analysis as summarized in Table 3. Only the 9-month interim sacrifice data were adequately fit by the multistage model (X^2 = 2.47, P = 0.78, $BMCL_{05}$ = 19.2 ppm). The 24-month data were adequately fit by the log probit model if the top dose was excluded. If the dose response used the average mixed venous concentration of diepoxybutane (DEB) from the mouse PBPK model an excellent fit to all the 24 months data was obtained (X^2 = 0.1, P = 0.99, $BMCL_{05}$ = 0.58µM DEB equivalent to 0.71 ppm BD). If the data were included in a time-adjusted model, all of the data could be fit (N = 435). Using the log probit model, a $BMCL_{05}$ of 1.01 ppm butadiene was obtained with a X^2 of 1.7, P = 0.64 (BMDS v 2.2). This study forms the basis of the derivation of 8-hour and chronic RELs described in sections 8.2 and 8.3, respectively.

While atrophy may be associated with normal reproductive senescence in rodents, it is noteworthy that butadiene-associated ovarian atrophy was observed in female mice as early as 9 months of age in association with butadiene exposure (NTP 1984). Depletion of ovarian follicles and ovotoxicity were also observed following intraperitoneal administration of butadiene monoepoxide or diepoxide (Doerr et al., 1996). Doerr et al. (1996) administered butadiene monoepoxide (BMO; 0.005-1.43 mmol/kg b.w.) or the diepoxide (DEB; 0.002-0.29 mmol/kg b.w.) to female $B6C3F_1$ mice (n = 10/dose) intraperitoneally daily for 30 days. The investigators analyzed the data on ovarian and uterine weight depression in mice (% body weight ± SD) using a PBPK model to simulate i.p. administrations and extrapolate internal dose metrics to external butadiene inhalation equivalents.

Table 3a. Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	X^2	P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMCL_{05}$ continuous, ppm	Comments
9 months	Multistage	2.47	0.78*	35.0	19.25	3.44	Full data set, N = 58
15 months	Log probit	10.74	0.030	11.2	3.66	0.654	Full data set, N = 52
	Log probit	10.64	0.014	11.1	3.45	0.616	Without top dose, N = 50
24 months	Log probit	6.47	0.091	0.056	0.0034	0.00054	Full data set, N = 325
	Log probit	2.80	0.42*	0.254	0.031	0.0055	Without top dose, N = 246
24 months#	Log logistic PBPK mean blood DEB concn	0.1	0.99	1.21 µM	0.56 µM, 0.71 ppm BD equivalent	0.51 ppm BD equivalent	Full data set, N = 325
9-24 mo time adjusted**	Log probit	1.7	0.64*	2.04	1.01	0.18	Full data, N = 435
9-24 mo time adjusted**	Log logistic	1.13	0.89*	2.03	1.58	0.28	Full data, N = 435

Note: * indicates adequate model fit to data, P ≥ 0.1; ** time adjustment by fitting % atrophy data to the hyperbola y (%) = a – b/(1 + c x dose)^d where a, b, c, and d are parameters, the dose was ppm butadiene (6hr/day, 5days/week), and the % atrophy response was weighted for time (PSI-Plot Hyperbola c, www.polysoftware.com). The fitted values were reconverted to quantal equivalents and analyzed by BMDS 2.2 with log probit, log logistic and multistage models (see Tables A8-A11 in appendix for additional details). # PBPK model simulation was for 24 hr and the 24 hr DEB AUC was divided by 24 to give average daily concentration so continuous adjustment is only 5/7 for 5 days/week exposure.

The values from Figure 2 of Doerr et al. (1996) were estimated as shown in Table 4. Internal dose metrics were then analyzed by benchmark dose methods using BMDS (versions 1.4.1c and 2.1.2). The benchmark dose BMD_{05} was the 0.05 relative deviation of a given dose metric typically using the Hill or Polynomial continuous dose response model and the $BMDL_{05}$ was the 95% lower confidence limit on that dose. The 5% response level is typically chosen by OEHHA for noncancer toxicity endpoints. The PBPK model was based on Kohn and Melnick (2001). The formation of hemoglobin adducts in the model was based in part on Csanady et al. (2003) with additional ACSL model code obtained from Prof. J.G. Filser (personal communication).

Table 4. Mean Ovarian and Uterine Weights (% Body Weight) in Mice Estimated from Figure 2 of Doerr et al. (1996).

Compound	Dose i.p., mmol/kg-d	Ovary	SD*	Uterus	SD*
BMO	0.005	0.0425	0.007	0.27	0.126
	0.02	0.0425	0.007	0.30	0.126
	0.09	0.038	0.007	0.28	0.126
	0.36	0.034	0.007	0.27	0.126
	1.43	0.020	0.007	0.10	0.126
DEB	0.002	0.0375	0.007	0.34	0.126
	0.009	0.034	0.007	0.33	0.126
	0.036	0.035	0.007	0.22	0.126
	0.14	0.020	0.007	0.11	0.126
	0.29	0.015	0.007	0.03	0.126

*****SD values assumed equal for BMD analysis since they appear similar for low doses and decrease for the high doses.

Following the 30 days of exposure, ovaries and uteri were weighed, fixed, stained, and the follicular populations were assessed microscopically. In mice receiving 1.43 mmol BMO /kg, body weights were slightly decreased (10%) while ovarian and uterine weights were significantly decreased ($p < 0.05$). Administration of DEB caused significant decreases in both ovarian and uterine weights at 0.14 and 0.29 mmol/kg b.w. ($p < 0.05$), along with a 15% decrease in body weights at the higher dose. A dose-dependent decrease in ovarian follicle numbers was observed with both epoxides. To facilitate comparison, the dose required to reduce follicle numbers by 50% relative to controls (ED_{50}) was estimated. For small and growing follicles, the ED_{50} values for BMO were 0.29 and 0.40 mmol/kg, respectively. DEB showed greater potency with ED_{50} values for these same follicle populations of 0.10 and 0.14 mmol/kg, respectively. These authors suggested that butadiene ovotoxicity may be associated with metabolism of the parent compound to reactive diepoxides in situ.

Perez et al. (1997) measured N-(2,3,4-trihydroxybutyl)valine (THBV) adducts of hemoglobin in rats exposed to 1,3-butadiene or butadiene epoxides. Sprague-Dawley rats (2 to 3/group) were treated with single i.p. doses of either epoxybutene (78.3 mg/kg), epoxybutanediol (30 mg/kg), or diepoxybutane (16,7, 33.4, 60 mg/kg). The animals were sacrificed and blood was collected after 48 hr. Rats (3/group) were exposed to 1,3-butadiene by inhalation for 6 hr/d for 5 days at concentrations of 0, 50, 200 or 500 ppm. Two humans occupationally exposed to 1 ppm butadiene were also analyzed. The HBI (hemoglobin binding index, pmol THBV adduct/g/µmol/kg for epoxides, or /ppm-hr for butadiene exposures) for rats was 0.07 for epoxybutene, 3.4 for epoxybutanediol, and 4.0 to 9.3 for diepoxybutane. For butadiene exposures in rats, THBV adducts ranged from 0.04 to 0.5 pmol/g/ppm-hr. For the exposed human subjects, the THBV adduct levels were 0.15 and 0.20 pmol/g. The results indicate that epoxybutanediol is comparable to DEB in it adduct forming ability and considerably more active than BMO.

In the Hazelton Laboratories study, Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hr/day, 5 day/week, for 105 weeks. Although investigators reported tumors in certain reproductive organs, there was no clear statement on the presence or absence of ovarian atrophy, or any other reproductive effect in females (Owen et al., 1987).

Male reproductive effects have also been observed in relation to chronic butadiene exposure, although generally at concentrations greater than those that elicited effects in female animals. In the NTP study, investigators observed a significant concentration-related decrease in testis weight at weeks 40 and 65 for males exposed to ≥ 62.5 ppm butadiene (Melnick et al., 1990). In addition, testicular atrophy (measured at weeks 40 and 60) was observed in males exposed to 625 ppm butadiene (Melnick et al., 1990). In the later NTP study (1993), investigators once again observed testicular atrophy at 625 ppm, and reduced testicular weights in the 200 ppm and 625 ppm exposure groups at the end of the study. However, no effect was seen at the lower butadiene concentrations, as noted in female mice (NTP 1993). In the Hazelton Laboratories study, there was no clear statement of the presence or absence of testicular atrophy in male rats exposed to up to 8000 ppm butadiene. However Leydig cell tumors were observed in 3 males in the 1000 ppm group and 8 males in the 8000 ppm group (Owen et al., 1987).

Kirman and Grant (2012) conducted a meta-analysis with dose-response data from rats and mice that were normalized using an internal dose estimate of diepoxybutane (DEB) in blood that is causally related to ovarian toxicity. A time-to-response (multistage-Weibull) model was used to fit the pooled data sets with exposures from 13 to 105 weeks. Reference concentration (RfC) values were derived for scenarios with average, low and high follicle counts at birth with differing durations of susceptibility to toxic action of DEB. The $BMCL_{01}$ points of departure for these scenarios in ppm continuous butadiene exposure were 17, 1.5, and 240 ppm, respectively. The authors applied a 10-fold uncertainty factor to these values to derive a range of presumably health protective values for human exposures.

7 Chronic Toxicity of 1,3-Butadiene

7.1 Chronic Toxicity to Adult Humans

Studies on the chronic effects of butadiene have been centered in the styrene-butadiene rubber manufacturing industry and the butadiene monomer industry. The Occupational Health Studies Group at the University of North Carolina, Chapel Hill, organized one of the earliest retrospective epidemiological studies conducted in the rubber industry. Investigators observed rubber workers during the period of 1964 – 1972 and reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and atherosclerotic heart disease) among the subjects (McMichael et al., 1976).

Penn and Snyder (2007) reviewed the evidence supporting an association between butadiene exposure and cardiovascular disease. Two independent longitudinal epidemiological studies have indicated an association. Matanoski et al. (1990) found that butadiene exposure in a styrene-butadiene manufacturing facility was associated with a significant increase in the standardized mortality ratio (SMR: the ratio for mortality in the workers compared to the general population) for arteriosclerotic heart disease in exposed black workers (SMR = 1.48 (95%CI, 1.23-1.76)). The SMRs were adjusted for age, calendar time, and race. Circulatory system disease was also significantly elevated in 214 of 1195 exposed black workers (SMR = 1.18 (95%CI, 1.03-1.35)). Significant differences in these diseases were not seen in 10,915 exposed white workers. In both cases the referent population was U.S. males. Devine (1990) and Devine and Hartman (1996) studied a cohort of nearly 2800 male workers employed at least 6 months at a 1,3-butadiene manufacturing facility. They found significantly elevated SMRs for all causes of death including arteriosclerotic heart disease in nonwhite workers (SMR = 1.28 (95%CI, 1.06-1.53)). For arteriosclerotic heart disease the SMR was 1.42 (95%CI, 0.95-2.04). The referent population was the U.S. nonwhite male mortality rates. Butadiene exposure was also associated with atherogenesis in an avian model (see 7.3 below).

Additional arguments have been made in regard to polymorphisms for xenobiotic metabolizing genes including GSTM1, GSTT1, CYP2E1, and microsomal epoxide hydrolase (EH) and susceptibility to atherosclerosis. Salama et al.(2002) studied 120 atherosclerotic subjects and 90 matched controls for associations between polymorphisms in metabolizing genes and atherosclerotic susceptibility. They found increased atherosclerotic susceptibility associated with the GSTT1 null allele and the fast allele of EH. Also individuals with one of three combined phenotypes (GSTM1 null/CYP2E1 5B, GSTM1 null/mEH YY and GSTT1 null/mEH) exhibited significantly increased atherosclerotic susceptibility (ORs = 3.4-15.42). The importance of EH as a potential cardiovascular disease susceptibility gene in population subgroups and as a key player in butadiene metabolism may influence the AR potential of butadiene in individuals exposed to ETS.

Johns Hopkins University and the National Institute for Occupational Safety and Health initiated two large cohort studies of North American synthetic rubber industry workers (Meinhardt et al., 1982; Matanoski et al., 1990). Investigators studied male workers at eight synthetic rubber plants and two plant complexes in Texas, Louisiana, Kentucky, and Ontario, and included men who began their employment as far back as 1943. Results from these studies were the first to report increased risk of hematologic neoplasms from workplace exposure to butadiene. The University of Alabama designed a series of follow-up epidemiological studies, and has since reported an excess of leukemia among hourly workers with long duration of employment and high butadiene exposure (Delzell et al., 1996), as well as a positive association between cumulative exposure to butadiene and leukemia risk (Macaluso et al., 1996). More recently, University of Alabama investigators evaluated all-cause mortality for the same synthetic rubber industry workers from 1944 – 1998 (Sathiakumar et al., 2005). The subjects had an overall mortality rate 14% lower than expected, including lower rates of cardiovascular, digestive, and genitourinary deaths than the general population.

However, the total group of 17,924 subjects had 16% more leukemia deaths than expected. While this increase was not limited to a single form of leukemia, it was concentrated in long-term hourly workers particularly those in polymerization, coagulation, and maintenance positions (Sathiakumar et al., 2005).

Results from studies of the genotoxic outcomes in workers exposed to butadiene have not been consistent. Begemann et al. (2001) analyzed blood samples from 17 workers in butadiene monomer production and 19 controls from a heat plant in the Czech Republic. Butadiene exposure was assessed by personal monitoring, with a median exposure concentration of 440 $\mu g/m^3$ for exposed workers and < 6 $\mu g/m^3$ for control subjects. The hemoglobin adduct HBVal, which is formed by a reaction of the *N*-terminal valine with the carbon-1 of epoxybutene, was measured with a limit of detection of 0.2 pmol/g globin. Results showed that the median butadiene-hemoglobin adduct level in monomer production workers was significantly higher than that of controls, with no consistent correlation with any other parameter except smoking (Begemann et al., 2001).

Hayes et al. (2000) also measured a spectrum of genotoxic outcomes, including aneuploidy and sister chromatid exchange, in 41 butadiene polymer production workers and 38 non-exposed controls in China. Full-shift personal exposure varied widely, and short-term breathing zone grab samples showed even greater extremes (median 6.5 ppm, range 0 – 1078 ppm). Hemoglobin adducts were significantly more common in butadiene exposed workers than controls ($p < 0.0001$). In addition, butadiene exposed workers had greater lymphocyte and platelet counts than controls. However, the frequency of total aneuploidy (chromosomes 1, 7, 8, 12) did not differ significantly between exposed and non-exposed workers, nor did structural or numerical chromosomal abnormalities. The authors found no correlation between hemoglobin adducts and any genotoxic markers, nor did they find a positive correlation between butadiene concentrations in air and sister chromatid exchanges (Hayes et al., 2000).

Lovreglio and colleagues (2005) conducted personal monitoring throughout an 8 hr work shift at a petrochemical plant where butadiene was produced and polymerized. Personal monitoring was conducted 3 to 4 times over a 6-week period, with the last session followed by blood sampling of the subjects. Twenty-seven healthy petroleum plant workers were matched with 26 controls not occupationally exposed. Results indicated significantly higher airborne butadiene levels for exposed workers when compared with controls, ranging from 0.2 to 69 $\mu g/m^3$. Blood samples were analyzed for sister chromatid exchange and percent chromosomal aberrations in peripheral blood lymphocytes. After correcting for cigarette smoking, the investigators found no genetic marker differences between butadiene exposed and control workers. In their conclusion, the authors stated that there was a lack of genotoxicity in workers exposed to very low concentrations of butadiene similar to ambient levels currently characterized in the general population (Lovreglio et al., 2005).

The Health Effects Institute reported on a genetic epidemiology study of Czech workers exposed to butadiene (Albertini et al., 2003). The study was designed to determine whether biomarkers of exposure could be correlated with in situ levels of butadiene and

personal workplace exposure. Blood and urine samples were collected from males, working in either a butadiene monomer production plant or in a butadiene-styrene polymerization facility. Samples were also collected from male administrative workers who had very limited occupational exposure to butadiene, and served as controls. In addition, full-shift personal monitoring was conducted on each of 10 days over a 60-day period for both groups of workers, including the days on which biological samples were collected. The mean concentrations ranged from 0.026 mg/m^3 for controls to 1.76 mg/m^3 for polymer workers, with maximum concentrations of 0.125 mg/m^3 and 39 mg/m^3, respectively (Albertini et al., 2003). Cytogenetic assays included chromosomal aberrations, breaks, translocations, and sister chromatid exchanges. No significant relations could be identified between group mean chromosomal aberrations, determined by the conventional method or by fluorescent in situ hybridization, and butadiene exposure levels after adjusting for age and smoking. Since tobacco smoke contains butadiene it is possible that this latter adjustment affected the results. In addition, there was no relationship found between butadiene exposure and aberrations associated with glutathione-S-transferase genotypes. Regression analyses showed no significant effects of butadiene exposure on sister chromatid exchanges, although the effect of smoking on sister chromatid exchanges was significant after adjusting for age and occupational butadiene exposure. Hemoglobin adducts of butadiene (to the valine residue) were analyzed as biomarkers for butadiene exposure. There was a significant correlation between the individual subject's exposure levels and two types of hemoglobin adducts. However, the authors cautioned that one adduct, trihydroxybutyl valine (THBVal), also appeared to be formed by an endogenous material other than butadiene and its metabolites, indicating relatively high natural background levels. Overall conclusions from the authors indicate that the cytogenetic endpoints did not prove to be sensitive indicators of butadiene exposure at the levels encountered in these facilities (Albertini et al., 2003).

