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Draft Human Health Hazard Assessment for 1,3-Butadiene

Technical Support Document for the Draft Risk Evaluation

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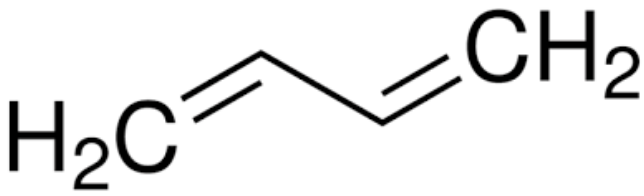

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ABBREVIATIONS AND ACRONYMS

ADAF	Age Dependent Adjustment Factor
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BMD(L)	Benchmark dose (lower 95%tile)
BMR	Benchmark response
CML	Chronic myeloid leukemia
CYP	Cytochrome P450
EB	3,4-Epoxy-1-butene (Epoxybutane)
EBD	3,4-Epoxybutane-1,2-diol
ER	Extra risk
DDEF	Data-derived extrapolation factor
DEB	1,2;3,4-Diepoxybutane
GD	Gestational day
Hb	Hemoglobin
HEC	Human Equivalent Concentration
IARC	International Agency for Research of Cancer
IRIS	Integrated Risk Information System
i.p.	Intraperitoneal
IUR	Inhalation unit risk
LO(A)EL	Lowest-observed-(adverse)-effect level
MOA	Mode of action
MOE	Margin of exposure
NHL	Non-Hodgkin lymphoma
NO(A)EL	No-observed-(adverse)-effect level
OCSPP	Office of Chemical Safety and Pollution Prevention
OEHHA	Office of Environmental Health Assessment (California)
OPPT	Office of Pollution Prevention and Toxics
OQD	Overall Quality Determination
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically-based pharmacokinetic
POD	Point of departure
RD	Relative deviation
RfC	Reference concentration

254	SBR	Styrene-butadiene rubber
255	SD	Standard deviation
256	STEL	Short-term exposure limit
257	SMR	Standardized mortality ratio
258	TCEQ	Texas Commission on Environmental Quality
259	TSCA	Toxic Substances Control Act
260	TWA	Time-weighted average
261	UF	Uncertainty factor
262	VCH	4-Vinyxlcyclohexene
263	VCD	4-Vinylcyclohexene dioxide

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As part of an intra-agency review, the draft 1,3-butadiene risk evaluation and associated technical support documents, including this one, were provided to multiple EPA Program Offices for review. Comments were submitted by Xabier Arzuaga, Catheryne Chiang, and Santhini Ramasamy from ORD and Timothy Anderson from the Office of Air.

Docket

Supporting information can be found in the public docket, Docket ID: EPA-HQ-OPPT-2024-0425.

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This draft technical support document was reviewed and cleared for release by OPPT and OCSPP leadership.

SUMMARY

1,3-butadiene exists as a colorless, volatile gas at room temperature. Based on physical and chemical properties and expected exposure scenarios, EPA quantitatively evaluated hazards via the inhalation route. Oral and dermal exposure is not expected. Inhalation hazards were assessed through systematic review of reasonably available evidence, which includes human epidemiology, animal toxicology, and mechanistic data (including *in vitro* studies). The Agency refined the systematic review approach for 1,3-butadiene by including previous authoritative reviews by federal agencies to better target the assessment. To this end, EPA's Integrated Risk Information System (IRIS) Health Assessment of 1,3-Butadiene (2002a) and the Agency for Toxic Substances and Disease Registry's (ATSDR), *Toxicological Profile for 1,3-Butadiene* (2012) to identify the primary hazards and key studies. Key studies from these assessments were supplemented with both literature that was "filtered" based on whether it was informative for dose-response analysis.

1,3-Butadiene is readily absorbed through the lungs and distributed throughout the body with higher partitioning to adipose tissue. The primary metabolites are reactive mono- or di-epoxides, which can interact with biomolecules and induce toxicity. Qualitatively, metabolic pathways are identical between mice, rats, and humans. However, they are quantitatively different, with mice producing much greater levels of metabolites—especially di-epoxides. 1,3-Butadiene is primarily eliminated through exhalation, with additional excretion via urination, and individual urinary metabolites corresponding to specific epoxy metabolites and/or pathways. These metabolites are considered to be the source of toxicity, so species-specific toxicokinetic differences can influence relative species sensitivity.

EPA began the assessment by focusing on the endpoints and studies considered for deriving hazard values in (U.S. EPA, 2002a) and (ATSDR, 2012). Ovarian atrophy was the basis of the chronic reference concentration (RfC) in (U.S. EPA, 2002a) while (ATSDR, 2012) due to uncertainty in how to accurately extrapolate the mouse data to humans. Following a mode of action analysis, EPA concluded that ovarian atrophy observed in mice is not appropriate for quantitative use in human health risk assessment due to evidence suggesting greatly increased susceptibility in mice and difficulty in confidently quantifying cross-species differences. Instead, the Agency determined that three other critical hazard outcomes were appropriate for dose-response analysis. These non-cancer health outcomes were (1) maternal and related developmental toxicity, (2) male reproductive system and resulting developmental toxicity, and (3) hematological and immune effects.

1,3-Butadiene is a potent multi-organ carcinogen in laboratory animals, notably inducing lymphomas in mice and exhibiting greater carcinogenic potential in mice than rats. Epidemiological evidence consistently links occupational 1,3-butadiene exposure to increased mortality from lymphatic and hematopoietic cancers. EPA determined that 1,3-butadiene "is carcinogenic to humans," based primarily on robust human, animal, and mechanistic evidence for lymphohematopoietic cancers, although varying evidence for other cancer types was also identified. Further, the weight of scientific evidence supports a mutagenic mode of action for carcinogenicity.

A hazard value was *not* derived for acute exposures because it is unlikely any adverse effects will result following a single exposure at concentrations relevant to human exposures. Candidate endpoints for an acute point of departure (POD) from repeat-dose studies were considered but have substantial uncertainties as to whether they are relevant to acute exposures and were also found to be less protective than the intermediate/chronic POD. EPA performed dose-response analysis for multiple repeated-dose non-cancer endpoints under each hazard domain. Decreased fetal weight associated with other developmental toxicity outcomes was selected as the most sensitive and robust human-relevant endpoint

for use in risk characterization of intermediate and chronic exposures. A human equivalent concentration (HEC) of 2.5 ppm (5.5 mg/m^3) was derived from benchmark dose modeling following dichotomization of male mouse fetal weight data. All other candidate PODs were within 2 to 4 times of this value.

EPA used an occupational epidemiological cohort with 50+ years of follow-up and subsequent exposure estimate updates to derive inhalation hazard values for leukemia applicable to general population and occupational exposures. Due to an identified mutagenic mode of action for cancer, the Agency applied an age-dependent adjustment factor (ADAF) to the inhalation unit risk (IUR) for leukemia for the general population; that is, risk scenarios where children or adolescents under 16 years old may be exposed. The IUR for general population risk estimation incorporating the ADAF is 0.0098 per ppm (4.4×10^{-6} per µg/m^3) and the chronic unit risk (UR) for occupational scenarios applied to adolescent and adult workers 16 years or older is 0.0062 per ppm (2.8×10^{-6} per µg/m^3).

EPA has robust overall confidence in the assessments and associated hazard values for maternal/developmental toxicity and leukemia, which will be used for risk estimation. These confidence ratings were based on the weight of scientific evidence considering evidence integration, selection of the critical endpoint and study, relevance to exposure scenarios, dose-response considerations, and incorporation of potentially exposed and susceptible subpopulations (PESS).

1 INTRODUCTION

This technical document presents the draft human health hazard assessment in support of the TSCA *Draft Risk Evaluation for 1,3-Butadiene* (U.S. EPA, 2024g), also referred to as the "draft risk evaluation," conducted under the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended TSCA on June 22, 2016. The law includes statutory requirements and deadlines for actions related to conducting risk evaluations of existing chemicals.