Since butadiene exposures rarely occur in the absence of other air toxic contaminants it is usually difficult to isolate the effects due to butadiene from those due to other chemicals or combinations. It is OEHHA policy to use human data in preference to animal data when they are of suitable quality. However, in the case of butadiene there are no human dose-response data that can be used to derive a chronic REL with confidence.

7.2 Chronic Toxicity to Infants and Children

Infants and children are rarely exposed to butadiene alone but rather experience long-term exposure to butadiene as one of the combustion products in vehicle exhaust, environmental tobacco smoke (ETS), and other combustion processes. As can be expected with many toxic air contaminants, it is difficult to elucidate the effects of one particular pollutant when it exists in the presence of multiple co-pollutants. Studies of the chronic effects of butadiene in children primarily focus on carcinogenesis as the endpoint. Butadiene has been associated with asthma exacerbation and increased respiratory tract infections, but only in the presence of other pollutants. While some recent studies have aimed to quantify children's exposure to butadiene, ascribing

chronic respiratory effects to butadiene alone remains problematic (see discussion of TEACH study in section 3 above).

7.3 Chronic Toxicity to Experimental Animals

Butadiene is considered a multi-site carcinogen in all identified long-term studies in mice and rats, although there appear to be strain and species differences in sensitivity and severity of effect (NTP 1984; Owen et al., 1987; Melnick et al., 1990; NTP 1993). Instances of tumors include malignant lymphomas, histiocytic sarcomas, cardiac hemangiosarcomas, as well as neoplasms of the liver, lung, mammary gland, ovary, and forestomach (NTP, 1993).

Far fewer data are available for the non-cancerous effects of long-term exposure to butadiene. However, based on these limited data, sensitivity to non-neoplastic events appears to be consistent with species variations in metabolism of butadiene and quantitative differences in the formation of butadiene metabolites (Hughes et al., 2001). For mice, National Toxicology Program (NTP) investigators exposed B6C3F1 males and females to 0, 625, or 1250 ppm butadiene for 6 hrs/day, 5 days/week, for up to 61 weeks (NTP 1984). Liver necrosis was observed in male mice at both doses and in female mice at 1250 ppm. At the highest dose, adverse changes were observed in the nasal cavity, including non-neoplastic lesions, chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. In a later study, investigators studied the effects of lower concentrations of butadiene in mice exposed for 6 hrs/day, 5 days/week, for up to 2 years (Melnick et al., 1990; NTP 1993). Two-year survival was significantly decreased in mice exposed to ≥ 20 ppm, which the investigators attributed to butadiene-induced malignant neoplasms. Beyond the neoplastic changes, there were increased incidences of angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia.

Chronic effects in rats were studied by Hazelton Laboratories and reported later by Owen and colleagues (1987; 1990). Groups of 110 male and female Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hrs/day, 5 days/week, for up to 2 years. After the first 52 weeks, investigators euthanized multiple female rats in both dose groups because of the high incidence of large subcutaneous masses. Increased mortality in males was observed in the highest dose group and associated with increased nephropathy. Body weight decreases were observed in the first twelve weeks in the high dose males and females, along with liver and kidney organ weight changes. Although there were minor clinical effects, including slight ataxia, no major changes in neuromuscular function could be definitively associated with butadiene exposure (Owen et al., 1987). Further reporting on this 2-year chronic investigation indicated that there were decreased survival rates at 105 weeks for females and 111 weeks for males (Owen and Glaister 1990). There were no effects on hematology, blood chemistry, and urinalysis, although changes in body and organs weights continued until study termination. Although somewhat equivocal, the rats displayed both common and uncommon tumor types, leading the investigators to suggest that butadiene might be a rat oncogen (Owen and Glaister 1990).

Other studies have identified hematological effects following chronic exposure to butadiene at concentrations at or below those that were associated with systemic toxicity (Irons et al., 1986a; Irons et al., 1986b; NTP 1993). Such hematotoxicity may be associated with the observed changes in organ weights (NTP, 1993). Butadiene exposure has also been associated with bone marrow changes, including bone marrow atrophy, bone marrow toxicity (regenerative anemia), and decreased cellularity as observed in mice exposed to ≥ 62.5 ppm (Melnick et al., 1990; NTP, 1993). Owen and colleagues (1987), however, found no hematological effects in rats exposed to as much as 8000 ppm butadiene in a 2-yr bioassay.

Penn and Snyder (2007) have identified vapor phase butadiene as the principal component in environmental tobacco smoke (ETS) promoting plaque development in an avian model of accelerated atherosclerosis (20 ppm butadiene x 16 weeks). The total butadiene doses that produced atherogenic effects were only 1/80th and 1/1000th the minimal doses causing cancer in mice and rats, respectively. The authors note that side-stream smoke, which represents about 90 % of ETS, contains butadiene at 205 to 400 µg/cigarette versus 16 to 77 µg/cigarette in main-stream tobacco smoke. Furthermore, Penn and Snyder (1996, 2007) found that the atherogenic effect of butadiene exposure was apparently not associated with either of the two principal epoxy metabolites of BD; 1,3-epoxybutene and 1,2:3,4-diepoxybutane. Neither of these metabolites was increased by butadiene exposure in avian liver in contrast to mouse liver.

7.4 Mode of Action

There is currently no accepted mode of action for the acute or chronic effects of butadiene exposure noted in this document. The metabolism of butadiene in all species studied produces epoxide, diepoxide and epoxydiol metabolites in addition to glutathione conjugates and 1,2-butene-3,4-diol. The epoxides all exhibit mutagenic activity in various test systems and react with DNA and proteins to form adducts. Butene diol has exhibited the ability to cause single strand breaks in DNA (Zhang et al., 2012). Adduct forming activity has also been observed for the glutathione conjugate of the butadiene metabolite DEB (Cho and Guengerich, 2012a). One of the DEB-conjugates (*S*-(2-Hydroxy-3,4-epoxybutyl)glutathione) was observed to be more mutagenic than DEB or other BD-epoxides in *Salmonella typhimurium* TA1535 (Cho and Guengerich, 2012b). The ability of DEB-GSH conjugates to form DNA adducts and cause mutations indicate a level of reactivity that may extend to protein binding. While mutagenicity of epoxide metabolites and other genetic toxicity is usually associated with oncogenic effects of butadiene and related chemicals, these genotoxic effects may also be associated with non-cancer effects such as cardiovascular disease, neurodegenerative disease, diabetes mellitus, rheumatoid arthritis, and aging (Burnet, 1974, Cooke et al., 2006). Recent reports indicate a specific mutation (*TREM2*) associated with increased risk of Alzheimer's disease in humans (Guerreiro et al., Jonsson et al., 2012).

In addition to the formation of pyr-Val-Hb and related hemoglobin adducts and their use as biomarkers of internal exposure to specific butadiene epoxy metabolites in

butadiene exposed human subjects, such adducts are also indicative of more general reaction of these metabolites with cellular proteins. The generalized organ and body adverse effects induced by butadiene in mice: fetal body weight decreases, relative organ weight decreases (lung, liver and kidney), and tissue atrophy (ovary, testes, nasal olfactory epithelium) are indicative of, or at least could be plausibly associated with, epoxy adduct formation of key cellular proteins and/or nucleic acids. Such adducts may lead to impaired cellular function and observed organ/tissue effects. The observed neurotoxic effects of butadiene exposure may also be due to reactive epoxide metabolites binding to key proteins and nucleic acids of the central nervous system.

Oxidative stress induced by BD metabolism may also play an important role in the observed tissue/organ toxicity and possible chronic effects in humans exposed to butadiene (Primavera et al., 2008; Yadavilli et al., 2007). Products of butadiene combustion have been observed to inhibit catalase activity and induce oxidative DNA damage repair enzymes in human bronchial epithelial cells in vitro (Kennedy et al., 2009). While the atherogenic effect of butadiene seen in an avian model does not have a clear link to the principal epoxide metabolites of butadiene, it may be related to ETS-induced oxidative stress as seen in mouse heart tissue (Yang et al., 2004). Alternatively, previously unidentified metabolites like the GSH-conjugates of Cho and Guengerich (2012a,b) may be involved in atherogenesis.

8 Derivation of Reference Exposure Levels

8.1 1.3-Butadiene Acute Reference Exposure Level (aREL)

Study	Hackett et al. (1987)
Study population	78 Pregnant CD-1 mice and offspring
Exposure method	Whole body inhalation of 0, 40, 200 or 1000 ppm on GD 6 through 15
Critical effects	Lowered male fetal weight at GD 18
LOAEL	200 ppm
NOAEL	40 ppm (based on Green re-analysis)
$BMCL_{05}$	17.7 ppm (Hill Model)
Exposure duration	6 hr
Time-adjusted exposure	n/a
Human Equivalent Concentration	29.7 ppm (17.7 ppm * 1.68 DAF)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	$\sqrt{10}$
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	$\sqrt{10}$
Cumulative uncertainty factor	100
Inhalation reference exposure level	297 ppb (660 µg/m^3)

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in noncancer adverse health effects (see Section 5 of the Technical Support Document).

The study by Hackett et al. (1987) was selected as it addressed the most sensitive noncancer endpoint associated with butadiene inhalation: developmental effects. A continuous benchmark dose analysis of the male mouse fetal weight data from Table 2 was conducted and the results are presented in Table 5. Both applied and PBPK doses were used but the latter showed little improvement in model fit or values derived. The best value based on the applied dose and the Hill model using the values from the Green (2003) re-analysis of the Hackett data was 17.7 ppm for the mouse and 29.7 ppm for the human equivalent concentration. This analysis in BMDS (v 2.3.1) was based on the male pup data and the homogenous variance assumption, due to the exact model fit and little evidence of differing variance in the exposed groups. We analyzed all the data (male and female) as well as the males only and the males consistently gave better model fits and lower $BMCL_{05}$ values (Table 12A). The nonhomogeneous variance assumption based BMC/BMCL values were about 50% lower than the homogeneous variance values and the model fit, while acceptable, was not exact (see Table A12). In the end we chose the lowest BMCL value giving the best model fit (male pups, Green, 17.7 ppm).

A second mouse PBPK model based on rat and human pregnancy (Gentry et al., 2002; Poet et al. 2010) allowed estimates of fetal exposure to BMO (mean fetal AUC GD 9-18). This metric also gave an exact fit to the fetal body weight data (Table 5). In the summary acute REL calculation above, we employed the standard intraspecies toxicokinetic uncertainty subfactor (UF_{H-k}) of 10: this is specifically justified to account for observed human variability due to polymorphisms in the microsomal epoxide hydrolase (mEH) gene reported by Abdel-Rahman, Wickliffe, Ward and co-workers (Abdel-Rahman et al., 2003, 2005; Wickliffe et al. 2003). When the interaction between BD exposure and inheritance of a variant ATT allele for mEH was evaluated in 49 exposed workers, the increase in HPRT variant mutant frequency was increased to $10.89 \pm 2.16 \times 10^{-6}$ (95% C.I. = 6.56-15.20 ($\times 10^{-6}$), p = 0.0027) over the background rate of $4.02 \pm 1.32 \times 10^{-6}$ (Abdel-Rahman et al. 2005). In this case, the background is not an unexposed general population but rather that portion of the worker study population (N= 33) with exposures < 150 (18.4 ± 5.5) ppb BD. Since mEH is a key enzyme in the detoxification of butadiene epoxide metabolites, low activities in this gene/enzyme translate to increased internal concentrations of these metabolites and risk of related toxic effects. The dosimetric adjustment factor (DAF) is related to the ratio of human and animal blood/air partition coefficients for butadiene and was estimated using mouse and human PBPK models. For example, the butadiene blood/air partition coefficient for rodents used by Kohn and Melnick (2001) was 1.95 and the butadiene blood/air partition coefficient for humans used by Brochot et al. (2007) was 1.22 ± 0.30. This would indicate a DAF based on these values of 1.60. OEHHA's estimates using rodent and human PBPK models was 1.68. That is:

DAF = PBPK predicted human blood concentration/PBPK predicted animal blood concentration and;

Human equivalent concentration (HEC) = Animal experimental concentration x DAF.

Table 5. Benchmark Dose Analysis of Male Mouse Fetal Weight Data of Hackett et al. (1987).

Dose Metric	N, male fetuses	Model	BMC_{05}	$BMCL_{05}$	$BMCL_{05}$ ppm BD equivalent	Human ppm equivalent 1.68 DAF
Applied BD ppm 6 hr/d						
0	109	Hill (Green reanalysis)	37.2**	17.7**	17.7**	29.7
40	118	Hill (Hackett et al. original values)	28.5**	13.4**	13.4**	22.5
200	133	Polynomial	48.8	41.1	41.1	69.0
1000	126	Power	261 n.s.	225 n.s	225 n.s.	378 n.s.
PBPK AUC Maternal BMO µMhr/d						
0	109	Hill	134**	70.1**	16.5**	27.8
166.4	118	Polynomial	100	69.2	14.9	25.0
371.9	133	Power	106	90.7	19.8	33.2
493.8	126					
PBPK AUC Fetal BMO µMhr/d[a]						
0	109	Hill	10.9**	5.1**	13.4**	22.5
15.2	118					
74.3	133					
356.7	126					

Note ** indicates exact model fit by graph and tabular output, P values were not applicable for exact fits of the Hill model to the continuous data sets or given as P <0.0001 for the other models despite obvious high degrees of fit visually and by tabular output of observed and predicted values; n.s. indicates insignificant model fit (P < 0.1)

[a] based on average fetal BMO AUC during gestation days 9-18.

8.2 1,3-Butadiene 8-Hour Reference Exposure Level

Study	NTP (1993) supported by Doerr et al. (1996)
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	758 ppb (1.01 ppm x 6/8 hr/d)
Human Equivalent Concentration	1.27 ppm (0.758 ppm x1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	300
Reference Exposure Level	4.2 ppb (9 µg/m^3)

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD). The study of NTP (1993) and the endpoint of ovarian atrophy was selected as the basis of both the 8 hr and chronic RELs. The benchmark concentration analysis of this study is summarized in Table 3 above. We selected the value of 1.01 ppm for the $BMCL_{05}$ based on the full data sets of 9, 15 and 24 months exposures as the best basis for the derivations. This study is supported by that of Doerr et al. (1996). The Doerr study involves PBPK modeling (Table 6) to estimate internal dose metrics for ovarian effects resulting from i.p. doses of mono- or diepoxide metabolites of BD. Dose-response modeling (BMDS v.2.1.2) was used to estimate $BMDL_{05}$'s for the various dose metrics evaluated.

The selected best point of departure, 1.27 ppm, is the $BMCL_{05}$ adjusted for time and human equivalent exposure. The interspecies uncertainty subfactor for toxicokinetics (UF_{A-k}) was reduced from √10 to 1 because we think that the mouse is more active in metabolizing butadiene to reactive epoxide metabolites than either the rat or human, thus reducing the uncertainty in the interspecies toxicokinetic extrapolation.

The other departure from defaults which we have made in this derivation is that we have increased the toxicodynamic subfactor (UF_{A-d}) from the default value of √10 to 10, based on the uncertainty arising from potentially greater human response to the ovotoxic effects of butadiene epoxide metabolites, particularly diepoxybutane (DEB), as compared to the mouse model from which the primary data for risk calculations were derived. Humans differ substantially from mice in lifespan and in the time available for

chronic exposure to effect ovotoxicity which is far longer in humans, and the generally greater robustness of the mouse reproductive system relative to the human (e.g., see He et al., 2007). Chemicals structurally similar to butadiene, specifically the butadiene dimer 4-vinylcyclohexene (VCH) and its diepoxide (VCD) are established rodent ovotoxicants. These related chemical species produce selective destruction of primordial and primary follicles, premature ovarian failure and increased risk of ovarian tumors (Smith et al., 1990; Ito et al., 2009; Mark-Kappeler et al., 2011). Mechanistic studies of the ovotoxic effects of VCD indicate that activation of proapoptotic signaling events in the Bcl-2 and mitogen-activated protein kinases play a role (Hu et al., 2001a, b, 2002). VCD causes a physiological form of ovotoxicity in which follicle loss is "silent" and mimics normal follicular atresia. Thus the damage caused by VCD, were this to be paralleled in humans, could go unnoticed in exposed and affected individuals leading one investigator to surmise that "As a result, because VCD only targets ovarian preantral follicles, chronic exposure in women to low levels of this chemical may represent a risk for early menopause without prior evidence of disrupted menstrual cycles" (Mark-Kappeler et al., 2011) (also see Hoyer and Sipes, 2007).

Table 6. Modeled Butadiene Inhalation Resulting in Ovarian and Uterine Atrophy Following I.P. Butadiene Mono- and Diepoxide Exposures

Data Set	Metric	Model	P for fit	BMD_{05}	$BMDL_{05}$	6 hr ppm mouse equivalent
Ovary						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.76	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.92	57.4	20.5	1.80
BMO i.p.	Hb adducts nmol/g/d	Poly	0.92	34.8	15.6	N.A.
DEB i.p.	Hb adducts nmol/g/d	Hill	0.43	183.3	85.1	N.A.
Uterus						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.36	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.84	103.1	37.9	3.47
BMO i.p.	Hb adducts nmol/g/d	Poly	0.85	799	117	N.A.
DEB i.p.	Hb adducts nmol/g/d	Poly	0.85	799	116.8	N.A.