Under TSCA section 6(b), EPA must designate chemical substances as high-priority substances for risk evaluation or low-priority substances for which risk evaluations are not warranted at the time. Upon designating a chemical substance as a high-priority substance, the Agency must initiate a risk evaluation. TSCA section 6(b)(4) directs EPA to conduct risk evaluations for existing chemicals to "determine whether a chemical substance presents an unreasonable risk of injury to health or the environment, without consideration of costs or other nonrisk factors, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified as relevant to the risk evaluation by the Administrator under the conditions of use."

TSCA section 6(b)(4)(D) and implementing regulations require that EPA publish the scope of the risk evaluation to be conducted, including the hazards, exposures, conditions of use (COUs), and PESS that the Administrator expects to consider, within 6 months after the initiation of a risk evaluation. In addition, a draft scope is to be published pursuant to 40 CFR 702.41. In December 2019, EPA published a list of 20 chemical substances that had been designated high priority substances for risk evaluations (Docket ID: EPA-HQ-OPPT-2019-0131) (84 FR 71924, December 30, 2019) as required by TSCA section 6(b)(2)(B), which initiated the risk evaluation process for those chemical substances. 1,3-Butadiene is one of the chemicals designated as a high priority substance for risk evaluation.

Considering the physical and chemical properties, along with anticipated exposure scenarios, EPA only evaluated hazards via inhalation route. This assessment includes EPA's assessment for both non-cancer (Section 4) and cancer (Section 5 outcomes. Section 2 presents EPA's approach and methodology for the human health hazard assessment, including refinement of systematic review processes. The toxicokinetics of 1,3-butadiene are discussed in Section 3. The hazard identification and evidence integration for each organ system are presented in Section 4.1 for non-cancer and Section 5.1 for cancer (with genotoxicity/mutagenicity and MOA analysis presented in Section 5.2). Evidence integration tables are presented in Appendix A. The dose-response analysis is in Sections 4.2.2 and 5.4 for non-cancer and cancer, respectively. The weight of scientific evidence conclusions are in Section 6, and a detailed analysis of PESS along with considerations for aggregate exposure are described in Section 7. Finally, the hazard values to be used for risk estimates are presented in Section 8.

2 APPROACH AND METHODOLOGY

EPA's OPPT utilized systematic review processes to search, screen, evaluate, extract, and integrate reasonably available information to make conclusions about relevant adverse health effects from 1,3-butadiene exposure. Following evidence integration, EPA performed dose-response analysis to derive hazard values for use in risk characterization. The Agency then evaluated the weight of scientific evidence for each aspect of the assessment and determined overall confidence ratings for each critical hazard outcome. The generalized process for conducting human health assessments under TSCA is presented below in Figure 2-1.

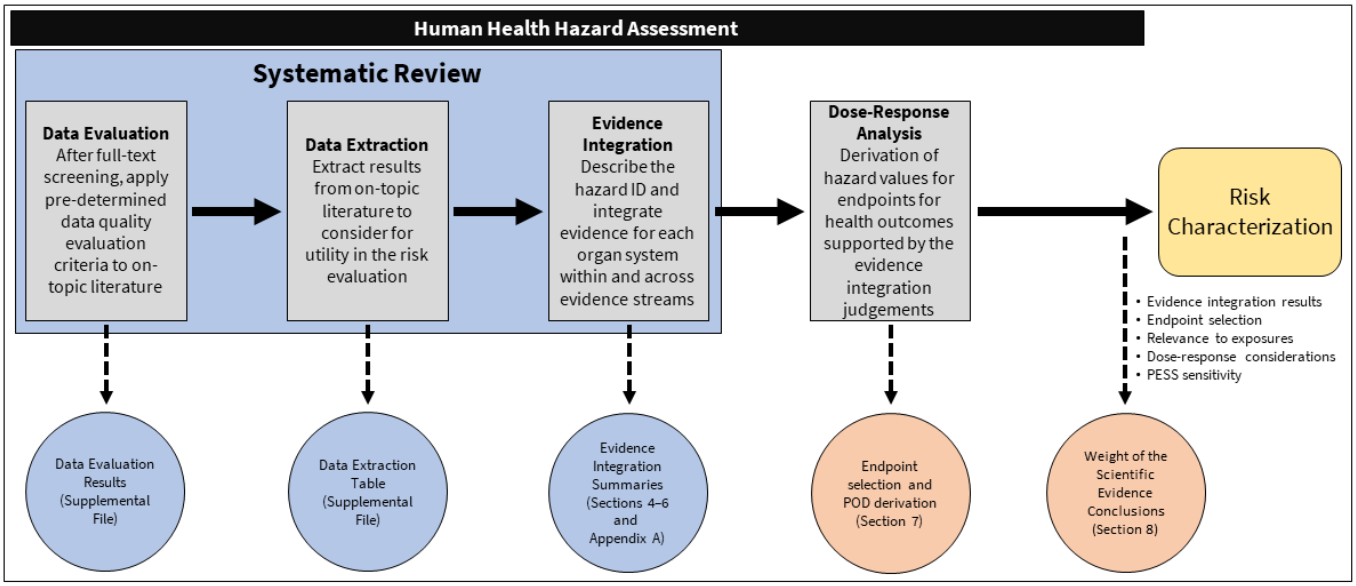

Figure 2-1. EPA Approach to Hazard Identification, Evidence Integration, and Dose-Response Analysis for 1,3-Butadiene

2.1 Systematic Review

The searching and screening steps of the systematic review process for 1,3-butadiene generally followed the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances, Version 1.0: A Generic TSCA Systematic Review Protocol with Chemical-Specific Methodologies* (also called the "Draft Systematic Review Protocol") (U.S. EPA, 2021) covering all reasonably available literature published through September 2019. Full details and screening results for all the identified studies are described in the *Draft Systematic Review Protocol for 1,3-Butadiene* (U.S. EPA, 2024h).

EPA used a refined approach to evaluate human health hazard information relevant to deriving hazard values through a filtering process to target data evaluation/extraction on key studies that may inform dose-response. Results of the filtering process can be found in *Draft Further Filtering Results for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024c). For all other Population Exposure Comparator and Outcome (PECO)-included studies that did not pass the filtering step, basic study-level information was extracted during the filtering process and was used to support evidence integration and weight of evidence analysis. The following steps were taken to filter for laboratory animal studies and epidemiological studies:

- Studies were included if they were considered and/or referenced for hazard value derivation in (U.S. EPA, 2002a) or (ATSDR, 2012); and

- Additional open literature studies and studies submitted to EPA not previously identified by ORD IRIS or ATSDR were also included if they contained at least two exposure groups plus a control.

For studies that went through data evaluation and extraction, formal extraction results can be found in *Draft Data Extraction Information for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024b)).

The Agency performed an initial investigation of the hazard identification, critical endpoints, and key scientific issues associated with 1,3-butadiene by reviewing previous assessments. The EPA IRIS Health Assessment of 1,3-Butadiene (2002a) and ATSDR *Toxicological Profile for 1,3-Butadiene* (2012) were the key federal government sources for this review. EPA also consulted U.S. state assessments—namely by the Texas Commission on Environmental Quality (TCEQ) (Grant et al., 2010) and California Office of Environmental Health Assessment (OEHHA, 2013). Additionally, EPA identified studies and analyses on metabolism and mechanisms/MOAs relevant to the human health risk assessment as well as other key studies and dose-response analyses provided by stakeholders or identified by EPA that were published after the original September 2019 literature cutoff date.

As part of the draft human health risk assessment, EPA incorporated all reasonably available information into the hazard identification, hazard characterization, evidence integration, and weight of evidence analyses.

2.2 Problem Formulation and Focus of Analysis

As mentioned in Section 2.1 above, the Agency used the EPA IRIS Health Assessment of 1,3-Butadiene (2002a) and ATSDR Toxicological Profile for 1,3-Butadiene (2012) as starting points to inform this draft human health hazard assessment. Through the systematic review process, EPA did not identify any additional laboratory animal studies examining non-cancer health effects published since the ATSDR assessment (2012) that would be considered for dose-response analysis. However, EPA did identify new 1,3-butadiene studies relevant for evaluation of MOA toxicokinetic differences across species. Recent non-cancer epidemiological studies were incorporated into the evidence integration for their respective hazard domains. The Agency began the assessment by focusing on the endpoints and studies considered for deriving hazard values in those assessments.

Ovarian atrophy was the basis of the chronic reference concentration (RfC) in the 2002 EPA IRIS Assessment (U.S. EPA, 2002a). Ovarian atrophy was also cited as the critical chronic endpoint in assessments by TCEQ (Grant et al., 2010) and California's (OEHHA) (OEHHA, 2013). In contrast, ATSDR in 2012 (ATSDR, 2012) did not derive a chronic-duration inhalation minimal risk level (MRL).

Fetal body weight was cited as the primary or co-critical acute endpoint by ORD IRIS, TCEQ, and OEHHA. ATSDR also did not derive an acute MRL.

Each of these four existing assessments acknowledge uncertainty in species extrapolation. For example, ATSDR cited "large species differences in the metabolism of 1,3-butadiene and the lack of chemical-specific data to adjust for these differences" in the decision to not derive any quantitative summary values for the assessment. Therefore, EPA performed a detailed examination of 1,3-butadiene toxicokinetics, mechanisms/MOAs, and quantitative consideration of species differences. The Agency also updated PODs and uncertainty factors in accordance with OPPT procedures and EPA guidance (*e.g.*, BMD modeling guidance (U.S. EPA, 2012b)) published since the original assessments.

With respect to cancer assessment, an IUR for leukemia was derived in (U.S. EPA, 2002a) based on an occupational cohort of male workers. Multiple updates to this epidemiological cohort have been published since 2002 that added additional follow-up years, female workers, and refined exposure analyses to the data set. EPA therefore focused the updated cancer assessment on the evaluation, evidence integration, and weight of scientific evidence of these newer studies. Based on this updated information, the Agency updated IURs for leukemia relevant to general population and occupational exposures. EPA also developed a mutagenic mode of action analysis for 1,3-butadiene. As with non-cancer PODs, the Agency also considered updates to EPA guidance (*e.g., Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b)) for quantitative analysis.

Based on the physical and chemical properties of 1,3-butadiene (see *Draft Physical Chemistry, Fate, and Transport Assessment for 1,3-Butadiene* (U.S. EPA, 2024f)) and expected exposure scenarios (see *Draft Occupational Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024e) and *Draft General Population Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024d)), EPA only evaluated hazards via the inhalation route. The most appropriate studies and specific endpoints for hazard value derivation relevant to intermediate, chronic, and/or lifetime exposure durations were then selected and points of departure (PODs)/inhalation unit risks (IURs) were derived (cancer values were specific either to the general population or workers).

3 TOXICOKINETICS

This section describes the absorption, distribution, metabolism, and elimination (ADME) data available for 1,3-butadiene. Because the primary route of exposure to 1,3-butadiene is via inhalation, and there are no data on ADME via oral and dermal routes, this section focuses on factors affecting its ADME through inhalation.

3.1 Absorption

As a highly volatile gas at room temperature, 1,3-butadiene is primarily absorbed through inhalation, where it readily diffuses from the lungs into the bloodstream. In both humans and animals, inhalation is recognized as the predominant exposure route (ATSDR, 2012; U.S. EPA, 2002a). The blood:air partition coefficient, which measures the propensity of a chemical to partition into blood from the lungs, provides insight into absorption efficiency. In rodents, the blood:air partition coefficient was determined to be 1.95 (Kohn and Melnick, 2001). The coefficient in humans has been measured as 1.22 (±0.30) (Brochot et al., 2007). Inhalation of 2 ppm 1,3-butadiene for 20 minutes resulted in an absorbed fraction ranging from 18 to 74 percent, with variations observed among ethnicities and potentially influenced by blood triglycerides levels (Lin et al., 2002; Lin et al., 2001). In *Macaca fascicularis* monkeys, uptake was calculated as 16.40 µmol/hour/10 ppm of inhaled and 3.20 µmol/hour/10 ppm of retained 1,3-butadiene (Dahl et al., 1990). Rodent studies have also demonstrated that absorption rates vary depending on the exposure level. In one study, rats and mice exposed to 20 ppm 14C-1,3-butadiene for 6 hours showed relatively low absorption, with only 2.2 and 1.6 percent of the total radioactivity absorbed, respectively (Swain et al., 2003). In addition, uptake in mice was linear up to 2,000 ppm, and in rats up to 1,000 ppm, indicating that metabolic saturation occurs at higher concentrations (Kohn and Melnick, 2001). Beyond rodents, studies in rabbits revealed rapid pulmonary absorption, with distribution coefficients between blood and air of 0.603 *in vitro* and 0.654 *in vivo* (Carpenter et al., 1944).

Absorption has also been confirmed by studies measuring various 1,3-butadiene metabolites. For example, 3,4-epoxy-1-butene or epoxybutane (EB), was detected in exhaled air and blood after exposure to 1 to 10,000 ppm in rats and 1 to 6,000 ppm in mice (Filser et al., 2007). In rats, EB levels in the test chamber plateaued at all exposure concentrations, while in mice, EB levels plateaued only up to 1,000 ppm, indicating potential species differences in metabolism (see Section 3.3). Additional studies detected metabolites in the blood and tissues of rats and mice exposed to 62.5 ppm of 1,3-butadiene, further confirming absorption (Thornton-Manning et al., 1995). Furthermore, variations in study protocols and limited data across exposure levels create some uncertainty in directly comparing absorption rates across species. Based on the limited available information quantifying absorption across varying exposure levels and longer durations, EPA assumes 100 percent absorption through the lungs in this risk evaluation.

3.2 Distribution

The distribution of 1,3-butadiene following inhalation exposure has been studied in various species. Due to its lipophilic nature, 1,3-butadiene is absorbed through the lungs into the bloodstream and rapidly distributed throughout the body, with notable accumulation in adipose tissue (ATSDR, 2012; U.S. EPA, 2002a). Consistent with this, PBPK studies show that adipose tissue exhibits the highest partition coefficient in humans, while well- and poorly perfused tissues show similar coefficients (0.69 and 0.72, respectively) (Brochot et al., 2007). Similarly, PBPK studies in rats demonstrate the highest partition coefficient from blood in adipose (21.9), followed by a decreasing trend in liver, kidney, muscle, and spleen (0.87–0.94), and the lowest in the brain (0.43) (Johanson and Filser, 1993). *In vivo* studies confirm these observations. Specifically, mice and rats exposed to up to 625 ppm of 1,3-butadiene

attained equilibrium within 2 hours, with mice showing three- to four-fold increased blood concentrations of 1,3-butadiene compared to rats at all times—potentially due to interspecies differences in metabolism rates and respiratory physiology (Himmelstein et al., 1994). Additionally, species-specific differences in the distribution of inhaled 1,3-butadiene were also observed. Studies comparing Sprague-Dawley rats and B6C3F1 mice found significantly higher molar tissue concentrations of ^{14}C-1,3-butadiene in mice, with up to 80-fold higher levels in the lung and 17-fold higher levels in the thyroid. Blood concentrations were also considerably higher in mice (57-fold) compared to rats, and intestinal radioactivity was 110- to 120-fold higher in mice (Bond et al., 1987; Bond et al., 1986). In human volunteers exposed to low levels (2 ppm) of 1,3-butadiene through inhalation, blood concentrations reached equilibrium within 5 minutes, demonstrating rapid absorption and distribution in humans (Smith et al., 2001).

3.3 Metabolism (Including Species Differences)

1,3-Butadiene undergoes a complex metabolic process involving oxidation, hydrolysis, and conjugation reactions, ultimately generating reactive epoxides with varying toxicological effects (ATSDR, 2012; U.S. EPA, 2002a). Initially, 1,3-butadiene is oxidized to 3,4-epoxy-1-butene (EB), primarily mediated by the cytochrome P450 (CYP) isozyme CYP2E1 (Figure 3-1). EB then undergoes further oxidation to produce 1,2,3,4-diepoxybutane (DEB). Concurrently, 1,3-butadiene is detoxified through conjugation with glutathione, facilitated by glutathione S-transferase (GST), and hydrolysis mediated by epoxide hydrolase (EH), resulting in 1,2-dihydroxy-3-butene (B-diol). These metabolites undergo further transformations. Specifically, DEB is hydrolyzed by epoxide hydrolase (EH) to 1,2-dihydroxy-3,4-epoxybutane (EBD), while B-diol is metabolized by alcohol dehydrogenase (ADH) and CYP2E1 to form hydroxymethylvinylketone (HMVK) (ATSDR, 2012; U.S. EPA, 2002a). Although the metabolic pathways of 1,3-butadiene are similar across species, including in humans, significant quantitative differences exist in how these metabolites are formed and detoxified (Kirman et al., 2010b).