Notes: BMO = butadiene mono epoxide; DEB = diepoxybutane; EBD = epoxybutenediol; AUC = area under the blood concentration x time curve; Hb = hemoglobin,
table BMD/L values are the average of 30 consecutive daily i.p. administrations,
ppm equivalent is inhalation exposure of butadiene (BD) giving the same internal BMDL dose,
N.A. indicates values unachievable by BD inhalation,
Poly is the polynomial model for continuous dose response in BMDS software,
BMD_{05} indicates 5% relative risk and BMDL the 95% lower confidence limit on the BMD value.

VCH is an industrial chemical released during the manufacture of rubber tires, plasticisers and pesticides, and human exposures to that chemical are likely to be limited to occupational settings. The similarity of the ovotoxcity of VCD, as a model system, however, indicates that of DEB may pose risk derived from mechanistic similarities. Should such mechanistic similarities exist, human females exposed chronically to DEB via butadiene may be at risk of silent ovotoxic effects that would not be manifest for many years. Because of these reproductive uncertainties, OEHHA believes it is adviseable to reject the lower and less conservative default value in favor of a larger $UF_{A\text{-}d}$ until further relevant information is available.

The PBPK modeling used two related models. PBPK Model 1 simulates butadiene plus three metabolites BMO, DEB, and butadiene diol (BDD) and estimates EBD as a metabolized dose (AMET). PBPK Model 2 added EBD as a circulating metabolite and erythritol as a metabolized dose (AMET). Both models use parameters and model structure from Kohn & Melnick, 2001. The ovary and uterus data sets (% of body weight vs. i.p. dose) are from Doerr et al. (1996) Fig 2. The general metabolic scheme is: BD →BMO→DEB→EBD→Erythritol and BMO→BDD→EBD→Erythritol. All metabolic steps have Michaelis-Menten kinetics for P450 oxidation, epoxide hydrolases (EH) or the ordered bi bi rate law for glutathione sulfotransferases (GST). Further details of the GST kinetics can be found in Kohn and Melnick (2000). Essentially, the GST reaction rate (moles/hr) is the product of the Vmax, substrate (Ci) and glutathione concentrations (CGSH) divided by the sum of the cross products of the Km's (Kmi, Kmgsh) and tissue concentrations of the substrate and glutathione:

$$V = Vmax*Ci*CGSH/(Kmi*Kmgsh + Kmi*CGSH + Kmgsh*Ci + Ci*CGSH)$$

All model simulations were conducted with Berkeley Madonna software. Additional details on the PBPK modeling including parameters, graphical and tabular model output, benchmark dose analysis, and sample model code are given in the Appendix.

8.3 1,3-Butadiene Chronic Reference Exposure Level (cREL)

Study	NTP (1993) supported by Doerr et al. (1996),
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	180 ppb (1.01 ppm x 6/24h x 5/7d)
Human Equivalent Concentration	302 ppb (180 ppb x 1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	$\sqrt{10}$
Cumulative uncertainty factor	300
Reference Exposure Level	1.0 ppb (2.2 µg/m^3)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 8 in the Technical Support Document). In the derivation of the chronic REL above, the $BMCL_{05}$ is the same as the 8-hour REL but the time adjustment is for potentially continuous exposure rather than 8 hours/day. The uncertainty factors applied are the same in both cases (i.e., 8-hour and chronic) and the proposed RELs are similar to those derived from the supporting study of Doerr et al. (1996), namely 23 ppb and 8 ppb for 8-hour and chronic RELs , respectively. This latter study uses the same toxicity endpoint but with parenteral administration of the butadiene epoxide metabolites BMO and DEB.

Butadiene causes non-cancer effects following chronic exposure, the most important of which appears to be reproductive toxicity. Inhalation exposure to relatively low concentrations of butadiene over a lifetime is associated with adverse effects in the reproductive organs of female and male mice (NTP, 1984; Melnick et al., 1990; NTP, 1993; Doerr et al., 1996). A PBPK model was developed to model the disposition of butadiene and its metabolites based on a rodent model described by Kohn and Melnick (2001), and data on the ovarian toxicity of 1,3-butadiene epoxide in rats and mice (Doerr et al.,1996).

As reported earlier, ovarian atrophy was observed at all concentrations in the NTP chronic bioassays (NTP, 1984; Melnick et al., 1990; NTP, 1993), and showed a dose-dependent effect associated with increased inhalation exposure. In all exposure groups, ovarian atrophy was associated with what might be considered premature

reproductive senescence. It is noteworthy that such senescence was observed as early as nine months in female mice exposed to 200 or 625 ppm, and after 15 months in female mice exposed to ≥20 ppm butadiene (NTP, 1984; NTP, 1993). It is currently unknown how ovarian atrophy in rodents relates to human reproductive senescence. Reproductive senescence is a general term referring to the loss of reproductive function with age. In the female, this involves a loss of normal ovarian, and hypothalamic or pituitary function (Valdez and Petroff, 2004). The correlation between the loss of oocytes and declining fertility with age is well characterized, and it is generally accepted that depletion of follicles results in the final loss of fertility. The actual end of ovarian activity is preceded by a period of irregular reproductive cycles and subfertility in both women and rodents (Valdez and Petroff, 2004). Age-related changes in the rodent hypothalamus and pituitary appear to bring about the final cessation of ovarian cyclicity before follicular depletion occurs (Wise et al., 2002). However, exhaustion of ovarian follicular reserves appears to drive menopause in women (vom Saal et al., 1994). Despite these apparent differences in final reproductive senescence, the biological mechanisms leading to prolonged cycles and aberrant endocrinology appear similar in primates and rodents (Wise et al., 2002; Bellino and Wise 2003).

Currently, there are few data detailing the potential role of butadiene on the etiology of ovotoxicity and late-stage infertility (Hughes et al., 2001). Potential mechanisms might involve the in situ formation of butadiene reactive intermediates, which may have a direct effect on ovarian function (Doerr et al., 1995; Hughes et al., 2001), or a more general role on the hypothalamic-pituitary-gonadal axis. Available data seem to suggest that humans potentially form qualitatively similar metabolites to experimental rodent species, although the rates of formation and the quantities of specific metabolites differ (Bond et al., 1986;Csanady, 1992). Mice appear to produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995). However there are conflicting in vitro data that suggest humans may form epoxides of butadiene at comparable rates to mice or at rates several fold less than mice (Csanady et al., 1992; Duescher and Elfarra 1994; Krause and Elfarra 1997). Dahl et al. (1991) reported that following equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in monkeys than in mice, and 4-14 times lower than in rats. Humans may more closely resemble the monkey than the mouse or the rat in their formation of epoxides from butadiene, and several models adjust for species differences in butadiene pharmacokinetics (Sweeney et al., 1997). In view of the fact that we have used a pharmacokinetic model to assess relevant dosimetry and the weight of published evidence that mice are more active butadiene metabolizers than other species studied, we used a UF of 1 to account for interspecies kinetic differences. However, an interspecies toxicodynamic adjustment for ovarian atrophy endpoint with butadiene is still needed to reflect the expected substantial toxicodynamic differences between mice and women (see the discussion in the derivation of the 8-hour REL). Therefore OEHHA used an interspecies toxicodynamic uncertainty factor of 10. We applied an intraspecies uncertainty factor of 30, 10 for toxicokinetics and $\sqrt{10}$ for toxicodynamics. The overall uncertainty factor was 300, and the cREL is 1.0 ppb.

For comparison, the U.S. EPA's RfC is 0.9 ppb. This value is based on a benchmark concentration (BMC) and a Weibull time-to response model for the ovarian atrophy endpoint in mice in the NTP 2-year inhalation study (NTP, 1993; U.S.EPA, 2002). U.S.EPA (2002) calculated a BMC_{10} of 1.05 ppm and a $BMCL_{10}$ of 0.88 ppm (continuous) without the top dose. They applied an overall uncertainty factor (UF) of 1000 to derive a final value of 0.88 ppb or 0.9 ppb rounded. The composite UFs included √10 for interspecies, 10 for effect level, 10 for intraspecies, and √10 for data deficiencies.

The Texas Commission on Environmental Quality (TCEQ) used a similar analysis employing the top dose in the same data set to calculate a $BMCL_{05}$ of 462 ppb. Their chronic reference value of 15 ppb employs an overall UF of 30, which includes 10 for intraspecies differences and √10 for data deficiencies (Grant et al., 2010).

Kirman and Grant (2012) conducted a meta-analysis of pooled rodent data on ovarian atrophy resulting from butadiene exposure. They used data in mice from NTP (1993), NTP (1984) and Bevan et al. (1996) together with negative data in rats from Owen et al. (1987) and Bevan et al. (1996). Instead of using a PBPK model to estimate internal dosimetry they used an empirical equation relating average blood concentration of diepoxybutane (DEB) to DEB-specific pyr-Val hemoglobin adducts with separate rate constants and erythrocyte lifespans for mice and rats (4.7×10^{-5}, 40.3; 5.5×10^{-5}, 64.4, L/g hr., days, respectively). This resulted in estimated DEB blood concentrations in mice of 11 to 823 nM for butadiene exposures of 6.25 to 1250 ppm. For rats, estimated blood concentrations of 2.9 to 8.1 nM DEB were obtained for exposure concentrations of 1000 and 8000 ppm butadiene, respectively. Dose response analysis was conducted using the Multistage-Weibull (MSW) time and dose model for incidental effects (ovarian atrophy, extra risk, non-fatal analysis). Analyses were conducted for the mouse data at 823, 686, 471, 186, 73, 29, and 11 nM DEB, for rat data at 2.9 nM DEB, and for pooled mouse and rat data at 0 nM DEB (controls).

The advantage of this approach was the prediction of effects at specific exposure durations that could be compared with human ovarian reserves at times prior to menopause. Three human scenarios for duration of susceptibility to ovarian effects were defined by human menopause at 60 yr (low susceptibility), 49.6 yr. (average susceptibility) and 38.7 yr. (high susceptibility). For these scenarios points of departure (PODs) were determined at the 10% and 1% effect levels with 95% confidence limits i.e., $BMCL_{10}$, $BMCL_{01}$. The $BMCL_{01}$ PODs for high, average and low susceptibility were 1.3, 0.88, and 0.67 nM DEB in blood, respectively. The human equivalent concentrations of butadiene (continuous exposure) $BMCL_{01}$s were 10, 17, and 39 ppm butadiene, respectively. Comparable rodent durations for the human scenario durations were 53.7, 68.8, and 83.2 weeks, respectively. The MSW predicted dose response curve for low follicle count at birth (high susceptibility scenario) was shifted by factors of 3 and 8.5 (25.5 total) to the left to reflect a sensitive individual due to variation in toxicokinetics and toxicodynamics. Similarly, the curve for high follicle count at birth (low susceptibility) was shifted to the right by the same factors. The dose-response curve was not shifted for the average scenario. For the POD $BMCL_{01}$s of 17 ppm BD (average), 1.5 ppm BD (high, 25.5 TK+TD shift UF) , and 240 ppm BD (low,1/25.5

TK+TD shift UF) the derived reference values including an additional net uncertainty factor of 10 were 2, 0.2, and 20 ppm butadiene rounded, respectively. The lowest value of 0.2 ppm or 200 ppb butadiene is over an order of magnitude higher than that proposed by Grant et al. (2010) for the Texas chronic reference value of 15 ppb butadiene and 67 times higher than the cREL proposed in this document.

This is a novel approach to butadiene non-cancer risk assessment. It rests on a number of assumptions which may not be justified. First of all, it assumes that the effects of butadiene in humans will be exactly as seen in mice, i.e. ovarian atrophy. Ovarian atrophy was the most sensitive non-neoplastic effect noted among several organ effects in mice (decreased lung, liver, and kidney weights) and uterine, testicular and nasal olfactory epithelial atrophies. Next it assumes that all the ovarian effects are due to DEB and that DEB acts the same in rats as in mice. An examination of their Table 3 shows some problems with this latter assumption since at a comparable estimated blood DEB concentration of 11 nM in mice 19/49 showed atrophy, whereas at 8.1 nM in rats no effect was seen (0/110). It appears that mice are much more sensitive to blood DEB than rats. There is also a concern that the effect of including the negative rat data (350 animals) may have reduced the overall response. The choice of a 1% response rate may also be problematic. Usually for non-cancer data the lower bound on the 5% response rate is chosen as the POD i.e., $BMCL_{05}$. While the authors note that several hundred animals were included in the analysis it is questionable if the analysis had the power to detect a 1% increase ($BMCL_{01}$) since the largest denominator for any specific dose level causing an effect was 79 mice. The $BMCL_{01}$ is an extrapolated response well beyond the bulk of observed values. There appears to be a discrepancy in Table 8 which lists a POD of 240 ppm and a reference level of 20 ppm (rounded) where the text gives a value of 255 ppm (10 x 25.5) which would give 26 ppm rounded.

In order to compare their method with ours we used the same TWA DEB internal dose estimates of Kirman & Grant (2012) and conducted a Multistage Weibull non-fatal time to effect analysis (MSW) using U.S.EPA software (U.S.EPA, 2010a) to estimate the benchmark concentration level time adjusted $BMCLt_{05}$ values for the largest and most important data set in their meta-analysis and our cREL derivation, namely the NTP (1993) 24 months data (N= 325). Using individual animal data (dose, time, incident, censored, number) we obtained a value of $BMCLt_{05}$ = 0.502 ppm butadiene equivalent (Table 7). This value is half the 1.01 ppm value based on our time-weighted analysis of 9, 15 and 24 months quantal data (dose, time, number with ovarian atrophy/total number exposed) and the $BMCL_{05}$ for a log probit model (N = 435). While there is no chi-square goodness of fit statistic for the $BMCLt_{05}$, graphical comparisons of the MSW parametric and nonparametric models in probability-time (pr), dose-response (dr), quantile-quantile (qq), and probability-probability (pp) plots indicate excellent fit of the MSW model to the 24 months ovarian atrophy data (Appendix B, U.S.EPA, 2010b). Since the $BMCLt_{05}$ is similar to the $BMCL_{05}$, but is based on fewer animals and conducted with new software with which we have limited experience, the MSW analysis is considered a supporting analysis for the cREL.

Table 7. Multistage Weibull Analysis of Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	AIC	$BMCt_{05}$ nM DEB	$BMCLt_{05}$ nM DEB	$BMCLt_{05}$ ppm BD equiv.	$BMCLt_{05}$ continuous ppm BD HEC	Comment
24 months	Multi-stage Weibull[1]	300.781[2]	1.56	1.28[3]	0.502[4]	0.151[5]	N = 325; good fit by pr, dr, qq, & pp plots[6]

Note: (1) P(response) = 1-exp{-(t-t0)^c * (beta_0 + beta_1*Dose^1 + beta_2*Dose^2 + beta_3*Dose^3)} run as nonfatal analysis, t0 = 0; (2) Akaike's Information Criterion; (3) $BMCLt_{05}$ 95% lower bound on the 5% response level by MSW with internal DEB dose metric of Kirman and Grant (2012); (4) external equivalent of 0.50 nM DEB in 6hr/d BD; (5) continuous human equivalent concentration of BD (6/24h x 5/7d x 1.68 dosimetric adjustment factor; (6) goodness of fit graphical tests: probability v. time (pr), probability v. dose (dr), time v time (qq), probability v. probability (pp) for parametric and nonparametric msw models (U.S.EPA, 2010b).

It is important to note that in deriving the cREL for butadiene, OEHHA makes no assumption concerning target site concordance in experimental animals and humans. We assume only that the most sensitive site and species with appropriate uncertainty factors will protect against any adverse effect in exposed humans including children.

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Appendix A

This appendix includes information on genotoxicity of butadiene, except discussion of dominant lethal mutations and germ cell mutagenicity, which are covered in the developmental and reproductive toxicity section of the main document. An overview of the genotoxicity of butadiene and its metabolites is presented in Table A1. Also included are data on PBPK modeling of butadiene employed in the derivation of the $BMCL_{05}$ and the 8-hr and chronic RELs. Table A2 gives a historical overview of the PBPK and related models that were more or less available for use.

While genetic toxicology generally provides key supporting documentation for cancer risk assessment rather than the present noncancer assessment, we believe that mutagenicity and other genetox effects, particularly oxidative DNA damage, may contribute to chronic diseases such as heart disease, neurodegenerative diseases, diabetes mellitus, rheumatoid arthritis, and aging, irrespective of their role in initiation and promotion of tumors (F.M.Burnet,1974; Cooke et al., 2006).

A.1 Genetic Toxicity: Introduction

This section includes information on genetic toxicity. Albertini et al. (2010) have provided a comprehensive review of 1, 3-butadiene genotoxicity. As noted above, the metabolism of butadiene produces the major electrophilic metabolites EB, EBD, and DEB. These epoxide metabolites are responsible both for the mutagenicity and carcinogenicity of 1, 3-butadiene. They are DNA reactive forming a variety of adducts, and all are genotoxic in vitro and in vivo. Also butadiene epoxide metabolites form adducts with proteins notably hemoglobin and may exert or influence noncancer endpoints. In general genotoxicity, specifically oxidative DNA damage, may influence a number of chronic diseases and aging in addition to cancer (Cooke et al., 2006; Evans & Cooke, 2006).

A.2 Microbial test systems

Butadiene has induced mutagenic responses in all microbial systems that were provided with exogenous microsomal activation (S-9) that allowed the formation of oxidative metabolites. The purified metabolites epoxybutene (EB) and diepoxybutane (DEB) have also been shown to be mutagenic in all microbial systems without exogenous activation (Albertini, 1999). Carmical et al. (2000) found evidence of stereospecific mutagenicity of diolepoxide derived adducts in an *E.coli* system. The BDE R,R isomer induced a mutantion frequency of 0.14% (A→G mutations exclusively), whereas the S,S isomer induced 0.25% (A→C mutations exclusively). The mutation frequencies induced by BMO isomers were not significantly different from controls (<0.05%). Curiously, a recent report by Cho et al. (2010) demonstrated the mutagenicity in *S. typhimurium* TA1535 of the glutathione conjugate of DEB, *S*-(2-hydroxy-3,4-epoxybutyl)GSH. In general GSH conjugates are regarded as detoxification products but in this case the conjugate was reported to have higher mutagenic potency than DEB.

A.3 Higher organism test systems

Butadiene and its diepoxide have been tested in the fruit fly *Drosophila melanogaster*. BD was negative in sex-linked recessive lethal mutations and the spot wing test whereas DEB was positive in these tests. Studies in mammalian cell systems parallel those in microbial systems with BD, EB, DEB and epoxybutane diol (EBD) showing positive results in systems provided with exogenous activation. In these systems the rank order of mutagenic potency was generally DEB> EB (BMO)>EBD>BD. DEB was generally 40 to 100-fold more potent than EDB. DEB is more likely to cause deletions, whereas all the epoxides induce base substitutions with a preference for A:T base pairs (Albertini, 1999). Both EB and DEB occur in different stereochemical configurations, which have been evaluated in the TK6 human lymphoblastoid cell line for increases in hypoxanthine-guanine phosphoribosyltransferase (HPRT) and thymidine kinase (TK) mutant frequencies. Meng et al. (2007) found no differences in cytotoxicity or mutagenicity among three isomers of DEB or the two isomers of EB. Thus, stereochemistry seems likely to play little role in the mutagenicity of BD. These findings were consistent with in vivo results, which showed little difference in the mutagenic efficiencies of racemic-DEB or meso-DEB in rodents. However, the results seem somewhat at odds with those of Carmical et al. (2000) above, where *S*,*S*-EBD derived adducts preferentially induced mutagenicity in *E.coli*.

Mutagenic studies in whole mammals have generally given positive responses when the animals (mice or rats) were exposed to butadiene by inhalation (Albertini et al., 2010). The tests included the mouse spot test, which involves in utero exposure of a developing embryo, which results in mutations in hair coloring in the offspring. Most of the assays were for mutations in the *hprt* locus in lymphocytes of exposed rodents.

A.4 Genotoxicity in humans

Genotoxicity in humans is usually assessed by the molecular epidemiology of exposed populations via the monitoring of genetic endpoints, including mutations. Several specific biomarkers of BD exposure are available which allow measurement of internal dosimetry of different BD metabolites. Two BD urinary metabolites: M1 (1, 2-dihydroxy-4-[*N*-acetylcysteinyl]-butane) and M2 (an isomeric mixture of 1-hydroxy-2-[*N*-acetylcisteinyl]-3-butene and 2-hydroxy-1-[*N*-acetylcisteinyl]-3-butene have frequently been used as measures of BD exposure (Albertini et al., 2010). N-terminal valine adducts of hemoglobin have also been used as in vivo BD dosimeters (Perez et al., 1997; Boogaard et al., 2001).

There have been several small studies involving one or more biomarkers of exposure and nine populations that have been subjected to comprehensive studies with biomarkers of BD exposure and specific genotoxicity endpoints including somatic gene mutations and chromosome aberrations (Albertini et.al., 2010). Overall the results of these investigations have been mixed. With respect to mutational endpoints, only studies of BD-exposed workers in Texas have reported significantly increased frequencies of *hprt* gene mutations in peripheral blood lymphocytes determined by autoradiography (Albertini et al., 2010; Wickliffe et al., 2009).

Albertini et al. (2007) conducted a molecular epidemiological study of 53 BD-exposed Czech workers and 51 controls. The mean 8-hr time weighted average (TWA) concentrations of BD were 0.008 mg/m^3 (3.5 ppb) and 0.397 mg/m^3 (180 ppb) for female controls and exposed, respectively, with individual 8-hr TWA values up to 9.8 mg/m^3 (4.45 ppm). For male workers, mean TWA values for control and exposed workers were 3.2 and 370 ppb, respectively with single individual values up to 5.72 ppm. For both sexes, the urinary BD metabolites M1 and M2 were elevated in exposed vs. control subjects, reaching statistical significance in males. *HPRT* mutations, via cloning assays, and multiple measures of chromosome level changes (SCEs) were not associated with BD exposures.

Wickliffe et al. (2009) evaluated the frequencies of HPRT mutant lymphocytes in BD polymer workers in a Southeast Texas facility. Thirty workers were exposed to BD concentrations of 93.5 ppb (mean and 2.5 ppb (median) with only one individual's exposure estimate (1684 ppb) exceeding the OSHA PEL of 1.0 ppm. The minimum detection level was 2.5 ppb and 50% of exposures were below this level. *HPRT* mutant frequencies by autoradiographic lymphocyte assay were not significantly associated with current exposures or age (N = 29). However *HPRT* mutant frequencies were significantly associated with occupational longevity (≥ 30 yr, N = 29, R^2 = 0.107, $P < 0.046$). The authors speculate that chronic or past high-level BD exposures may have produced retention of mutant long-term memory T-cells.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Procaryotic			
BD	*Salmonella typhimurium* TA 1530, TA1535	+	Rat and human S-9, multiple tests
BD	*Salmonella typhimurium* TA 1530, TA1535	-	Without S-9, usually negative, positive results attributed to volatile metabolites
EB (BMO)	*Salmonella typhimurium* TA 1530, TA1535,TA100	+	Without S-9, LEC = 2431 µM
EB (BMO)	*Escherichia coli*	+	Without S-9
EB N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	-	Neither isomer adduct was significantly mutagenic, Carmical et a. 2000
EB (BMO)	*Klebsiella pneumoniae*	+	Without S-9
DEB	*Salmonella typhimurium*	+	Without S-9, more potent than EB, LEC = 302µM
DEB	*E.coli*	+	Without S-9
DEB	*Bacillus megaterium*	+	Without S-9
DEB	*Pseudomonas pyocyanea*	+	Without S-9
DEB	*K. pneumonia*	+	Without S-9
DEB-GSH	*Salmonella typhimurium* TA1535	+	*S*-(2-hydroxy-3,4-epoxybutyl)GSH reported more mutagenic than DEB (Cho et al., 2010)
EBD	*Salmonella typhimurium*	+	With or without S-9, LEC = 980 µM
EBD N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	+	S,S- stereoisomer of EBD gave higher mutagic frequency than the R,R isomer, Carmical et al., 2000

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Eucaryotic			
DEB	*Neurospora crassa*	+	Without S-9
DEB	*Schizosaccharomyces pombe*	+	Without S-9
DEB	*Saccharomyces cerevisiae*	+	Without S-9
DEB	*Aspergillus nidulans mitotic crossive over and recombinant selection, gene mutations*	+	20µM, 1 min; LEC = 50 mM, 13 min
DEB	*Penicillium multicolor*	+	Both stereoisomers
Insect			
BD	*Drosophila melanogaster*	-	Sex-linked recessive lethal mutations, spot wing test somatic mutations
ED (BMO)	*Drosophila melanogaster* adult males exposed to EB by inhalation for 24 hr. Gene mutations scored	+	Sex-linked recessive lethal mutations induced highest exposure level on 3800 ppm EB (91,200 ppm-hr) w/ DNA repair or at 2000 ppm (24,000 ppm-hr) w/o DNA repair.
DEB	*Drosophila melanogaster,* gene mutations, deletions, CAs	+	Sex-linked recessive lethal mutations, semi lethal and visible mutations, LEDs 100-800 µg/kg by injection.
DEB	*Drosophila melanogaster,* heritable gene deletions, point mutations	+	500 µM (single dose), high frequency of deletions vs. point mutations. Males fed DEB progeny phenotypes scored, or adults from treated larvae.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Mammalian cells in vitro			
BD	Mouse Lymphoma	+	S-9
DEB	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 40-100x more potent than BDE, LECs = 3.5, 2.3 µM, respectively.
EB (BMO)	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 3.5x more potent than BDE, LECs = 122, 243 µM, respectively.
BDE	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, LECs = 200, 300 µM, respectively.
DEB	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
EB (BMO)	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
Mammalian, whole animal			
BD inhalation	Mouse melanocyte mutations, mouse spot test	+	500 ppm, 6hr/d on gestation days 8-12, offspring inspected for color spots at 2-3 weeks of age.
BD inhalation	Mouse B6C3F1 *hprt* splenic lymphocytes	+	Mutagenic potency mouse/rat = 5
BD inhalation	Mouse B6C3F1 *hprt* thymic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* splenic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* thymic lymphocytes	+	
BD inhalation	Mouse 102XC3h *hprt* splenic lymphocytes	+	
BD inhalation	Mouse CD1 *hprt* splenic lymphocytes	-	
BD inhalation	Mouse MM *lac I*	+, -	Lung positive, bone marrow and liver negative
BD inhalation	Mouse BB *lac I*	+	

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
BD inhalation	Mouse 2-year Bioassay K-ras mutation	+	Lung and liver tumors, lymphoma
BD inhalation	Mouse 2-year Bioassay H-ras mutation	+	Liver tumors
BD inhalation	Mouse 2-year Bioassay p53, Rb, Chr.4 gene, allele loss	+	Lung and mammary tumors
EB (BMO)	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
EB (BMO)	Mouse 102XC3h *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Fischer 344 *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Lewis *hprt* splenic lymphocytes	-	
DEB	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
DEB	Mouse 102XC3h *hprt* splenic lymphocytes	-	
DEB	Mouse C57B1 *hprt* splenic lymphocytes	-	
DEB	Rat Fischer 344 *hprt* splenic lymphocytes	+	
DEB	Rat Lewis *hprt* splenic lymphocytes	-	
Mammalian cytogenetics in vitro			
BD	Chinese hamster ovary cells, sister chromatid exchanges (SCEs)	+	S-9
BD	Human lymphocytes, SCEs	±	± S-9, S-9 not necessary
EB (BMO)	Mouse, rat and human lymphocytes, Cas	-	EB negative for all species tested

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EB (BMO)	Mouse and rat splenocytes, Cas	-	EB negative in both species, HIC = 931 µM EB
EB (BMO)	Human peripheral blood lymphocytes, MN induction	+	LEC = 1.0 µM
EB (BMO)	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	Negative at 1000 µM
DEB	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	+	LEC = 5 µM
DEB	Human peripheral blood lymphocytes, MN induction	+	LEC = 0.5 µM
DEB	Mouse and rat splenocytes, Cas	+	DEB positive in both species, 0.47 and 0.36 aberrant metaphases/100 metaphases/µM, respectively. LEC mouse = 40µM, LEC rat = 80µM.
DEB	Mouse, rat and human lymphocytes, Cas	+	DEB positive for all species tested, aberrant metaphases = 0.36/100/µM DEB for mice and rats.
DEB	Rat embryo fibroblasts, chromosome aberrations	+	Induction of Cas and high degree of tetraploidy, LEC = 93 µM
DEB	Rat liver cells, cytological scoring	+	Concentration not stated.
DEB	Human skin fibroblasts	+	Cas positive in Fanconi's anemia heterozygotes, LEC = 0.1 µM.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EBD	Rat spermatid, MN induction. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	HIC = 100 µM
Mammalian cytogenetics in vivo			
BD inhalation	Mouse Chromosome aberrations (Cas)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse induced micronuclei (MN)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse, induced MN in lung fibroblasts, 500 ppm (males) and 1300 ppm, 6hr/d x 5d	+	Significant increase in kinetochore (+) MN in males at 500 ppm, and kinetochore (+) and (-) MN in females at 1300 ppm.
BD inhalation	Mouse SCEs	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Rat Cas	-	
BD inhalation	Rat MN	-	
BD inhalation	Rat SCEs	±	
EB (BMO), i.p. injection	Mouse, Cas in bone marrow cells	+	Positive dose response 25-150 mg/kg
EB (BMO), i.p. injection	Mouse and Rat, MN in splenic lymphocytes	+	Positive in both species, LED mice = 40 mg/kg, LED rats = 80 mg/kg
DEB, s.c. injection	Rat, peripheral blood lymphocytes Cas	+	Stickiness and clumping of chromosomes at 1 mg/kg, chromosome fragmentation at 3 mg/kg
DEB, i.p. injection	Mouse and Rat, MN in splenic lymphocytes by cytokinesis-block method; characterization of induced MN by FISH.	+	Positive in both species, LED mice = 15 mg/kg, LED rats = 30 mg/kg
DEB, i.p. injection	Mouse, Cas in spermatogenic cells	+	Significant increase in chromosome breaks.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EBD, i.p. injection	Rat, MN in bone marrow cells at 30 and 60 mg.kg	±	Weak positive at 30 mg/kg only.
EBD, i.p. injection	Rat, MN in spermatids at 30 amd 60 mg/kg	+	Significant induction of MN on days 2 and 3 post-exposure (diploteme and late pachyteme)
Mammalian germ cell genotoxicity in vivo			
BD inhalation	Mouse dominant lethals	+	Multiple tests
BD inhalation	Mouse heritable translocation	+	2 studies
BD inhalation	Mouse sperm head abnormalities	+	Increases at 200 ppm, statistically significant at 1,000 and 5,000 ppm, 6hr/d x 5 d
BD inhalation	Mouse spermatid micronuclei	+	
BD inhalation	Mouse testicular cells Comet Assay	+	DNA fragmentation
BD inhalation	Rat dominant lethal	-	
EB (BMO), i.p. injection	Mouse splenic lymphocytes, SCEs	+	Statistically significant increases at 48.8 and 73.2 mg/kg. HID = 24.4 mg/kg
DEB, i.p., i.v. injection	Mouse SCEs in bone marrow cells, alveolar macrophages and liver cells	+	Positive SCE induction in all cell types.
DEB, 1 hr aerosol inhalation, i.p. injection.	Mouse and Chinese hamsters, SCEs in bone marrow cells	+	Positive in mice, LED = 20-30 mg/kg (based on blood concentration). Hamster less sensitive.
EBD, i.p. injection	Mouse, dominant lethal mutations	-	Single EBD dose of 120 mg/kg

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

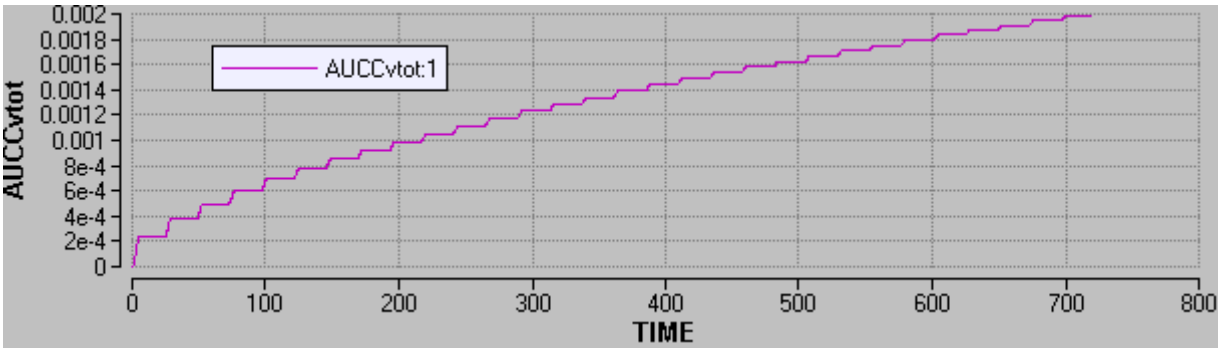
Compound	Test System	Results	Comments
Human cytogenetics in vivo			
BD occupational exposure	Cas -	±	+ for $GST^{-/-}$ genotype
BD occupational exposure	SCEs	±	
BD occupational exposure	MN	-	
BD occupational exposure	Comet Assay	-	
BD occupational exposure	*hprt* T-cell (Autoradiography)	+	Variant frequencies significantly associated with occupational longevity (N = 29, R^2 = 0.107, P < 0.046), Wickliffe et al., 2009.
BD occupational exposure	*hprt* T-cell (Cloning Assay)	-	
BD occupational exposure	*ras* oncoprotein	-	

A.5 PBPK Modeling Data

A general summary of the PBPK models and related reports in the literature is given in Table A2. Of the available models, the one that seemed the most developed in view of relevant metabolites covered in mice and rats was that of Kohn and Melnick (2001). Unfortunately, due to the untimely death of the senior author we were unable to secure the actual model code for this model and were limited to the published report. The values given for the physiological parameters for mice and rats are reproduced in Table A3. Average reported values for alveolar ventilation rates of 20.2 and 20.0 L/hr/$kg^{0.7}$ for mice and rats, respectively were used in the model. For cardiac output the values used were 15.3 and 14.7 L/hr/$kg^{0.7}$, for mice and rats respectively. Typical body weights were 0.025 to 0.030 kg for mice and 0.215 to 0.360 kg for rats. Model partition coefficients are shown in Table A4. The biochemical parameters are listed in Table A5. The activities of P450 and epoxide hydrolase (EH) in mice and rats were multiplied by 30, 9, and 9 mg of microsomal protein per gram of tissue for liver, lung, and kidney, respectively. The glutathione *S*-transferase activities were multiplied by 82.8 or 108 mg of cytosolic protein per gram of tissue for mice and rats, respectively. Michaelis-Menten kinetics were used for P450 and EH and the ordered bi-bi rate law was used for GST.