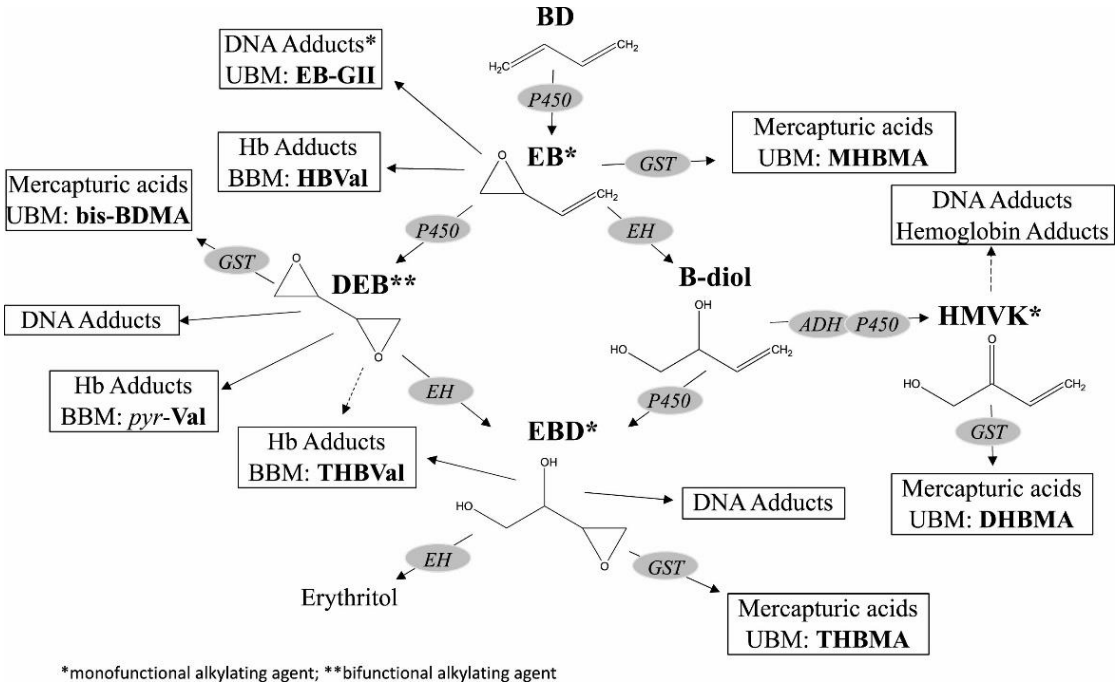

Figure 3-1. Schematic of 1,3-Butadiene Metabolism

ADH = alcohol dehydrogenase; B-diol = butenediol; bis BDMA = bis-butanediol-mercapturic acid; DEB = diepoxybutane; EB = epoxybutane; EBD = epoxybutane diol; EH = epoxide hydrolase; GSH = glutathione; GST = glutathione-S transferase; HBVal = N-(2-hydroxy-3 butenyl)valine; HMVK = hydroxymethylvinyl ketone; DHBMA = 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane; MHBMA = 1-(N-acetylcysteinyl)-2-hydroxy-3-butene; P450 = cytochrome P450; pyrVal = N,N-(2,3-dihydroxy-1,4-butadiyl)-valine; THBMA = 1,2,3,-trihydroxy-4-(N-acetylcysteinyl)-butane; THBVal = N (2,3,4 trihydroxybutyl)-valine. (Source: Kirman et al (**2022**), obtained under a Creative Commons license.)

In vitro studies demonstrate the significance of these quantitative differences, revealing variations in metabolic rates across species (ATSDR, 2012; U.S. EPA, 2002a). Studies using microsomal and cytosolic preparations from the livers of mice, rats, and humans indicated that mice exhibit the highest rate of 1,3-butadiene oxidation to EB, with a Vmax of 2.6 nmol/mg protein/minute, compared to 1.2 in humans and 0.6 in rats (Csanady et al., 1992). Notably, only mouse liver microsomes demonstrated a quantifiable rate of EB metabolism to DEB. In contrast, human liver microsomes showed the highest rate of EB hydrolysis to B-diol via epoxide hydrolase, with Vmax values at least twice those measured for rats and mice (Csanady et al., 1992). Additionally, the rate of EB glutathione conjugation was found to be highest in mouse cytosol. The overall activation/ detoxification ratios determined from these experiments were significantly higher in mice (72) compared to rats (5.8) and humans (5.9), underscoring the interspecies differences in 1,3-butadiene oxidation to DEB (Csanady et al., 1992). Further studies using liver homogenates from various species revealed that mice formed the most EB from 1,3-butadiene compared to rats, humans, and rhesus monkeys (Schmidt and Loeser, 1986). Additionally, epoxide hydrolase activity was higher in humans and monkeys compared to both rodent species (Schmidt and Loeser, 1986). Human cytochrome P450 (CYP) 2E1, expressed from complementary DNA, was efficient in metabolizing EB to DEB (Krause et al., 1997), though with considerable variability (~60-fold) across human samples. In studies using rodent microsomes and c-DNA CYP isozymes, the enzyme efficiency (defined as Vmax/Km) for metabolism of EB to DEB was 15 in mice, 11 in rats, and 8 in humans (Kreuzer et al., 1991). Motwani et al. (2014) also observed higher enzymatic efficiency *in vitro* for the oxidative steps of EB to DEB and B-diol to EBD in mice

compared to rats or humans. Furthermore, they noted that the hydrolysis of DEB to EBD was substantially faster than the oxidation of B-diol for all species, being 2.5-, 11-, and 25-fold faster than formation of EBD from B-diol in mice, rats, and humans, respectively. More recently, *in vitro* studies have identified additional bifunctional metabolites of 1,3-butadiene. These include a chlorinated metabolite formed via myeloperoxidase and hypochlorous acid as well as ketone/aldehyde metabolites of EBD formed via alcohol dehydrogenase (Nakamura et al., 2021; Wu et al., 2019; Wang et al., 2018; Elfarra and Zhang, 2012). Importantly, a recent study (Nakamura et al., 2021) demonstrated that EBD damages DNA and exhibits toxicity to cells lacking Fanconi anemia (FANC) genes. This cytotoxic effect, similar to the more potent DEB, suggests that EBD likely forms bifunctional DNA interstrand crosslinks upon metabolic activation by ADH, thus contributing to its potential role in leukemia and lymphoma development. Collectively, *in vitro* studies reveal that mice exhibit greater metabolic efficiency in oxidizing 1,3-butadiene to EB and from EB to DEB, compared to both rats and humans.

These *in vitro* findings further support observations in *ex vivo* and *in vivo* studies in mice and rats that consistently demonstrate greater metabolic efficiency in mice than in rats. For instance, isolated perfused liver studies showed that following 1,3-butadiene exposure, mouse livers produced EB, DEB, EBD, and B-diol, while rat livers primarily produced EB and B-diol (Filser et al., 2010; Filser et al., 2001). The lower levels of DEB in rats compared to mice suggest species-specific differences in the formation of DEB. Several animal studies have confirmed this trend, with DEB detected in the blood of exposed mice but not rats (Filser et al., 2007; Himmelstein et al., 1994). Similarly, hemoglobin adducts related to DEB exposure (pyr-Val) were substantially higher in mice than rats at equivalent 1,3-butadiene exposure concentration (Swenberg et al., 2011; Georgieva et al., 2010). In contrast, primates appear to metabolize 1,3-butadiene more similarly to rats than mice (Henderson et al., 2001; Henderson et al., 1996; Dahl et al., 1991). Dal et al (Dahl et al., 1991) demonstrated that total 1,3-butadiene metabolites in the blood of monkeys were 5 to 50 times lower than in mice and 4 to 14 times lower compared to rats. Furthermore, primates demonstrate significantly higher epoxide hydrolase activity compared to rodents. This increased enzyme activity results in the rapid conversion of EB to B-diol. This point is evident from the higher levels of the B-diol-derived metabolite M-I in primate urine (Sabourin et al., 1992).