The results of PBPK simulations of Doerr et al (1996) are shown in Table A6. Daily i.p. doses of BMO are administered to a young mouse over 30 days (720 hr). The

cumulative AUC for DEB in the mixed venous blood (Cvtot) is divided by 30 to give the dose metric of µMhr/d, which is then analyzed by the benchmark dose method (Table A7) to obtain a $BMDL_{05}$. A Berkeley Madonna graph of the 30 days simulation of the 0.09 mmol/kg mid dose is shown in the Figure below. The units are AUC_{DEB} in mixed venous blood Mhr versus time in hours. The terminal value (0.002Mhr) corresponds to the 1985 µMhr value in Table A5 obtained from the tabular output of the same simulation.



An example of code for the OEHHA implementation of the Kohn and Melnick (2001) butadiene model is shown after the tables.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Hattis & Wasson, 1987	Mice, Rats	BD	Pb = 0.35 Pf = 118.2 Plvrg = 5.4 Pm = 5.26 P_{450} Vmax = 400µmol/kg-hr (mice), 200µmol/kg-hr (rats) Km = 5.0 µM	3 compartment model liver combined with vessel rich group. Model runs in moles, minutes, liters and Vmax = 1.46 x 10^{-6} mol/min (rat)
Csanady et al., 1992	Mice, Rats, Humans	BD, BMO	P_{450} Vmax/Km = 157-1295; EH Vmax/Km = 3.6-35; GST Vmax/Km = 4.3-17 nmol/mg protein/min/mM, all values for liver	2-compartment kinetic model for BD and BMO metabolism in vitro: BD gas and liquid, BMO gas and liquid.
Johanson & Filser, 1993	Mice, Rats	BD, BMO, GSH (liver)	BD Pb = 3.03; Pf = 7.2; Pmusc = 0.24; Pl = 0.31; Pkid = 0.30; Pbrain = 0.14; BMO Pb = 83.4; Pf = 1.85; Pmusc = 0.72; Pl = 0.65; Pkid = 0.84; Pbrain = 0.62.	Closed chamber experiments simulated.
Evelo et al., 1993	Mice, Rats	BD	Pb = 1.184, 0.603 Pf = 32.36; Pl = 2.675; Pmusc = 1.871; Pkidney = 1.69; Plung = 1.272; Pbrain = 2.355; Pvrg = 2.02	2-compartment lung with gas exchange in alveoli, metabolism in bronchi and alveoli; Mice Vmax liver = 318, pulmonary = 70, bronchi = 77 µmol/hr-kg, Km = 8 µM

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Kohn & Melnick, 1993	Mice, Rats, Humans	BD, BMO	BD Pb = 1.5; BD Pf = 118.2; BD Pl = 5.49; BD Pmusc = 5.26; BD Pviscera = 5.34; BMO Pb = 60; BMO Pf = 1.81; BMO Pl = 0.6545; BMO Pmusc = 0.6533; BMO Pviscera = 0.6348	Sensitivity Analysis; Vmax BD P_{450} = 155.4 (mouse), 35.4 (rat), 70.8 (human) nmol/hr-mg prot., liver microsomal protein = 11.6, 16.8, 14.5 g/L, Km = 2, 3.75, 5.14 µM, respectively.
Medinsky et al., 1994	Mice, Rats	BD, BMO	BD Pb = 1.49, 1.34; BD Pf = 14.9, 14.3; BD Pl = 0.799, 1.01 BD Pm = 0.99, 2.99 BMO Pb = 50.4, 36.6; BMO Pf = 2.74, 2.49, BMO Pl = 1.43, 1.15 BMO Pm = 0.39, 0.64 (rats and mice, respectively.)	BD liver and lung oxidation; BMO liver oxidation, hydrolysis, and conjugation. Liver oxidation Vmax/Km = 16.5, 169 µmol/hr/kg/µM, rats and mice, respectively.
Johanson & Filser,1996	Mice, Rats, Humans	BD,BMO,GSH (liver)	BD Km = 83 µM GST Km(BMO) = 100 mM	Intrahepatic oxidation of BD and hydrolysis of BMO
Csanady et al., 1996	Mice, Rats, Humans	BD,BMO,DEB	Rats BD Vmax = 220 µmol/kg-hr, Km = 36 µM; Mice BD Vmax = 400 µmol/kg-hr, Km = 26 µM; BMO k_{P450} = 0.00126 L/hr; DEB k_{elim} = 0.0046 L/hr	Hemoglobin adducts from BMO reaction with N-terminal valine:2.9E-5 L/hr/g Hb

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 1997	Mice, Rats	BD, BMO, DEB in Liver and Lung;	DEB P_{liver} = 1.53, P_{kidney} = 1.41, P_{muscle} = 1.82, P_{fat} = 2.20; Mice BD Liver Vmax = 338 µmol/kg-hr, Km = 2.0µM, Lung Vmax = 21.6 µmol/kg-hr, Km = 5.01 µM; Liver DEB EH Vmax = 4193µmol/kg-hr, Km = 8100 µM; GST Vmax = 5.03 x 10^4 µmol/kg-d, Km = 6400 µM	Mice BMO Liver P_{450} Vmax = 176.6 µmol/kg-hr, Km = 145 µM, EH Vmax= 754 µmol/kg-hr, Km = 1590 µM, GST = 1.54 x 10^5 µmol/kg-hr, Km = 3.53 x 10^4 µM
Kohn & Melnick, 2000	Mice, Rats	BD, BMO, DEB	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BD liver Vmax/Km = 1292, 578 nmol/min/mg protein/mM for mice and rats, respectively	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.
Kohn & Melnick, 2001	Mice, Rats	BD, BMO, DEB, BDD, EBD	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BDD Pf = 0.573 EDB Pf = 0.496	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 2001	Human	BD,BMO, DEB,BDD,EBD	Vmax/Km: BD→BMO, 0.0132/0.7; BD→DEB, 0.031/880; BMO→BDD, 1.4/540; BMO→$GS_{conj,}$ 2.7/10,400; DEB→GS_{conj}, 0.4/3390; BDD→EBD, 0.031/880; DEB→EBD, 9.2/4605; EBD→Erythritol, 4.6/4605; EBD→GS_{conj}, 0.2/339, µmol/mg prot.-hr/µM.	Tissue/blood partition coefficients for BDD and EBD assumed to be 1.0. Sensitivity analysis (SA) for exhaled BD (2hr human exposure to 5 ppm BD): BD blood/air = 0.36; alveolar ventilation = -0.36; cardiac output = 0.28; Qliver = -0.40; Qfat = -0.23. SA for DEB AUC: Vmax EB→DEB = -0.98;Vliver = -0.96; Km DEB→EBD = 0.94; Vmax DEB→EBD = -0.93; Vmax BMO→BDD = -0.87, microsomal protein liver = -0.80; BD blood/air = 0.78.
Smith et al. 2001	Humans	BD 3 compartment model fit to data from human subjects, N = 133	BD uptake µg/kg; kmet /min, CYP2E1 genotype, phenotype	BD uptake and rate of metabolism not related in this analysis. No significant differences in uptake or kmet with genetic makeup.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Mezzetti et al., 2003	Humans	BD 3-compartment model fit to data from human subjects, N= 130 8 parameters analyzed	1120 parameters sampled, the most sensitive: W_{wp_i} the weight fraction of the well perfused tissues; P_{fat}, the fat/blood partition coeff; P_{wp} = wp/blood partition coeff.	Bayesian Analysis Model, MCMC analysis. Subgroups analyzed: males, females; age<30yr; age ≥30 yr; African-American; Hispanic; Asian, Caucasian
Brochot et al., 2007	Humans	BD, BMO, DEB, BDD, EBD	BD Pb = 1.22 BD Pf = 18.4 BD P_{450} Kmet = 0.19 ± 0.06 /min	Global sensitivity analysis
Pery & Bois, 2009	Human	BD	Pb = 0.653 Pf = 22.0 Kmet = 0.3 L/min	23 tissue compartments PBPK model coupled to PD model of carcinogenicity
Beaudouin et al., 2010	Humans, N = 133	BD	BD P_{450} Vmax = 2.28 x 10^{-5} mmol/min/mg microsomal protein, Km = 0.39 mM	Acute and chronic (lifetime) exposures modeled. Human inter-individual variability in exhaled BD well described, within 95% CI: median x 0.3 to 1.6.

Table A3. Physiological Parameter Values for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Tissue compartment volumes, % body weight	Mouse	Rat
Liver	5.5	3.7
Lung (bronchi)	0.6	0.52
Alveolar	0.5	0.515
Kidney	1.67	1.48
GI Tract	7.5	7.5
Viscera	3.93	14.3
Fat	6.0	5.4
Muscle and skin	64.5	54.2
Blood	6.0	5.4
Capillary blood volume, % tissue volume		
Liver	11.0	13.8
Lung	11.0	18.0
Kidney	10.2	16.0
GI tract	2.9	2.65
Viscera	7.1	7.1
Fat	3.0	2.0
Muscle and skin	1.3	2.0
Blood flow rate, % cardiac output		
Liver (hepatic artery only)	4.4	3.9
Kidney	16.3	13.3
GI tract	18.1	18.1
Viscera	22.4	24.8
Fat	5.0	6.5
Muscle and skin	33.8	33.4

Table A4. Partition Coefficients for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Partition Coefficient	Butadiene	Epoxybutene	Butenediol	Epoxybutanediol	Diepoxybutane
Blood:air	1.95	56.8	-	-	-
Liver:blood	0.595	0.984	1.04	0.903	1.41
Lung:blood	0.615	0.977	1.107	0.958	1.41
Kidney:blood	0.472	0.842	0.962	0.833	1.54
GI tract:blood	0.446	0.908	1.22	1.06	1.41
Viscera:blood	0.446	0.908	1.22	1.06	1.41
Fat	10.8	2.25	0.573	0.496	2.19
Muscle and Skin:blood	0.564	0.736	1.139	0.986	1.82

Table A5. Biochemical Parameters for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

	Mouse Liver	Rat Liver	Mouse Lung	Rat Lung	Mouse Kidney	Rat Kidney
Butadiene						
P450 Vmax	155	130	139	9.6	1430	30
P450 Km	0.002	0.00375	0.00501	0.00775	0.00501	0.00216
Epoxybutene						
P450 Vmax	45.1	24.3	10.2	9.84	48.6	12.6
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
EH Vmax	347	584	34.8	42.8	113	14.7
EH Km	1.59	0.26	1.59	0.7	1.59	0.7
GST Vmax	6420	4260	720	196	960	494
GST Kmx, adjusted	3.59	2.59	3.59	4.94	3.59	4.39
Butenediol						
P450 Vmax	16.3	67.1	1.0	31.5	1.0	85.0
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
GST Vmax	3280	1230	491	276	1070	658
GST Kmx	34	34	34	34	34	34
Epoxybutanediol						
EH Vmax	363	1150	69.5	169	10.0	152
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	2260	271	50.0	100	50.0	138
GST Kmx	6.40	4.17	6.40	4.17	6.40	4.17
Diepoxybutane						
EH Vmax	1920	3170	10.0	1160	35.2	1000
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	9720	1940	100	100	100	100
GST Kmx, adjusted	6.40	4.17	6.40	4.17	6.40	4.17
Cysteine						
Tissue cysteine	0.193	0.195	0.171	0.127	0.280	0.326
γ-GCS Vmax	420	396	54	50	7920	6080

Table Vmax values in nmol/hr/mg protein, Km values in mM.

Table A6. PBPK Modeling ot the Doerr et al. (1996) Data on Ovarian Atrophy in Mice (Model based on Kohn and Melnick, 2001)

BMO i.p. dose mmol/kg-d x 30 d	AUC DEB in blood µM hr (30 d)	AUC DEB in blood µM hr/day	Hb adducts nmol THBV/g globin-day
0.005	449	15.0	2.43
0.02	1046	34.9	21.8
0.09	1985	66.2	41.4
0.36	2717	90.6	56.7
1.43	3971	132.4	82.9

Table A7. Benchmark Dose Analysis of AUC DEB and Hb Adduct Dose Metrics with the Doerr et al. (1996) Data Set on Ovarian and Uterine Atrophy in Mice Induced by Parenteral Administration of Epoxybutene (BMO).

Model, continuous, site	Fit Statistic, P	BMD_{05} µMhr/d	$BMDL_{05}$ µMhr/d
Polynomial, ovary	0.919	57.4	20.5
Power, ovary	0.0284 n.s.	2688	1765
Polynomial, uterus	0.84	103.1	37.9
		BMD_{05} nmol Hb/g-d	$BMDL_{05}$ nmol Hb/g-d
Hill, ovary	0.661	31.75	18.3
Polynomial, ovary	0.9225	34.81	15.60
Polynomial, uterus	0.80	58.2	23.9

Table A8 Ovarian Quantal Toxicity Data (NTP, 1993)

Dose group	9 months	15 months	24 months	N
0	0/10	0/10	4/49	69
6.25	0/10	0/10	19/49	69
20.0	0/10	0/10	32/48	68
62.5	0/10	9/10	42/50	70
200.0	9/10	7/10	43/50	70
625.0	8/8	2/2	69/79	89
$BMCL_{05}$	3.44	0.65	0.00054	435

Table A9 Ovarian Toxicity Continuous Data for Weighted Analysis (Time Adjustment)

Dose group, Xi, ppm	Yi, %	Wi, weight time in months*
0	0	9
0	0	15
0	8.46	24
6.25	0	9
6.25	0	15
6.25	38.78	24
20	0	9
20	0	15
20	66.67	24
62.5	0	9
62.5	90.0	15
62.5	84.0	24
200	90.0	9
200	70.0	15
200	86.0	24
625	100.0	9
625	100.0	15
625	87.3	24

Note:* weighting by $X^2 = \sum_i W_{i(Yi - Y(Xi, P1 \ldots Pm))}^2$, $Y = a - b/(1 + c(X))^d$, fitted parameters $a = 93.52 \pm 0.236$, $b = 89.23 \pm 0.2933$, $c = 0.01558 \pm 0.000632$, $d = 1.7097 \pm 0.05887$ (SD).

Table A10 Ovarian Continuous to Quantal Conversion

Dose group	Y predicted ,% with parameters a, b, c, d	Adjusted quantal response for BMC analysis	Observed at 24 months, %	Observed average 9-24 months, %
0	4.29	3/69	8.16	2.8
6.25	17.4	12/69	38.78	12.5
20.0	37.4	25/68	66.67	22.2
62.5	65.6	46/70	84.0	58.0
200.0	85.6	60/70	86.0	82.0
625.0	91.98	82/89	87.3	95.8

Table A11. Continuous Benchmark Analysis of Adjusted Quantal Response from Time Weighted Regression*

Model	X^2, P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMLC_{05}$ ppm continuous
Log Logistic	1.13, 0.8896	2.05	1.58	0.28
Log Probit	**1.70, 0.6364**	**2.04**	**1.009**	**0.18**
Multistage	58.35, -	7.54	6.16	n.s.
Multistage	10.19, 0.017	4.10	3.37	Without top dose, n.s.

Note:* BMDS version 2.2

Table A 12. Benchmark Dose Analysis of Fetal Weight Data from Hackett et al. (1987) and Green (2003) Re-Analysis.

Data Set	BMC_{05}	$BMCL_{05}$	Variance assumption	Fit*	Model	Comment
Hackett et al. (1987), total males and females	44.4 183.9	20.0 111.2	Equal Unequal	Exact No	Hill Hill	Best fit
	57.6 89.6	45.9 62.2	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	39.5 28.5	19.3 13.4	Equal Unequal	Exact Yes	Hill Hill	Best fit
Green (2003), total males and females	40.3 172.5	19.2 48.0	Equal Unequal	Exact No	Hill Hill	Best fit
	58.2 54.4	46.7 44.4	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	37.2 22.6	**17.7** 9.6	Equal Unequal	Exact Yes	Hill Hill	**Best fit overall**

Note * Exact = predicted values equal observed by table and plot, all absolute value residuals <3E-7, Yes = test 4 for fit, A3 vs. fitted, all absolute value residuals <2 . All values obtained with BMDS version 2.3.1.