Findings from animal models, supported by studies of hemoglobin (Hb) adducts in workers exposed to 1,3-butadiene, provide valuable evidence for human 1,3-butadiene metabolism. Hemoglobin (Hb) adducts were identified in workers occupationally exposed to 1,3-butadiene at monomer production and polymerization facilities in the Czech Republic using liquid chromatography-mass spectrometry (LC-MS) (Boysen et al., 2022; Boysen et al., 2012; Vacek et al., 2010; Albertini et al., 2007; Albertini et al., 2003). Exposure concentrations were measured on 10 occasions over a 2- to 4-month period using personal monitoring devices worn for an 8-hour work shift. Boysen et al. (2012) specifically measured the concentrations of certain Hb adducts (HB-Val, pyr-Val, and THB-Val) in male workers including administrative controls, monomer workers, and polymerization workers. Interestingly, Hb adducts were also detected in control workers, likely due to background exposure to acrolein from cigarette smoke or vehicle exhaust (ATSDR, 2012; Albertini et al., 2003). The amount of the DEB adducts (pyr-Val) increased with higher 1,3-butadiene exposure levels in polymerization workers compared to controls and monomer workers. Also, pyr-Val adduct levels exhibited high variability in male workers, and no clear exposure-response relationship was observed (Boysen et al., 2012). Notably, THB-Val, linked to EBD exposure, was the dominant adduct in all worker groups (>99%), highlighting EBD as a primary 1,3-butadiene metabolite in humans. While THB-Val may also form as a direct adduct of DEB, metabolism data suggest that DEB hydrolysis to yield EBD occurs rapidly in humans via epoxide hydrolase (Motwani and Törnqvist, 2014). This extensive EB hydrolysis is further supported by the finding that in humans, greater than 97 percent of urinary mercapturic acid formed after 1,3-butadiene inhalation is

DHBMA, indicating that most EB is hydrolyzed via EH rather than forming the diepoxide (Henderson et al., 1996). The low levels of DEB-specific hemoglobin adduct observed in exposed workers and the significantly higher levels of EBD-specific hemoglobin adducts, suggest high epoxide hydrolase activity in humans.

Overall, studies across various models, including *in vitro*, *ex vivo*, animal and human demonstrate significant interspecies differences in 1,3-butadiene metabolism. Although the metabolic pathways of 1,3-butadiene are similar across species, there are significant quantitative difference in the formation and detoxification of these metabolites. Mice exhibit a greater capacity for oxidizing 1,3-butadiene to its more reactive epoxide forms, EB and DEB, compared to rats and humans. DEB has been identified as the primary metabolite in mice, whereas EBD is the predominant metabolite observed in humans. Rats, by contrast, metabolize 1,3-butadiene at lower overall levels. Furthermore, recent studies have identified novel 1,3-butadiene metabolites, such as chlorinated and ketone/aldehyde bifunctional metabolites, but whose role in species-specific differences and potential health effects remains to be evaluated. Despite the unique effect of DEB on ovarian atrophy in mice, there is currently insufficient evidence to attribute specific health outcomes directly to any individual 1,3-butadiene metabolites.

3.4 Elimination

The main route of elimination of 1,3-butadiene and its metabolites in rodents and primates is through exhalation and urinary excretion, with minor biliary excretion also observed (ATSDR, 2012; U.S. EPA, 2002a). Studies in rats, mice, and monkeys have quantified the routes and amounts of excretion following inhalation. In rodents, exhalation and urinary excretion are the primary routes of elimination, with similar elimination patterns observed across different exposure concentrations and species (Bond et al., 1986). Rapid elimination of radioactivity was observed in mice and rats following exposure to ^{14}C-1,3-butadiene, with 77 to 99 percent of the initial tissue amount cleared within 2 to 10 hours (Bond et al., 1987). Elimination kinetics in both blood and tissues were biphasic, exhibiting rapid initial clearance followed by a slower elimination phase (Bond et al., 1986). In addition, exhalation of radiolabeled carbon was a major pathway for the elimination of ^{14}C-1,3-butadiene in mice and rats, particularly at higher concentrations (Bond et al., 1986). This result was further corroborated by a substantial decrease in blood 1,3-butadiene concentration within minutes after a 6-hour inhalation exposure, although the rate of decline was slower in rats compared to mice (Himmelstein et al., 1996). Similar to rodents, non-human primates also exhibit efficient elimination of 1,3-butadiene. Studies in Cynomolgus monkeys showed that approximately 2 percent of inhaled 1,3-butadiene was excreted as metabolites, regardless of exposure concentration. The composition of these metabolites exhibited dose-dependent variation, with carbon dioxide being predominantly exhaled at lower concentration and epoxy metabolites become more prominent at higher exposure levels (Sun et al., 1989). In a separate study with *Macaca fascicularis* monkeys exposed to low concentration (10 ppm) of 1,3-butadiene, 39 percent of the total metabolite radioactivity was eliminated in urine, 0.8 percent in feces, and 56 percent as exhaled carbon dioxide within 70 hours post-exposure (Dahl et al., 1990). In humans, the primary urinary metabolite of 1,3-butadiene is dihydroxybutenylmercapturic acid (DHBMA), accounting for over 97 percent of the excreted mercapturic acids (ATSDR, 2012; Henderson et al., 1996). A minor pathway involving glutathione-S-transferase (GST) conjugation also contributes to the formation of monohydroxybutenylmercapturic acid (MHBMA).

The relative abundance of MHBMA and DHBMA in urine has been established as a biomarker of exposure in both environmental and occupational monitoring of 1,3-butadiene (ATSDR, 2012). Studies have revealed sex-based differences in 1,3-butadiene metabolism, with women excreting lower levels of both DHBMA and MHBMA compared to men following 1,3-butadiene exposure (Albertini et al., 2007).

However, the ratio of DHBMA to MHBMA remains consistent between the sexes, suggesting a difference in metabolic activity rather than a shift in the metabolic pathway.

3.5 PBPK Modeling Approach

Although several PBPK models have been developed to simulate the 1,3-butadiene kinetics in mice, rats, and humans, the models are limited in their ability to predict internal doses of all the key metabolites (ATSDR, 2012; U.S. EPA, 2002a). Major uncertainties persist—including the incomplete understating of alternative oxidation pathways, the validity of *in vitro* metabolic data, the omission of intrahepatic first-pass metabolism, and poorly characterized factors such as ventilation rates, stereoselective metabolism, and the kinetics of key metabolites, including newly identified chlorinated and ketone/aldehyde bifunctional metabolites. Consequently, these significant uncertainties in the existing PBPK models prevent their use in human risk assessment at this time.

4 NON-CANCER HAZARD ASSESSMENT

4.1 Hazard Identification

The sections below describe adverse outcome and mechanistic data available, evidence integration, and weight of scientific evidence conclusions for relevant human health hazard outcomes for 1,3-butadiene. Full details on all evaluated health outcomes from all key studies are in *Draft Data Extraction Information for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024b). Additional hazard information supporting evidence integration is presented in *Draft Further Filtering Results for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024c).

For complete details on evidence integration judgements within and across evidence streams, see the evidence profile tables for data-rich organ systems in Appendix A. Evidence integration judgements were determined based on considerations described in Chapter 7 of the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021). In short, strength of the evidence judgements (robust, moderate, slight, indeterminate, or compelling evidence of no effect) for individual evidence streams (*i.e.*, human, animal, mechanistic) were determined by expert judgement based on quality of the database, consistency, magnitude and precision, dose-response, and biological significance. These were then integrated into an overall summary classification (see Appendix A for overall judgement classifications).

As described in Section 2, EPA used previous governmental assessments as the starting point for focusing hazard identification efforts. EPA used results from systematic review, existing analyses, and additional metabolite/analog studies to independently evaluate the weight of scientific evidence for each hazard outcome. Hazard outcomes with sufficient confidence and quantitative study data then underwent dose-response analysis (Section 4.2).

This section begins with an evaluation of ovarian atrophy in Section 4.1.1 as this was the critical chronic endpoint in the prior assessments by EPA IRIS (2002a) TCEQ (Grant et al., 2010) and California OEHHA (OEHHA, 2013). EPA has proposed a mode of action for ovarian atrophy in accordance with the IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans (Boobis et al., 2008). The Agency then performed evidence integration and considers the weight of scientific evidence based on human, animal, and mechanistic data for other critical hazard outcomes described in Section 4.1.2. Other hazard outcomes with more limited evidence are in Appendix D. As discussed in Section 2.2, toxicokinetic species differences—especially relative rates of metabolism and the significance of individual metabolites—were an important consideration in the evaluation of all hazard outcomes.

4.1.1 Ovarian Atrophy and Associated Female Reproductive System Toxicity

As mentioned in Section 2.2, ovarian atrophy based on observations in mice was the basis of the chronic RfC and the most sensitive endpoint in the 2002 EPA IRIS Assessment (U.S. EPA, 2002a). It was also cited as the critical chronic endpoint in assessments by TCEQ (2010) and California OEHHA (2013). There has been extensive scientific discussion concerning the relevance of mouse data for this endpoint to humans in both these assessments and other publications. The sections below outline the reasonably available evidence, proposed MOA, and overall conclusions for the endpoint.

4.1.1.1 Human Evidence

EPA did not identify any human studies that examined the female reproductive system or any measurements related to ovarian atrophy.

4.1.1.2 Laboratory Animal Evidence

Toxicity of 1,3-butadiene to female reproductive organs, especially the ovaries, has been examined in multiple studies covering a wide range of durations and doses. No histopathological changes were observed in a 5-day study where mice were exposed to 1,3-butadiene at 0, 625, 1,250, 2,500, 5,000 or 8,000 ppm (NTP, 1984). However, this examination was rated as uninformative due to reliability questions about the associated contract laboratory and the result is not given any consideration in the weight of scientific evidence. Ovarian atrophy was observed in mice following 13 weeks of exposure to 1,3-butadiene at 980 ppm (no other doses tested) for 5 hours/day, 5 days/week (Bevan et al., 1996), and in multiple studies following 40 weeks to 2 years of exposure (NTP, 1993, 1984; Battelle PNL, 1982) Ovarian atrophy was observed at all doses in a 2-year mouse study with concentrations of 6.21, 19.8, 61.4, 199, and 619 ppm (NTP, 1993). Thirty-nine percent of mice demonstrated atrophy at the lowest concentration following 2 years of exposure, while statistically significant increases to 90 percent of mice were observed at 62.5 ppm for 15 months and 200 ppm for 9 months of exposure, respectively. Ovarian toxicity was accompanied in these studies by an absence of oocytes, follicles, and corpora lutea, along with angiectasis and uterine involution.

In contrast to mice, no signs of ovarian atrophy or other toxicity to female reproductive organs were observed in a chronic rat study following up to 2 years of 1,000 or 8,000 ppm exposure (Hazleton Labs, 1981b).

4.1.1.3 Mechanistic Evidence and Mode of Action Analysis

Given the extensive discussion about the human relevance in previous authoritative assessments, EPA evaluated the MOA for ovarian toxicity and how it may inform the human relevance of the mouse-specific observations. Kirman et al (2012) suggested an MOA for ovarian toxicity to support a proposed data-derived uncertainty factor for extrapolation of the mouse data to humans. EPA below proposes a modified MOA from that of Kirman et al. based on consideration of all reasonably available mechanistic and toxicokinetic evidence.

The proposed key events are

- KE1: Bioactivation of 1,3-butadiene to DEB and other epoxide metabolites.
- KE2: Distribution of metabolites into ovarian tissue.
- KE3: Destruction of ovarian follicles via apoptosis.
- KE4: Premature ovarian failure and atrophy.

Figure 4-1 presents the proposed MOA for ovarian toxicity, including a summary of available evidence for each step and underlying mechanisms.

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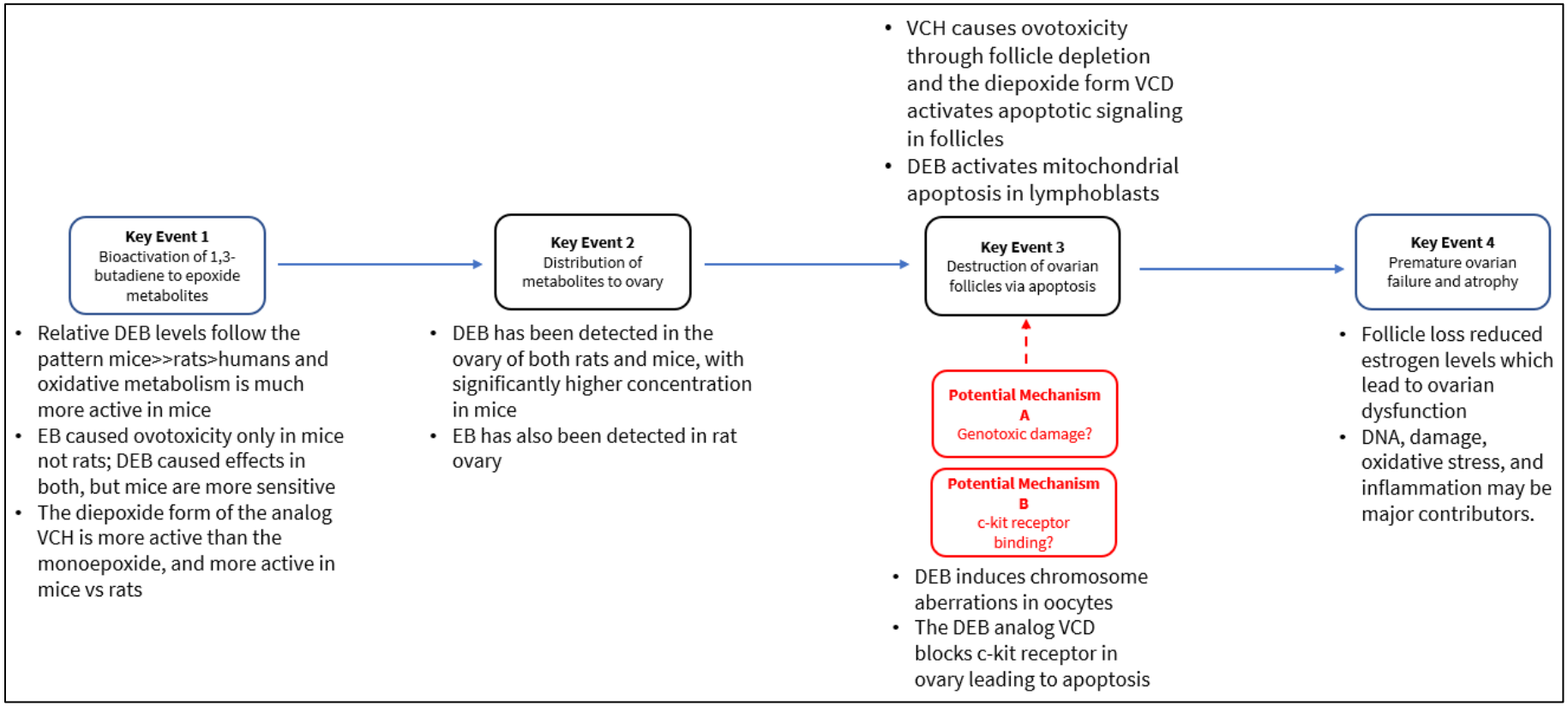

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803 **Figure 4-1. Proposed MOA for Ovarian Toxicity and Associated Mechanisms**
804 Red boxes represent potential mechanisms for metabolite-induced destruction of ovarian follicles.