The following model code is an example of OEHHA implementation of the Kohn and Melnick (2001) butadiene model in Berkeley Madonna v. 8.3.9. In Berkeley Madonna lines preceded by semi colons or enclosed in curved brackets are not executed.

```
METHOD Stiff

STARTTIME = 0
STOPTIME = 720
DT = 0.0005
DTOUT = 0.25
;Butadiene multimetabolite mouse model based on Kohn & Melnick (Chemico-Biol. Interact. 135-136:285-301(2001)). Symbols: A = mass of BD in moles, AB = BMO, AC = DEB, AD = BDD; AE = BDE; f = fat, l = liver, m = muscle, vrg = vessel rich group, br = bronchi, pu = alveoli, uo = uterus-ovary, kid, k = kidney, cap = tissue capillary bed; exh = exhaled, perit = peritoneum,C = concentration, Cv = concentration leaving tissue, Cvtot = mixed venous concentration, AUC = area under the time x concentration curve, V = volume, Q = flow, P = partition coeff.,Vmax = metabolic rate, Km = affinity constant, P450 = oxidase; EH = epoxide hydrolase, GST = glutathione sulfotransferase, BW = body weight for adult or young mouse for 30 day simulations, Amet = amount metabolized
{butadiene, moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Abr = 0
Limit Abr >= 0
init Apu = 0
Limit Apu >= 0
init Auo = 0
Limit Auo >= 0
init AUCvtot = 0
Limit AUCvtot >= 0
init Aexh = 0
Limit Aexh >= 0
init Akid = 0
Limit Akid >= 0

init Afcap = 0
Limit Afcap >= 0
init Alcap = 0
Limit Alcap >= 0
init Amcap = 0
Limit Amcap >= 0
init Avrgcap = 0
Limit Avrgcap >= 0
;init Abrcap = 0
;init Apucap = 0
init Auocap = 0
```

```
Limit Auocap >= 0
init Akidcap = 0
Limit Akidcap >= 0

{butadienemonoxide moles}
init ABperit = 0
init ABf = 0
Limit ABf >= 0
init ABl = 0
Limit ABl >= 0
init ABlfree = 0
init ABm = 0
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABbr = 0
Limit ABbr >= 0
init ABbrfree = 0
init ABpu = 0
Limit ABpu >= 0
init ABpufree = 0
init ABuo = 0
Limit ABuo >= 0
init AUCBuo = 0
Limit AUCBuo >= 0
init AUCBvtot = 0
Limit AUCBvtot >= 0
init ABkid = 0
Limit ABkid >= 0
init ABkidfree = 0

init ABfcap = 0
Limit ABfcap >= 0
init ABlcap = 0
Limit ABlcap >= 0
init ABmcap = 0
Limit ABmcap >= 0
init ABvrgcap = 0
Limit ABvrgcap >= 0
;init ABbrcap = 0
;init ABpucap = 0
init ABuocap = 0
Limit ABuocap >= 0
init ABkidcap = 0
Limit ABkidcap >= 0

{diepoxybutane moles}
init ACperit = 0
init ACf = 0
Limit ACf >= 0
init ACl = 0
Limit ACl >= 0
init AClfree = 0
init ACm = 0
Limit ACm >= 0
init ACvrg = 0
```

```
Limit ACvrg >= 0
init ACbr = 0
Limit ACbr >= 0
init ACbrfree = 0
init ACpu = 0
Limit ACpu >= 0
init ACpufree = 0
init ACuo = 0
Limit ACuo >= 0
init AUCCuo = 0
Limit AUCCuo >= 0
init AUCCvtot = 0
Limit AUCCvtot >= 0
init ACkid = 0
Limit ACkid >= 0
init ACkidfree = 0

init ACfcap = 0
init AClcap = 0
init ACmcap = 0
init ACvrgcap = 0
;init ACbrcap = 0
;init ACpucap = 0
init ACuocap = 0
init ACkidcap = 0

{dihydroxybutene}
init ADf = 0
Limit ADf >= 0
init ADl = 0
Limit ADl >= 0
init ADm = 0
Limit ADm >= 0
init ADvrg = 0
Limit ADvrg >= 0
init ADbr = 0
Limit ADvrg >= 0
init ADpu = 0
Limit ADpu >= 0
init ADuo = 0
Limit ADuo >= 0
init AUCDvtot = 0
init ADkid = 0
Limit ADkid >= 0

init ADfcap = 0
init ADlcap = 0
init ADmcap = 0
init ADvrgcap = 0
;init ADbrcap = 0
;init ADpucap = 0
init ADuocap = 0
init ADkidcap = 0

{epoxybutanediol}
init AEf = 0
```

```
Limit AEf >= 0
init AEl = 0
Limit AEl >= 0
init AElfree = 0
init AEm = 0
Limit AEm >= 0
init AEvrg = 0
Limit AEvrg >= 0
init AEbr = 0
init AEbrfree = 0
Limit AEvrg >= 0
init AEpu = 0
Limit AEpu >= 0
init AEpufree = 0
init AEuo = 0
Limit AEuo >= 0
init AUCEvtot = 0
init AEkid = 0
Limit AEkid >= 0
init AEkidfree = 0

init AEfcap = 0
init AElcap = 0
init AEmcap = 0
init AEvrgcap = 0
;init AEbrcap = 0
;init AEpucap = 0
init AEuocap = 0
init AEkidcap = 0

{moles of butadiene metabolized}
init Ametl = 0
init Ametpu = 0
init Ametbr = 0
init Ametk = 0

{moles of butadienemonoxide metabolized}
init ABmetl1 = 0
Limit ABmetl1 >= 0
init ABmetl2 = 0
Limit ABmetl2 >= 0
init ABmetpu1 = 0
Limit ABmetpu1 >= 0
init ABmetpu2 = 0
Limit ABmetpu2 >= 0
init ABmetbr1 = 0
Limit ABmetbr1 >= 0
init ABmetbr2 = 0
Limit ABmetbr2 >= 0
init ABmetl3 = 0
Limit ABmetl3 >= 0
init ABmetpu3 = 0
Limit ABmetpu3 >= 0
init ABmetbr3 = 0
Limit ABmetbr3 >= 0
```

```
{moles of dihydroxybutene metabolized}
init ADmetpu6 = 0
init ADmetbr6 = 0
init ADmetl6 = 0

{moles of diepoxybutane metabolized}
init ACmetpu4 = 0
init ACmetbr4 = 0
init ACmetl4 = 0

{Cysteine metabolism, moles, mol/L}
init AFl = Ccysl*Vl
init AFlu = Ccyslu*Vlu
init AFkid = Ccyskid*Vkid
Ccysl = 1.93E-4
Ccyslu = 1.71E-4
Ccyskid = 2.8E-4
Vmax10lC = 420E-9
Vmax10l = Vmax10lC*CPL*Vl
Vmax10luC = 54E-9
Vmax10lu = Vmax10luC*CPLu*Vlu
Vmax10kidC = 7920E-9
Vmax10kid = Vmax10kidC*CPLu*Vkid
Kmcys = 3.5E-4
Kl = 2.3E-3
Kl0 = 1.5*Vl
Klu0 = 1.5*Vlu
Kkid0 = 1.5*Vkid

{Glutathione metabolism, moles, mol/L}
init AGl = CGSHl*Vl
Limit AGl >= 0
init AGlu = CGSHlu*Vlu
Limit AGlu >= 0
init AGkid = CGSHkid*Vkid
Limit AGkid >= 0
CGSHl = 5E-3
CGSHlu = 1E-3
CGSHkid = 2E-3
CGl = AGl/Vl
Limit CGl >= 0
CGlu = AGlu/Vlu
Limit CGlu >= 0
CGkid = AGkid/Vkid
Limit CGkid >= 0
Ka = 3E-3

{Adduct formation}
init Adduct = 0

{tissue flows L/hr}
Qtot = 15.3*BW^0.7
Qalv = 20.2*BW^0.7
Qf = 0.05*Qtot
Ql = 0.044*Qtot
Qkid = 0.163*Qtot
```

```
Qm = Qtot - (Qf + Ql + Qvrg + Quo + Qkid)
Qvrg = 0.389*Qtot
Qpu = 0.928*Qtot
Qbr = 0.072*Qtot
Quo = 0.016*Qtot

{tissue volumes, L}
Vf = 0.06*BW
Vl = 0.055*BW
Vkid = 0.0167*BW
Vm = BW - (Vf + Vl + Vvrg + Vlu + Vuo + Vkid)
Vvrg = 0.1143*BW
Vlu = 0.011*BW
Vpu = 0.454*Vlu
Vbr = 0.545*Vlu
Vuo = 0.001*BW
;BW = 0.030
BW = 0.01263 + 3.69E-4*(T/24) - 5.59E-6*(T/24)^2

{capillary blood volumes, L}
Vfcap = 0.03*Vf
Vlcap = 0.11*Vl
;Vbrcap = 0.11*Vbr
;Vpucap = 0.11*Vpu
Vkidcap = 0.102*Vkid
Vvrgcap = 0.071*Vvrg
Vuocap = 0.071*Vuo
Vmcap = 0.013*Vm

{blood/air and tissue/blood partition coefficients, butadiene}
Pb = 1.95
Pl = 0.595
Pf = 10.8
Pkid = 0.472
Pm = 0.564
Pvrg = 0.472
Ppu = 0.615
Pbr = 0.615
Puo = 0.446
Pibd = 1.183

{blood/air and tissue/blood partition coefficients, butadienemonoxide}
PBb = 56.8
PBl = 0.984
PBkid = 0.842
PBf = 2.25
PBm = 0.736
PBvrg = 0.842
PBpu = 0.977
PBbr = 0.977
PBuo = 0.908
Pibmo = 2.125

{blood/air and tissue/blood partition coefficients, diepoxybutane}
PCl = 4.41
PCkid = 1.54
```

PCf = 2.19
PCm = 1.82
PCvrg = 1.54
PCpu = 1.41
PCbr = 1.41
PCuo = 1.41
Pideb = 1.174

{blood/air and tissue/blood partition coefficients, dihydroxybutene}
PDl = 1.04
PDkid = 0.833
PDf = 0.573
PDm = 1.139
PDvrg = 0.962
PDpu = 1.107
PDbr = 1.107
PDuo = 1.22
Pidhb = 1.198

{blood/air and tissue/blood partition coefficients, epoxybutanediol}
PEl = 0.903
PEkid = 1.06
PEf = 0.496
PEm = 0.986
PEvrg = 1.06
PEpu = 0.833
PEbr = 0.833
PEuo = 1.06
Piebd = 1.237

{butadiene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxlu = 139E-9*9.0E3*Vlu
Vmaxbr = 0.50*Vmaxlu
Vmaxpu = 0.50*Vmaxlu
Kmlu = 5.01E-6
Vmaxl = 155.0E-9*30.0E3*Vl
Km = 2.0E-6
VmaxkC = 1430E-9
Vmaxk = VmaxkC*Vkid*30.0E3
Kmk = Kmlu
MPL = 3.0E4
MPLu = 9.0E3

{butadienemonoxide metabolic parameters, mol/hr, mol/L, /hr; 1 = hydrolysis EH, 2 = conjugation GST, 3 = oxidation P450}
;EH
Vmaxl1 = 347.0E-9*30.0E3*Vl
Km1 = 1.59E-3
Vmaxlu1C = 34.8E-9
Vmaxbr1 = Vmaxlu1C*Vbr*9.0E3
Vmaxpu1 = Vmaxlu1C*Vpu*9.0E3
Kmlu1 = 1.59E-3
Vmaxk1C = 113E-9
Vmaxk1 = Vmaxk1C*Vkid*9.0E3
Kmk1 = Kmlu1
krel = 3.76

```
R = 0.052

;GST
Vmaxl2C = 6420E-9
Vmaxl2 = Vmaxl2C*Vl*8.28E4
Km2 =3.59E-3
Vmaxlu2C = 720.0E-9
Vmaxbr2 = Vmaxlu2C*Vbr*8.28E4
Vmaxpu2 = Vmaxlu2C*Vpu*8.28E4
Kmlu2 = 3.59E-3
Vmaxk2C = 960.0E-9
Vmaxk2 = Vmaxk2C*Vkid*8.28E4
Kmk2 = 3.59E-3
CPL = 8.28E4
CPLu = 8.28E4
Kmgsh = 1.0E-3

;P450
Vmaxl3 = 45.1E-9*30.0E3*Vl
Vmaxlu3C = 10.2E-9
Vmaxpu3 = Vmaxlu3C*Vpu*9.0E3
Vmaxbr3 = Vmaxlu3C*Vbr*9.0E3
Km3 = 1.56E-5
Kmlu3 = Km3
Kmk3 = Km3
Vmaxk3C = 48.6E-9
Vmaxk3 = Vmaxk3C*Vkid*9.0E3

{Diepoxybutane (DEB) metabolic parameters, 4 = EH, 5 = GST, mol/hr, mol/L}
Vmaxl4C = 1920E-9
Vmaxl4 = Vmaxl4C*Vl*30E3
Km4 = 8.1E-3
Vmaxlu4C = 10E-9
Vmaxbr4 = Vmaxlu4C*Vbr*9.0E3
Vmaxpu4 = Vmaxlu4C*Vpu*9.0E3
Kmlu4 = 7.5E-3
Vmaxk4C = 35.2E-9
Vmaxk4 = Vmaxk4C*Vkid*9.0E3
Kmk4 = Kmlu4
k4rel = 9.75

Vmaxl5C = 9720E-9
Vmaxl5 = Vmaxl5C*Vl*CPL
Km5 = 6.4E-3
Vmaxlu5C = 100E-9
Vmaxbr5 = Vmaxlu5C*Vbr*CPlu
Vmaxpu5 = Vmaxlu5C*Vpu*CPlu
Vmaxk5C = 100E-9
Vmaxk5 = Vmaxk5C*Vkid*CPlu

{Dihydroxybutene (DHB) metabolic parameters, 6 = P450, 7 = GST, mnol/hr, mol/L}
Vmaxl6C = 16.3E-9
Vmaxl6 = Vmaxl6C*Vl*30E3
Km6 = 1.56E-5
Vmaxlu6C = 1.0E-9
Vmaxbr6 = Vmaxlu6C*Vbr*9.0E3
```

```
Vmaxpu6 = Vmaxlu6C*Vpu*9.0E3
Vmaxk6C = 1.0E-9
Vmaxk6 = Vmaxk6C*Vkid*9.0E3

Vmaxl7C = 3480E-9
Vmaxl7 = Vmaxl7C*Vl*CPL
Km7 = 34E-3
Vmaxlu7C = 491E-9
Vmaxbr7 = Vmaxlu7C*Vbr*CPlu
Vmaxpu7 = Vmaxlu7C*Vpu*CPlu
Vmaxk7C = 1070E-9
Vmaxk7 = Vmaxk7C*Vkid*CPlu

{Epoxybutanediol metabolic parameters 8 = EH, 9 = GST}
Vmaxl8C = 363E-9
Vmaxl8 = Vmaxl8C*Vl*30E3
Km8 = 8.1E-3
Vmaxlu8C = 69.5E-9
Vmaxbr8 = Vmaxlu8C*Vbr*9.0E3
Vmaxpu8 = Vmaxlu8C*Vpu*9.0E3
Kmlu8 = 7.5E-3
Vmaxk8C = 10E-9
Vmaxk8 = Vmaxk8C*Vkid*9.0E3
Kmk8 = Kmlu8
k8rel = 50.6

Vmaxl9C = 2260E-9
Vmaxl9 = Vmaxl9C*Vl*CPL
Km9 = 6.4E-3
Vmaxlu9C = 50E-9
Vmaxbr9 = Vmaxlu9C*Vbr*CPlu
Vmaxpu9 = Vmaxlu9C*Vpu*CPlu
Vmaxk9C = 50E-9
Vmaxk9 = Vmaxk9C*Vkid*CPlu

Kadduct = 1E-4

Kip = 1.25
T = TIME
{exposure in ppm converted to moles}
Cair = IF TIME <= 6 THEN 0*(1E-6/25.45) ELSE 0

{calculated concentrations of butadiene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvkid = Akid/(Vkid*Pkid)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvuo = Auo/(Vuo*Puo)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Quo*Cvuo + Qkid*Cvkid)/Qpu
Cvtotg = Cvtot*MWBD*1000
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
```

MWBD = 54.1

{calculated concentrations of butadienemonoxide}
CBart = (Qpu*CBvpu + Qbr*CBvbr)/Qtot
CBvf = ABf/(Vf*PBf)
CBvl = ABl/(Vl*PBl)
CBvkid = ABkid/(Vkid*Pkid)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvpu = ABpu/(Vpu*PBpu)
CBvbr = ABbr/(Vbr*PBbr)
CBvuo = ABuo/(Vuo*PBuo)
CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Quo*CBvuo + Qkid*CBvkid)/Qtot
CBvtotg = CBvtot*MWEB*1000
CBair = CBvtot/PBb
CBvipu = (Qalv*CBair + Qpu*CBvtot)/((Qalv/PBb) + Qpu)
CBexh = CBvipu/PBb
MWEB = 70.1

{calculated concentrations of diepoxybutane}
CCart = (Qpu*CCvpu + Qbr*CCvbr)/Qtot
CCvf = ACf/(Vf*PCf)
CCvl = ACl/(Vl*PCl)
CCvkid = ACkid/(Vkid*PCkid)
CCvm = ACm/(Vm*PCm)
CCvvrg = ACvrg/(Vvrg*PCvrg)
CCvpu = ACpu/(Vpu*PCpu)
CCvbr = ACbr/(Vbr*PCbr)
CCvuo = ACuo/(Vuo*PCuo)
CCvtot = (Ql*CCvl +Qf*CCvf + Qm*CCvm + Qvrg*CCvvrg + Quo*CCvuo + Qkid*CCvkid)/Qtot
CCvtotg = CCvtot*MWDEB*1000
CCvipu = (Qalv + Qpu*CCvtot)/(Qalv + Qpu)
MWDEB = 86.0

{calculated concentrations of dihydroxybutene}
CDart = (Qpu*CDvpu + Qbr*CDvbr)/Qtot
CDvf = ADf/(Vf*PDf)
CDvl = ADl/(Vl*PDl)
CDvkid = ADkid/(Vkid*PDkid)
CDvm = ADm/(Vm*PDm)
CDvvrg = ADvrg/(Vvrg*PDvrg)
CDvpu = ADpu/(Vpu*PDpu)
CDvbr = ADbr/(Vbr*PDbr)
CDvuo = ADuo/(Vuo*PDuo)
CDvtot = (Ql*CDvl + Qf*CDvf + Qm*CDvm + Qvrg*CDvvrg + Quo*CDvuo + Qkid*CDvkid)/Qtot
CDvtotg = CDvtot*MWDHB*1000
CDvipu = (Qalv + Qpu*CDvtot)/(Qalv +Qpu)
MWDHB = 72.0