4.1.1.3.1 Key Event 1: Bioactivation of 1,3-Butadiene to DEB and Other Epoxide Metabolites

The metabolism of 1,3-butadiene is described in detail in Section 3.3. In short, 1,3-butadiene is oxidized to the mono-epoxide EB which is then either detoxified into the alcohol B-diol or further oxidized to the di-epoxide DEB. B-diol can then be subsequently oxidized to EBD. Oxidative metabolism is much more active in mice compared to rats or humans (Motwani and Törnqvist, 2014) and mice appear to have greater metabolic efficiency overall as perfused mouse livers produce all the aforementioned metabolites while rats primarily produce only EB and B-diol (Filser et al., 2010; Filser et al., 2001). Consistent with this data, blood DEB levels are estimated to be 40 to 100 times higher in mice compared to rats and 100 to 300 times or more higher in mice compared to humans (Motwani and Törnqvist, 2014; ATSDR, 2012; Csanády et al., 2011; Swenberg et al., 2011; Georgieva et al., 2010) for the same administered dose of 1,3-butadiene.

A key study for understanding the impact of different metabolites and species sensitivity is Doerr et al., (1996), which exposed mice and rats intraperitoneally for 30 days to either the mono-epoxide EB or di-epoxide DEB. While the EB induced ovotoxicity in mice (but not rats), the DEB caused effects in both (in contrast to parental 1,3-butadiene), with a much stronger effect in mice. A similar response is seen with the 1,3-butadiene analog 4-vinylcyclohexene (VCH). Similar to 1,3-butadiene, vinylcyclohexene can be metabolized into either a mono- or di-epoxide form. As with 1,3-butadiene, VCH induces ovarian atrophy only in mice but not rats. In a study mirroring the design of Doerr et al., the di-epoxide form (4-vinylcyclohexene dioxide, VCD) was 2 to 3 times more potent at inducing follicle loss than the mono-epoxide form, and both epoxides were 2 to 3 times more active in mice compared to rats when directly administered (Hoyer and Sipes, 2007). The results from these two studies demonstrate that mice are not only toxicokinetically more sensitive from producing more epoxide metabolites, but they are also more toxicodynamically sensitive than rats to the same metabolite exposure.

4.1.1.3.2 Key Event 2: Distribution of Metabolites into Ovarian Tissue

Distribution of the toxic metabolites to the ovary is assumed based on the observed ovarian toxicity. DEB has been measured in the ovary of both mice and rats, with over an order of magnitude higher concentration observed in mice (Thornton-Manning et al., 1997). Both EB and DEB have been detected in the ovary of rats, although DEB concentrations range from 200 to 400 times more than EB (Thornton-Manning et al., 1998).

4.1.1.3.3 Key Event 3: Destruction of Ovarian Follicles via Apoptosis

Follicle depletion appears to be a key event in ovarian atrophy resulting from exposure to the 1,3-butadiene analog VCH and DEB analog VCD, which destroys primary and primordial follicles (Hoyer and Sipes, 2007). However, the mechanism for how 1,3-butadiene metabolites lead to follicle depletion is unclear. Mechanistic evidence suggests that the di-epoxide form VCD inhibits the c-kit signaling pro-survival pathway in oocytes (Kappeler and Hoyer, 2012), leading to induction of apoptosis in follicles (Hu et al., 2001).

The vinylcyclohexene mechanism of c-kit-mediated induction of apoptosis plausibly applies to DEB-induced ovotoxicity; DEB induces apoptosis in lymphocytes (Yadavilli and Muganda, 2004). However, the available mechanistic evidence is all indirect and there is no direct, quantifiable data demonstrating DEB- induced apoptosis in primordial ovarian follicles. In a potential alternative mechanism, DEB has been shown to induce chromosome aberrations in oocytes (Tiveron et al., 1997).

4.1.1.3.4 Key Event 4: Premature Ovarian Failure and Atrophy

Multiple mechanisms may contribute to the progression of follicle destruction to premature ovarian failure. As the ovary loses follicles, its ability to produce essential hormone, like estrogen, is compromised, ultimately leading to ovarian dysfunction (Torrealday et al., 2017). Data on VCH demonstrates that destruction of small ovarian follicles leads to subsequent increase in serum follicle stimulating hormone and loss of estrous cycling (Hoyer and Sipes, 2007), suggesting the connection between follicle loss, hormonal imbalance, and ovarian failure. The observed ovarian damage may in fact be caused by a combination of DNA damage, oxidative stress, and inflammation (Liu et al., 2015; Hoyer and Sipes, 2007), ultimately leading to atrophy.

4.1.1.3.5 Uncertainties

Bioactivation of 1,3-butadiene to epoxide metabolites is a critical component in the proposed MOA based on the large differences in metabolism across species, although the quantification of individual metabolites is indirect and variable. While multiple lines of evidence indicate lower levels of epoxide metabolites in rats and humans compared to mice (*e.g.*, relative rates of activating oxidation vs detoxification, see Section 3.3), the absolute measurements of metabolites are indirect based on Hb adducts and the human data demonstrate high variability across sexes, exposure levels, and studies (Boysen et al., 2022; Boysen et al., 2012; Vacek et al., 2010; Albertini et al., 2007; Albertini et al., 2003).

Mice appear to be both toxicokinetically *and* more toxicodynamically sensitive to ovotoxicity (see evidence for Key event 1 above in Section 4.1.1.3.1). The Kirman et al. (2012) MOA proposes that only DEB is responsible for ovarian toxicity. While mechanistic studies on 1,3-butadiene metabolites and the VCH analog clearly demonstrate that DEB is more potent than EB, they do not indicate that DEB alone is ovotoxic. The presence of both metabolites in rat ovaries and evidence of some toxicity from mono-epoxides suggests that the induction of ovotoxicity may be based on relative epoxide dose delivered to the ovary (of which mice would have more of across all metabolites) rather than a specific metabolite, while later key events may contribute to the increased toxicodynamic sensitivity.

The underlying mechanisms for the destruction of ovarian follicles via apoptosis (Key event 3) are also uncertain. EPA has identified plausible mechanisms for this key event based on the molecular interactions of DEB, however this evidence is only indirect. Additionally, it is unclear if follicular apoptosis (Key event 3) is a required event upstream of ovarian failure (Key event 4), although they are both likely connected to hormonal dysregulation. Overall, this complexity makes it harder to pinpoint a single, precise mechanism or define a specific order of required key events.

4.1.1.3.6 Conclusions for Proposed MOA

EPA has preliminarily concluded that the evidence is sufficient to conclude that the proposed MOA is operational in rodents and that mice are particularly sensitive.

- Exposure to 1,3-butadiene results in ovarian atrophy and other associated reproductive toxicity in mice (Section 4.1.1.2).
- 1,3-Butadiene is oxidized into bioactive epoxide metabolites that appear to be responsible for the ovarian toxicity (Section 4.1.1.3.1 and 4.1.1.3.3).
- Mice appear to be uniquely sensitive to 1,3-butadiene-induced ovarian toxicity, both due to greater oxidative metabolism and increased toxicodynamic sensitivity (Section 4.1.1.3.1).
- Inhibition of the c-kit pro survival signaling pathway and/or cytogenetic damage represent potential mechanisms leading to ovarian follicle destruction (Section 4.1.1.3.3).

4.1.1.3.7 Applicability of Ovarian Atrophy to Human Health Risk Assessment

EPA applied the IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans (Boobis et al., 2008) in considering how to interpret the human relevance of ovarian toxicity. In building off a set of papers establishing the mode of action framework and how it can inform human relevance (Seed et al., 2005; Meek et al., 2003; Sonich-Mullin et al., 2001; U.S. EPA, 1999), the framework poses a series of questions to help *organize decision-making into a step-wise process "to determine whether to apply the default assumption that all effects seen in animals are relevant to humans."*

1. **Is the weight of scientific evidence sufficient to establish an MOA in animals?**

Yes. While there are several uncertainties as to the specific mechanisms and whether a specific metabolite is required, the general steps and key events as described above are supported. The proposed key events are sufficiently supported by the evidence to support an MOA for ovarian toxicity in rodents, especially mice.

2. **Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?**

No. Metabolism pathways are qualitatively the same across species and DEB does form in humans (Section 3.3). Further, mono-epoxides can induce ovarian atrophy (Hoyer and Sipes, 2007; Doerr et al., 1996) and humans produce substantial amounts of the mono-epoxide EBD, although there are no studies examining EBD in relation to ovarian toxicity. Additionally, the c-kit receptor and kit ligand have been detected in human ovaries (Tuck et al., 2015), indicating that this potential mechanism supporting key event 3 may function similarly in humans.