{calculated concentrations of epoxybutanediol}
CEart = (Qpu*CEvpu + Qbr*CEvbr)/Qtot
CEvf = AEf/(Vf*PEf)
CEvl = AEl/(Vl*PEl)
CEvkid = AEkid/(Vkid*PEkid)
CEvm = AEm/(Vm*PEm)

```
CEvvrg = AEvrg/(Vvrg*PEvrg)
CEvpu = AEpu/(Vpu*PEpu)
CEvbr = AEbr/(Vbr*PEbr)
CEvuo = AEuo/(Vuo*PEuo)
CEvtot = (Ql*CEvl + Qf*CEvf + Qm*CEvm + Qvrg*CEvvrg + Quo*CEvuo + Qkid*CEvkid)/Qtot
CEvtotg = CEvtot*MWEBD*1000
CEvipu = (Qalv + Qpu*CEvtot)/(Qalv +Qpu)
MWEBD = 102.1

{differential equations for butadiene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu) - Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr) - Vmaxbr * Cvbr/(Kmlu + Cvbr)
d/dt(Alcap) = Ql*(Cart - Alcap/Vlcap) - Ql*Pibd*(Cart - Cvl)
d/dt(Al) = Ql*Pibd*(Alcap/Vlcap - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Akidcap) = Qkid*(Cart - Akidcap/Vkidcap) - Qkid*Pibd*(Cart - Cvkid)
d/dt(Akid) = Qkid*Pibd*(Cart - Cvkid) - Vmaxk*Cvkid/(Kmk + Cvkid)
d/dt(Afcap) = Qf*(Cart - Afcap/Vfcap) - Qf*Pibd*(Cart - Cvf)
d/dt(Af) = Qf*Pibd*(Cart - Cvf)
d/dt(Amcap) = Qm*(Cart - Amcap/Vmcap) - Qm*Pibd*(Cart - Cvm)
d/dt(Am) = Qm*Pibd*(Cart - Cvm)
d/dt(Avrgcap) = Qvrg*(Cart - Avrgcap/Vvrgcap) - Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Avrg) = Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Auocap) = Quo*(Cart - Auocap/Vuocap) - Quo*Pibd*(Cart - Cvuo)
d/dt(Auo) = Quo*Pibd*(Cart - Cvuo)
d/dt(AUCvtot) = Cvtot
d/dt(Aexh) = Cexh*0.15

{amount of butadiene metabolized in liver and lung}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(Ametpu) = Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Ametbr) = Vmaxbr*Cvbr/(Kmlu + Cvbr)
d/dt(Ametk) = Vmaxk*Cvkid/(Kmk + Cvkid)

{differential equations for BMO metabolism}
d/dt(ABperit) = PULSE(1.31*9.0E-5*BW,0,24) -ABperit*Kip
init AUCABperit = 0
d/dt(AUCABperit) = ABperit

d/dt(ABpufree) = ABpu*krel

d/dt(ABpu) = Qpu*(CBart - CBvpu) + Vmaxpu*Cvpu/(Kmlu + Cvpu) - Vmaxpu1*ABpufree/(Km1*Vpu +
ABpufree) - Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu) - Vmaxpu2*CBvpu*CGSHlu/(Kmlu2*Kmgsh +
CBvpu*Kmgsh + CGSHlu*Kmlu2 + CBvpu*CGSHlu) - Vmaxpu3*CBvpu/(Kmlu3 + CBvpu)

d/dt(ABbrfree) = ABbr*krel

d/dt(ABbr) = Qbr*(CBart - CBvbr) + Vmaxbr * Cvbr/(Kmlu + Cvbr) - Vmaxbr1*ABbrfree/(Km1*Vbr +
ABbrfree) - Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) -Vmaxbr2*CBvbr*CGSHlu/(Kmlu2*Kmgsh +
CBvbr*Kmgsh + CGSHlu*Kmlu2 + CGSHlu*CBvbr) - Vmaxbr3*CBvbr/(Kmlu3 + CBvbr) + ABperit*Kip

d/dt(ABlcap) = Ql*(CBart - ABlcap/Vlcap) - Ql*Pibmo*(CBart - CBvl)

d/dt(ABlfree) = ABl*krel
```

d/dt(ABl) = Ql*Pibmo*(CBart - CBvl) + Vmaxl*Cvl/(Km + Cvl) - Vmaxl1*ABlfree/(Km1*Vl + ABlfree) - Vmaxl1*ABl/(R*Km1*Vl + ABl) - Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 + CGSHl*CBvl) - Vmaxl3*Cbvl/(Km3 + CBvl)

d/dt(ABkidcap) = Qkid*(CBart - ABkidcap/Vkidcap) - Qkid*Pibmo*(CBart - CBvkid)

d/dt(ABkidfree) = ABkid*krel

d/dt(ABkid) = Qkid*Pibmo*(CBart - CBvkid) + Vmaxk*Cvkid/(Kmk + Cvkid) - Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) - Vmaxk1*ABkid/(R*Km1*Vkid + ABkid) - Vmaxk2*CBvkid*CGSHkid/(Kmk2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Kmk2 + CGSHkid*CBvkid) - Vmaxk3*CBvkid/(Kmk3 + CBvkid)

d/dt(ABfcap) = Qf*(CBart - ABfcap/Vfcap) - Qf*Pibmo*(CBart - CBvf)
d/dt(ABf) = Qf*Pibmo*(CBart - CBvf)

d/dt(ABmcap) = Qm*(CBart - ABmcap/Vmcap) - Qm*Pibmo*(CBart - CBvm)
d/dt(ABm) = Qm*Pibmo*(CBart - CBvm)

d/dt(ABvrgcap) = Qvrg*(CBart - ABvrgcap/Vvrgcap) - Qvrg*Pibmo*(CBart - CBvvrg)
d/dt(ABvrg) = Qvrg*pibmo*(CBart - CBvvrg)

d/dt(ABuocap) = Quo*(CBart - ABuocap/Vuocap) - Quo*Pibmo*(CBart - CBvuo)
d/dt(ABuo) = Quo*Pibmo*(CBart - CBvuo)

{Hb and DNA adducts based on styrene oxide induced formation rates}
init HB1 = 0
d/dt(HB1) = CCart*kher ; mole /hr/g Hb
kher = 3.2E-5 ; L/hr/g Hb
Ter = 960 ; lifetime of RBC in hr
HB = (1E9)*HB1*(1 - (T/(2*Ter))) ; nmol/g Hb
init DNA1 = 0
d/dt(DNA1) = CCart*kfdna - DNA*keldna/1E9
DNA = DNA1*1E9 ; nmol/gDNA
kfdna = 9.6E-5 ; N7 DNA adducts/gDNA/hr
keldna = 0.0077 ; elimination of DNA adducts /hr

{AUCs for butadienemonoxide}
d/dt(AUCBuo) = CBvuo
d/dt(AUCBvtot) = CBvtot

{amounts of butadienemonoxide metabolized}
d/dt(ABmetl1) = Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl + ABl)
d/dt(ABmetl2) = Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 + CGSHl*CBvl)

d/dt(ABmetpu1) = Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) + Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu)
d/dt(ABmetpu2) = Vmaxpu2*CBvpu*CGSHlu/(Km2*Kmgsh + CBvpu*Kmgsh + CGSHlu*Km2 + CGSHlu*CBvpu)

d/dt(ABmetbr1) = Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) + Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr)
d/dt(ABmetbr2) = Vmaxbr2*CBvbr*CGSHlu/(Km2*Kmgsh + CBvbr*Kmgsh + CGSHlu*Km2 + CGSHlu*CBvbr)

d/dt(ABmetl3) = Vmaxl3*CBvl/(Km3 + CBvl)
d/dt(ABmetpu3) = Vmaxpu3*CBvpu/(Km3 + CBVpu)
d/dt(ABmetbr3) = Vmaxbr3*CBvbr/(Km3 + CBvbr)

```
init ABmetk1 = 0
init ABmetk2 = 0
init ABmetk3 = 0
d/dt(ABmetk1) = Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) + Vmaxk1*ABkid/(R*Km1*Vkid + ABkid)
d/dt(ABmetk2) = Vmaxk2*CBvkid*CGSHkid/(Km2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Km2 +
CGSHkid*CBvkid)
d/dt(ABmetk3) = Vmaxk3*CBvkid/(Km3 + CBvkid)


{differential equations for diepoxybutane}
d/dt(ACperit) = PULSE(0*BW,0,24) -ACperit*Kip

d/dt(ACpufree) = ACpu*k4rel

d/dt(ACpu) = Qpu*(CCart - CCvpu) + Vmaxpu3*CBvpu/(Km3 + CBvpu) - Vmaxpu4*ACpufree/(Kmlu4*Vpu
+ ACpufree) - Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) - Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh +
CCvpu*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvpu)

d/dt(ACbrfree) = ACbr*k4rel

d/dt(ACbr) = Qbr*(CCart - CCvbr) + Vmaxbr3*CBvbr/(Km3 + CBvbr)  - Vmaxbr4*ACbrfree/(Kmlu4*Vbr +
ACbrfree) - Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) - Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh +
CCvbr*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvbr) + ACperit*Kip

d/dt(AClfree) = ACl*k4rel

d/dt(AClcap) = Ql*(CCart - AClcap/Vlcap) - Ql*Pideb*(CCart - CCvl)
d/dt(ACl) = Ql*Pideb*(CCart - CCvl) + Vmaxl3*CBvl/(Km3 + CBvl) - Vmaxl4*AClfree/(Km4*Vl + AClfree) -
Vmaxl4*ACl/(R*Km4*Vl + ACl) - Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 +
CGSHl*CCvl)

d/dt(ACkidfree) = ACkid*k4rel

d/dt(ACkidcap) = Qkid*(CCart - ACkidcap/Vlcap) - Ql*Pideb*(CCart - CCvkid)
d/dt(ACkid) = Qkid*Pideb*(CCart - CCvkid) + Vmaxk3*CBvkid/(Km3 + CBvkid) -
Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) - Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid) -
Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 + CGSHkid*CCvkid)

d/dt(ACfcap) = Qf*(CCart - ACfcap/Vfcap) - Qf*Pideb*(CCart - CCvf)
d/dt(ACf) = Qf*Pideb*(CCart - CCvf)

d/dt(ACmcap) = Qm*(CCart - ACmcap/Vmcap) - Qm*Pideb*(CCart - CCvm)
d/dt(ACm) = Qm*Pideb*(CCart - CCvm)

d/dt(ACvrgcap) = Qvrg*(CCart - ACvrgcap/Vvrgcap) - Qvrg*Pideb*(CCart - CCvvrg)
d/dt(ACvrg) = Qvrg*Pideb*(CCart - CCvvrg)

d/dt(ACuocap) = Quo*(CCart - ACuocap/Vuocap) - Quo*Pideb*(CCart - CCvuo)
d/dt(ACuo) = Quo*Pideb*(CCart - CCvuo)

{metabolism of diepoxybutane to epoxybutanediol}
d/dt(ACmetpu4) = Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) + Vmaxpu4*ACpu/(R*Kmlu4*Vpu +
ACpu)

d/dt(ACmetbr4) = Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) + Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr)
```

```
d/dt(ACmetl4) =  Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl + ACl)
init ACmetk4 = 0
d/dt(ACmetk4) = Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) + Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid)

{AUCs for diepoxybutane}
d/dt(AUCCuo) = CCvuo
d/dt(AUCCvtot) = CCvtot

{metabolism of diepoxybutane to DEB-SG}
init ACmetpu5 = 0
init ACmetbr5 = 0
init ACmetl5 = 0
Limit ACmetl5 >= 0
init ACmetk5 = 0

d/dt(ACmetpu5) = Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh + CCvpu*Kmgsh + CGSHlu*Km5 +
CGSHlu*CCvpu)
d/dt(ACmetbr5) = Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh + CCvbr*Kmgsh + CGSHlu*Km5 +
CGSHlu*CCvbr)
d/dt(ACmetl5) = Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 + CGSHl*CCvl)
d/dt(ACmetk5) = Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 +
CGSHkid*CCvkid)


{differential equations for dihydroxybutene}
d/dt(ADpu) = Qpu*(CDart - CDvpu) + Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) +
Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu)  - Vmaxpu6*CDvpu/(Km6 + CDvpu) -
Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvpu)

d/dt(ADbr) = Qbr*(CDart - CDvbr) + Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) +
Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) - Vmaxbr6*CDvbr/(Km6 + CDvbr) -
Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvbr)

d/dt(ADlcap) = Ql*(CDart - ADlcap/Vlcap) - Ql*Pidhb*(CDart - CDvl)
d/dt(ADl) = Ql*Pidhb*(CDart - CDvl) + Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl +
ABl)  - Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 +
CGSHl*CDvl)

d/dt(ADkidcap) = Qkid*(CDart - ADkidcap/Vkidcap) - Qkid*Pidhb*(CDart - CDvkid)
d/dt(ADkid) = Qkid*Pidhb*(CDart - CDvkid) +  Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) +
Vmaxk1*ABkid/(R*Km1*Vkid + ABkid)  - Vmaxl6*CDvkid/(Km6 + CDvkid) -
Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 + CGSHkid*CDvkid)

d/dt(ADfcap) = Qf*(CDart - ADfcap/Vfcap) - Qf*Pidhb*(CDart - CDvf)
d/dt(ADf) = Qf*Pidhb*(CDart-CDvf)

d/dt(ADmcap) = Qm*(CDart - ADmcap/Vmcap) - Qm*Pidhb*(CDart - CDvm)
d/dt(ADm) = Qm*Pidhb*(CDart - CDvm)

d/dt(ADvrgcap) = Qvrg*(CDart - ADvrgcap/Vvrgcap) - Qvrg*Pidhb*(CDart - CDvvrg)
d/dt(ADvrg) = Qvrg*Pidhb*(CDart - CDvvrg)

d/dt(ADuocap) = Quo*(CDart - ADuocap/Vuocap) - Quo*Pidhb*(CDart - CDvuo)
d/dt(ADuo) = Quo*Pidhb*(CDart - CDvuo)
```

```
d/dt(AUCDvtot) = CDvtot
init (AUCDvuo) = 0
d/dt(AUCDvuo) = CDvuo

{metabolism of DHB to EBD }
d/dt(ADmetpu6) = Vmaxpu6*CDvpu/(Km6 + CDvpu)
d/dt(ADmetbr6) = Vmaxbr6*CDvbr/(Km6 + CDvbr)
d/dt(ADmetl6) = Vmaxl6*CDvl/(Km6 + CDvl)
init ADmetk6 = 0
d/dt(ADmetk6) = Vmaxk6*CDvkid/(Km6 + CDvkid)

{metabolism of DHB to DHB-SG conjugate}
init ADmetpu7 = 0
init ADmetbr7 = 0
init ADmetl7 = 0
init ADmetk7 = 0

d/dt(ADmetpu7) = Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 +
CGSHlu*CDvpu)
d/dt(ADmetbr7) = Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 +
CGSHlu*CDvbr)
d/dt(ADmetl7) = Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 + CGSHl*CDvl)
d/dt(ADmetk7) = Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 +
CGSHkid*CDvkid)


{differential equations for epoxybutanediol}
d/dt(AEpufree) = AEpu*k8rel

d/dt(AEpu) = Qpu*(CEart - CEvpu) +  Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) +
Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) + Vmaxpu6*CDvpu/(Km6 + CDvpu) -
Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) - Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu) -
Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvpu)

d/dt(AEbrfree) = AEbr*k8rel

d/dt(AEbr) = Qbr*(CEart - CEvbr) + Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) +
Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) +  Vmaxbr6*CDvbr/(Km6 + CDvbr)  - Vmaxbr8*AEbrfree/(Km8*Vbr
+ AEbrfree) - Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr) - Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh +
CEvbr*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvbr)

d/dt(AElfree) = AEl*k8rel

d/dt(AElcap) = Ql*(CEart - AElcap/Vlcap) - Ql*Piebd*(CEart - CEvl)
d/dt(AEl) = Ql*Piebd*(CEart - CEvl) + Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl +
ACl) + Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl8*AElfree/(Km8*Vl + AElfree) - Vmaxl8*AEl/(R*Km8*Vl + AEl) -
Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)

d/dt(AEkidfree) = AEkid*k8rel

d/dt(AEkidcap) = Qkid*(CEart - AEkidcap/Vkidcap) - Qkid*Piebd*(CEart - CEvkid)
d/dt(AEkid) = Qkid*Pidhb*(CEart - CEvkid) +  Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) +
Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid)  + Vmaxl6*CDvkid/(Km6 + CDvkid) -
Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) - Vmaxk8*AEkid/(R*Km8*Vkid + AEkid) -
Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 + CGSHkid*CEvkid)
```

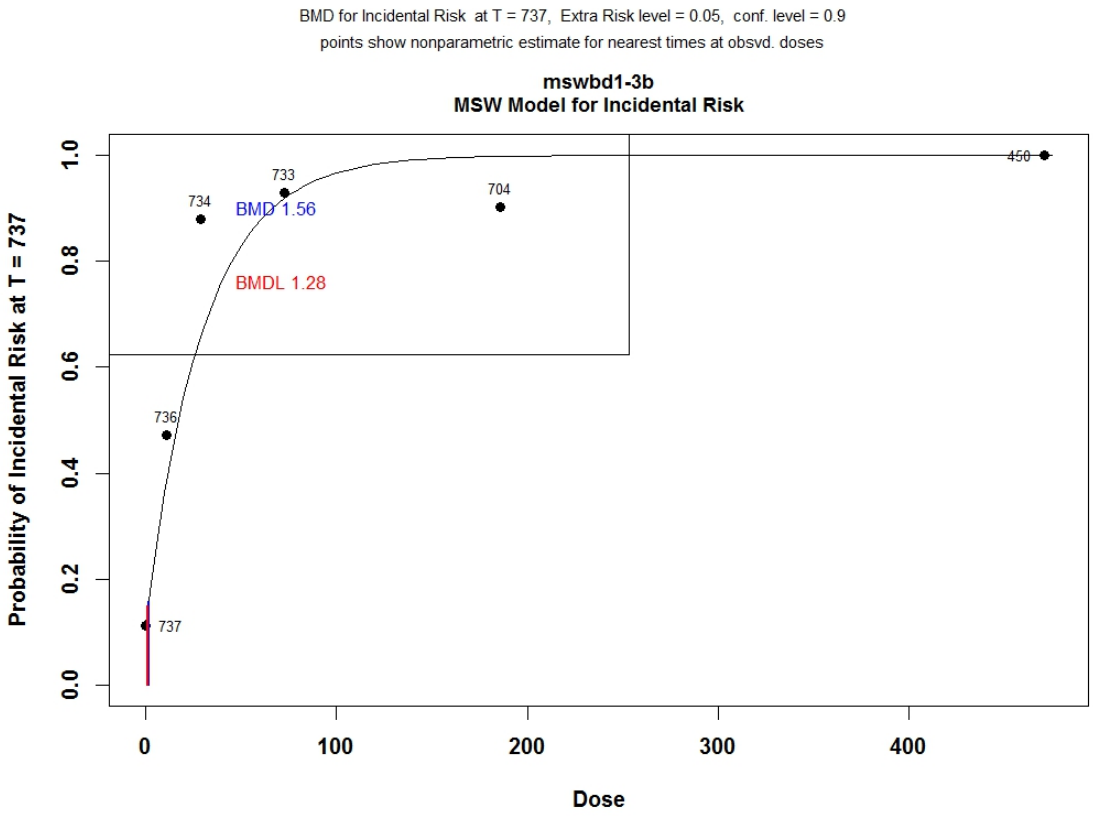
```
d/dt(AEfcap) = Qf*(CEart - AEfcap/Vfcap) - Qf*Piebd*(CEart - CEvf)
d/dt(AEf) = Qf*Piebd*(CEart-CEvf)

d/dt(AEmcap) = Qm*(CEart - AEmcap/Vmcap) - Qm*Piebd*(CEart - CEvm)
d/dt(AEm) = Qm*Piebd*(CEart - CEvm)

d/dt(AEvrgcap) = Qvrg*(CEart - AEvrgcap/Vvrgcap) - Qvrg*Piebd*(CEart - CEvvrg)
d/dt(AEvrg) = Qvrg*Piebd*(CEart - CEvvrg)

d/dt(AEuocap) = Quo*(CEart - AEuocap/Vuocap) - Quo*Piebd*(CEart - CEvuo)
d/dt(AEuo) = Quo*Piebd*(CEart - CEvuo)

d/dt(AUCEvtot) = CEvtot
init (AUCEvuo) = 0
d/dt(AUCEvuo) = CEvuo

{metabolism of  epoxybutanediol to erythitol}
init AEmetpu8 = 0
init AEmetbr8 = 0
init AEmetl8 = 0
init AEmetk8 = 0
d/dt(AEmetpu8) =  Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) + Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu)
d/dt(AEmetbr8) = Vmaxbr8*AEbrfree/(Km8*Vbr + AEbrfree) + Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr)
d/dt(AEmetl8) = Vmaxl8*AElfree/(Km8*Vl + AElfree) + Vmaxl8*AEl/(R*Km8*Vl + AEl)
init AEmetk8 = 0
d/dt(AEmetk8) = Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) + Vmaxk8*AEkid/(R*Km8*Vkid + AEkid)

{metabolism of EBD to EBD-SG}
init AEmetpu9 = 0
init AEmetbr9 = 0
init AEmetl9 = 0
init AEmetk9 = 0

d/dt(AEmetpu9) = Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvpu)
d/dt(AEmetbr9) = Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh + CEvbr*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvbr)
d/dt(AEmetl9) = Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)
d/dt(AEmetk9) = Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 +
CGSHkid*CEvkid)

{differential equations for cysteine metabolism}
d/dt(AFl) = Kl0 - Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(KI*Vl)))
d/dt(AFlu) = Klu0 - Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(KI*Vlu)))
d/dt(AFkid) = Kkid0 - Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(KI*Vkid)))

{differential equations for glutathione metabolism}
d/dt(AGl) = Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(KI*Vl))) - ABmetl2 - ACmetl5 - ADmetl7- AEmetl9
d/dt(AGlu) = Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(KI*Vlu))) - ABmetpu2 - ABmetbr2 -
ACmetpu5 - ACmetbr5 - ADmetpu7 - ADmetbr7 - AEmetpu9 - AEmetbr9
d/dt(AGkid) = Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(KI*Vkid))) - ABmetk2 - ACmetk5 -
ADmetk7 - AEmetk9

{differential equation for  Hb adduct formation, pmol/g Hb-ppm-hr}
d/dt(Adduct) = CEvtot*Kadduct*1E12/(476.7*8000)
; Mass balance
```

```
MassA = Af + Al + Am + Avrg + Abr + Apu + Auo + Aexh + Akid
MassB = ABf + ABl + ABm + ABvrg + ABbr + ABpu + ABuo + ABkid
MassBfree = ABlfree + ABpufree + ABbrfree + ABkidfree
MassC = ACf + ACl + ACm + ACvrg + ACbr + ACpu + ACuo + ACkid
MassCfree = AClfree + ACpufree + ACbrfree + ACkidfree
MassD = ADf + ADl + ADm + ADvrg + ADbr + ADpu + ADuo + ADkid
MassE = AEf + AEl + AEm + AEvrg + AEbr + AEpu + AEuo + AEkid
MassEfree = AElfree + AEpufree + AEbrfree + AEkidfree
Massfree = MassBfree + MassCfree + MassEfree
MassG2 = ABmetl2 + ABmetpu2 + ABmetbr2 + ABmetk2
MassG5 = ACmetl5 + ACmetpu5 + ACmetbr5 + ACmetk5
MassG7 = ADmetl7 + ADmetpu7 + ADmetbr7 + ADmetk7
MassG9 = AEmetl9 + AEmetpu9 + AEmetbr9 + AEmetk9
MassG = MassG2 + MassG5 + MassG7 + MassG9
MassE8 = AEmetl8 + AEmetpu8 + AEmetbr8 + AEmetk8
TotMass = MassA + MassB + MassC + MassD + MassE + MassG + MassE8 + Massfree
init Inh = 0
d/dt(Inh) = Cair*Qalv
```

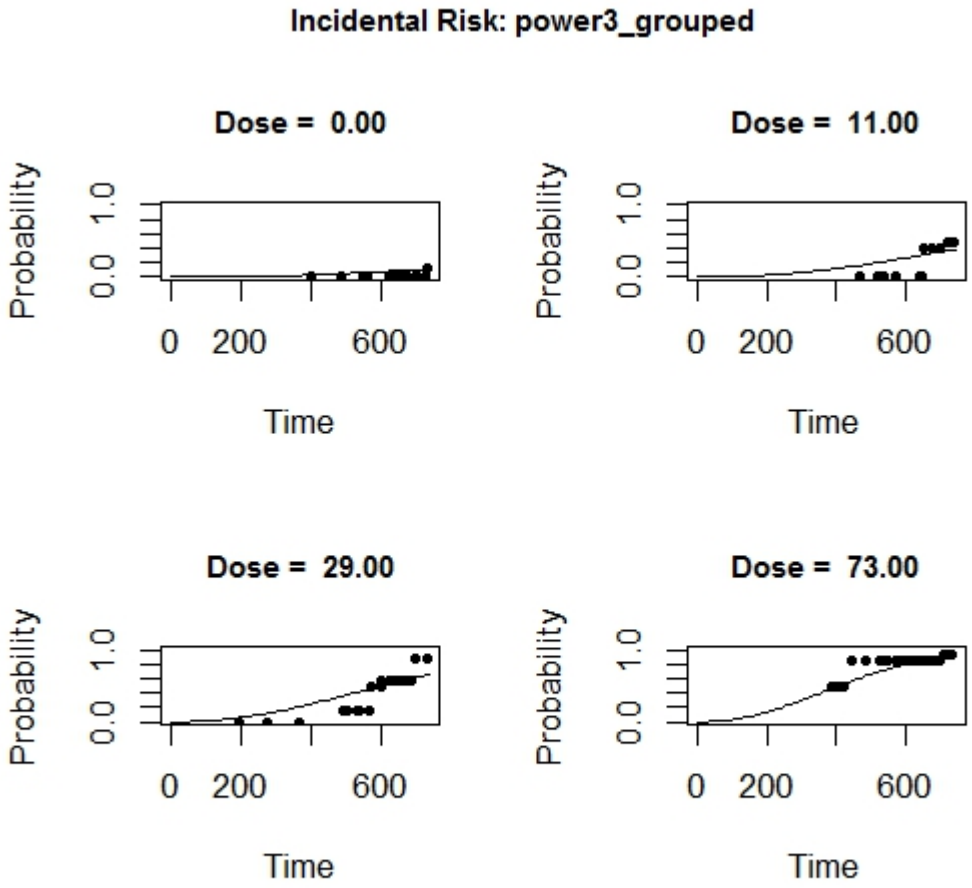
APPENDIX B

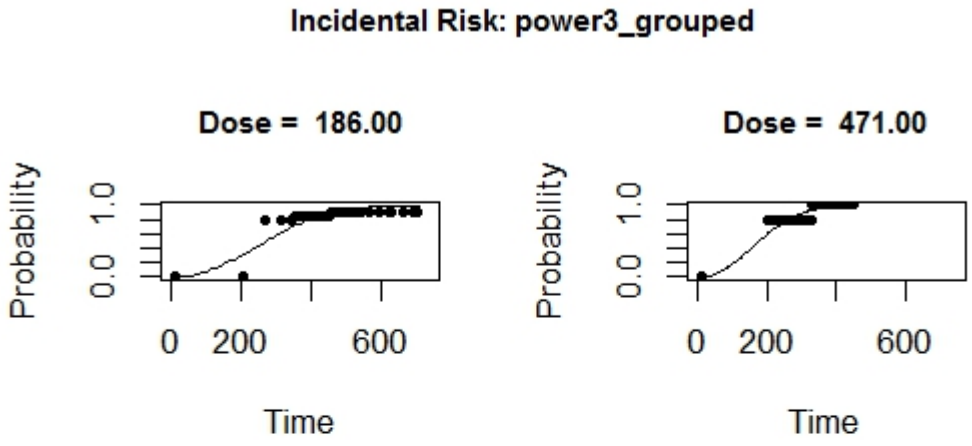
This appendix contains the goodness of fit (gof) plots for the Multistage Weibull model (MSW) fits to the 24 month mouse ovarian atrophy data in NTP (1993). The plots are generated in the statistical program R using slightly modified input files that were used in the main MSW analysis.

Appendix B1. GOFPLOT: DR Plot for Incidental Risk of Ovarian Atrophy in Mice Versus Butadiene Exposure ppm for 24 months. Solid line is Multistage Weibull Model Prediction, Point are Nonparametric Model Estimates. BMD and BMDL are for 95% Lower Bounds on 5% Response Level. Points Closer to Line Indicate Better Model Fit.

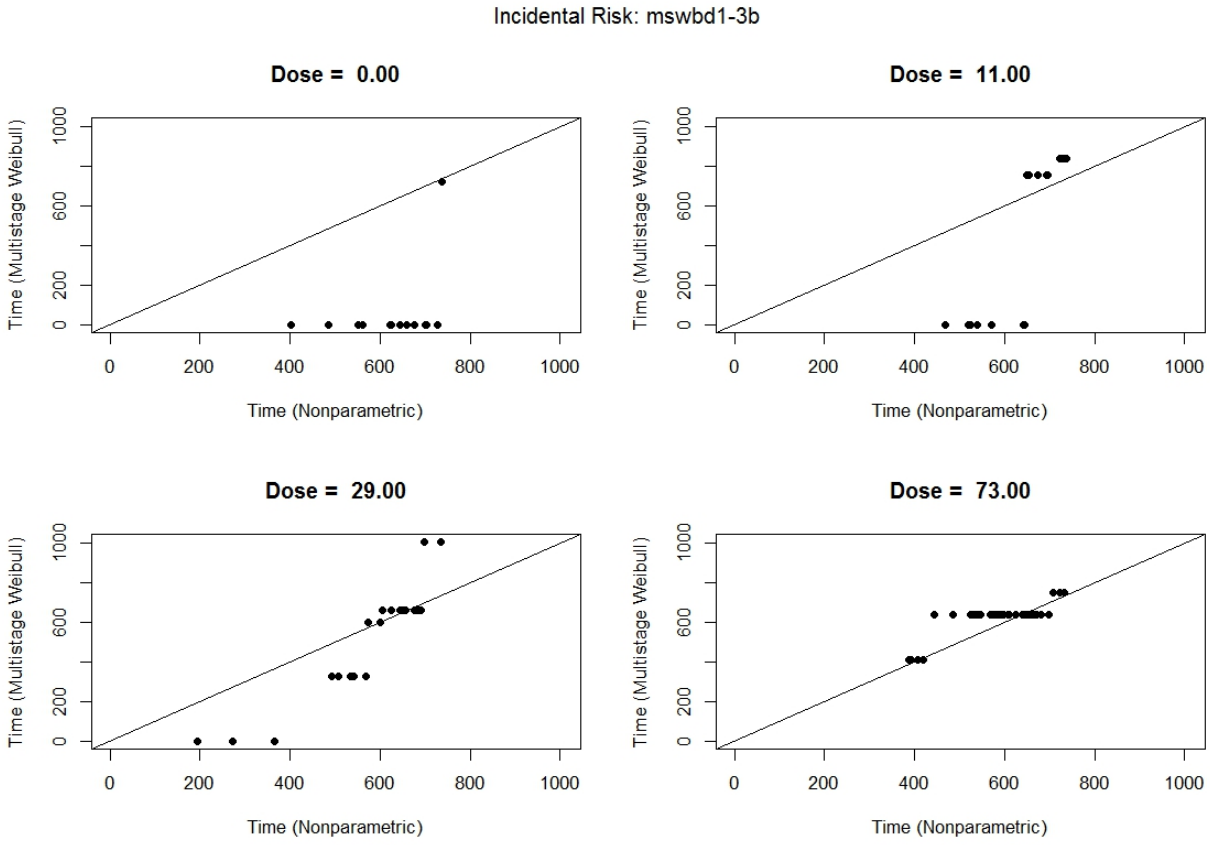

BMD for Incidental Risk at T = 737, Extra Risk level = 0.05, conf. level = 0.9
points show nonparametric estimate for nearest times at obsvd. doses
mswbd1-3b
MSW Model for Incidental Risk
Probability of Incidental Risk at T = 737
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
Dose
734
733
704
450
736
737
BMD 1.56
BMDL 1.28

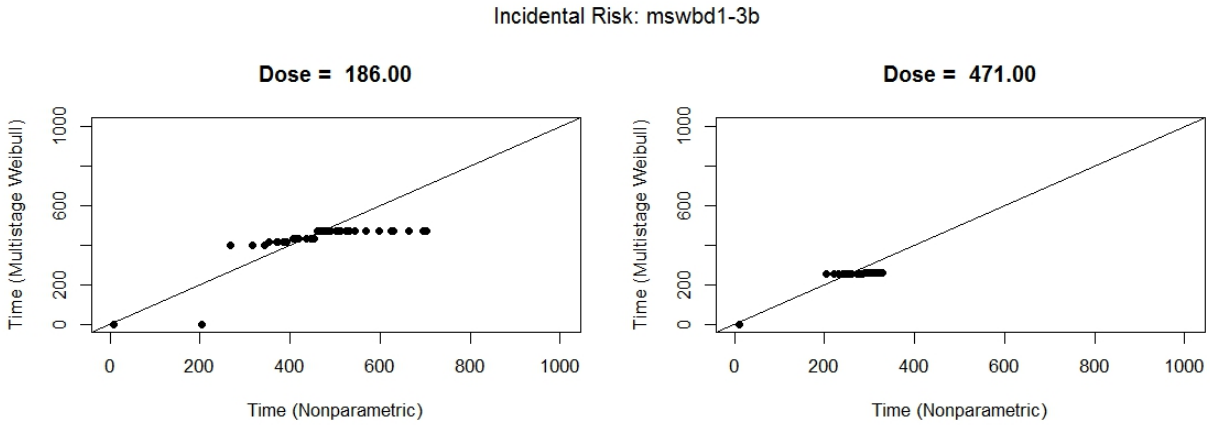
Appendix B2. GOFPLOT: PR Plot. Probability of Ovarian Atrophy in Butadiene Exposed Mice by Multistage Weibull Model (Solid Line) versus Time for Butadiene Internal Doses nM Diepoxybutane in Blood. Points Represent Nonparametric Model Estimates. More Points Closer to the Line Indicates Better Model Fit.




Incidental Risk: power3_grouped
Dose = 186.00
Probability
0.0 1.0
0 200 600
Time
Dose = 471.00
Probability
0.0 1.0
0 200 600
Time

Appendix B3. GOFPLOT: QQ Plot. Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Time-Multistage Weibull Model versus Time-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.




Incidental Risk: mswbd1-3b
Dose = 186.00
Dose = 471.00
Time (Multistage Weibull)
Time (Nonparametric)
0
200
400
600
800
1000

Appendix B4. GOFPLOT: PP Plot. . Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Probability-Multistage Weibull Model versus Probability-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.