3. **Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in key events between experimental animals and humans?**

No. Ovarian atrophy is observed in mice in a dose- and duration-responsive manner in multiple studies, including all medium and high quality studies. As discussed above, strong evidence indicates that mice are both toxicokinetically and toxicodynamically more sensitive than rats, and humans may be even less toxicokinetically sensitive than rats based on estimates of relative DEB levels; DEB rapidly hydrolyzes to yield EBD occurs rapidly in humans via epoxidase hydrolase (Motwani and Törnqvist, 2014). Therefore, any DEB-mediated mode of action for ovarian atrophy in humans would likely require orders of magnitude higher exposure to 1,3-butadiene to produce a comparable level of ovotoxicity, albeit the relative quantification of metabolites involves several uncertainties. While the evidence indicates that mice are likely more sensitive to ovarian toxicity both kinetically and dynamically, much of this evidence is based on an analog that overall has uncertain toxicological similarity to 1,3-butadiene and it is difficult to precisely quantify metabolite levels in humans (Section 3.3). Additionally, the framework states that "since quantitative exposure assessment is part of the subsequence risk characterization… the difference would have to be of such a magnitude that human exposure could not possibly be envisaged to reach such levels."

Based on this stringent threshold, ovarian toxicity cannot be explicitly ruled out for humans. However, the framework does recommend bringing forward any quantitative differences into the dose-response analysis, and EPA concludes that there is very low confidence in the direct applicability of the hazard values from mice studies to human exposures. The Agency considered whether these issues could be accounted for as part of the dose-response analysis, which leads to the fourth question of the framework.

4. **Are there any quantitative differences in the key events such that default values for uncertainty factors for species or individual differences could be modified?**

Yes, but the appropriate adjustment cannot be determined. TCEQ did apply a reduced uncertainty factor for interspecies extrapolation, and some recent analyses have attempted to quantitatively address these species differences by calculating a data-derived extrapolation factor based on relative hemoglobin adduct levels (Kirman et al., 2022; Kirman and Grant, 2012) that results in an adjusted reference concentration many orders of magnitude higher than earlier assessments. Although EPA considered deriving a data-derived extrapolation factor (U.S. EPA, 2014) to dosimetrically adjust the mouse results based on human metabolism, there is substantial uncertainty in quantifying an appropriate human equivalent concentration for the endpoint. DEB levels are estimated to be at least 100 times lower and possibly 300 times or more lower in humans compared to mice however they may have more similar levels of EBD ((Swenberg et al., 2011) and Section 3.3); any toxicodynamic differences are unknown. Determining an appropriate quantitative adjustment with any confidence is therefore not reasonable. Regardless of the most appropriate adjustment, it is evident that any potential data-derived extrapolation factor (DDEF) would result in a hazard value likely orders of magnitude higher than the animal POD and would probably be less protective and with much greater uncertainty than any POD derived from the other critical hazard outcomes (see subsequent sections). Therefore, EPA did not utilize the ovarian atrophy endpoint for dose-response analysis.

There is indeterminate human evidence for ovarian toxicity due to an absence of relevant studies, moderate animal evidence due to consistent results of a strong effect observed in only mice, and indeterminate mechanistic evidence, including an MOA analysis suggesting there may be greatly reduced sensitivity in humans. See Table_Apx A-1 for the evidence integration table for this outcome. Based on the weight of scientific evidence, while the possibility of 1,3-butadiene-induced ovarian atrophy in humans cannot be ruled out, EPA has concluded that ovarian atrophy observed in mice is not appropriate for quantitative use in human health risk assessment.

4.1.2 Critical Non-cancer Hazard Outcomes

The sections below summarize the hazard identification and evidence integration of maternal and related developmental toxicity, male reproductive system and resulting developmental toxicity, and hematological and immune effects, which are the most relevant human health hazard outcomes associated with exposure to 1,3-butadiene. Details on the evidence database and evidence integration judgements are presented in Appendix A. See Full data extraction for all relevant studies in *Data Extraction Information for Human Health Hazard Animal Toxicology and Epidemiology* (U.S. EPA, 2024b) and *Further Filtering Results for Human Health Hazard Animal Toxicology and Epidemiology* (U.S. EPA, 2024c)).

4.1.2.1 Exposure During Gestation: Developmental and Maternal Toxicity

4.1.2.1.1 Human Evidence

An epidemiological study examined the risks for autism in children associated with a location relative to an air monitor measuring ambient 1,3-butadiene concentrations (von Ehrenstein et al., 2014). That study found that *in utero* exposure to 1,3-butadiene was positively associated with autism, with higher risk observed at closer distance to the air monitor. However, exposure levels were not directly quantified in the study. Furthermore, EPA did not identify any other studies assessing the effects of 1,3-butadiene exposure on maternal and related developmental toxicity in humans.

4.1.2.1.2 Laboratory Animal Evidence

Several studies have investigated the effects of 1,3-butadiene exposure on maternal and developmental toxicity in laboratory animals. One study investigated the effects of 1,3-butadiene exposure in pregnant mice, exposing them to concentrations of 0, 40, 200, or 1,000 ppm for 6 hours per day from GD 6 through 15 (Battelle PNL, 1987b). The measured mean (± SD) concentrations were 39.9 (± 0.06), 199.8 (±3.0), and 1,000 (±13.1 ppm). Significant maternal toxicity was observed at 1,000 ppm—including three mortalities, two due to dehydration, and early parturition in the third. Additional signs of maternal included decreased maternal weight gain at greater or equal to 199.8 ppm. Body weight gain between GD 11 and 16, as well as gravid uterine weight and extra gestational weight gain, were significantly reduced at 1,000 ppm. Weight gain reductions were also observed at 199.8 ppm, while no significant maternal toxicity was observed at 39.9 ppm.

In contrast, fetal body weight reductions were observed at lower concentrations than those that caused maternal toxicity. Overall fetal body weights (males and females combined) were reduced by 4.5 percent at 39.9 ppm, 15.7 percent at 199.8 ppm, and 22.4 percent at 1,000 ppm. A significant does-response relationship was identified, with reductions in feal body weights being significant for males at all exposure levels (39.9, 199.8, and 1,000 ppm) and for females at 199.8 and 1,000 ppm. In addition to fetal effects, placental weights were reduced across exposed groups compared to controls. Although sporadic malformations occurred infrequently across all exposure groups, significant increases in skeletal variations, such as supernumerary ribs and reduced ossification of the sternebrae, were observed at 199.8 and 1,000 ppm. Certain skeletal malformations/variations such as fused sternebrae were observed only at 1,000 ppm, while “abnormal sternebrae”—including misaligned, scrambled, or cleft sternebrae—demonstrated a statistically significant linear dose response with a statistically elevated incidence at the highest dose.

Similar findings of maternal and developmental toxicity were observed in rats but at higher concentrations. Female rats exposed for 10 days (GD 6–15) to less than or equal to 7,647 ppm for 6 hours/day (Hazleton Labs, 1981a) and less than or equal to 1,005 ppm for 6 hours/day (Battelle PNL, 1987a) showed decreased maternal body weight gain during exposure at greater than or equal to 200 ppm and 1,005 ppm, respectively. Fetuses showed significant 6.1 percent decreases in body weight and crown-rump length at 7,647 ppm, with dose responsive skeletal defects at greater or equal to 990 ppm. These skeletal defects included wavy ribs, increasing in a dose-dependent manner, ranging from minor at lower doses to more pronounced at higher concentrations. At 7,647 ppm, major fetal abnormalities were noted, including severe skeletal malformation such as fused ribs and angiectasis.

In a separate reproductive toxicity screening study conducted according to OECD 421 guideline in rats, males were exposed for 83 to 84 days, and females for 60 to 70 days, with one group of F1 pups sacrificed at weaning and others exposed for 7 days post-weaning (WIL Research, 2003). In that study, female rats exposed before and during mating and throughout gestation and lactation showed clinical signs of toxicity (chromodacryorrhea, chromorhinorrhea, and increased salivation) in the 1 hour after exposure at greater than or equal to 1,507 ppm. In addition, body weight was statistically significantly reduced in female F1 pups (males had a biologically but not statistically significant reduction) exposed for 7 days to greater than or equal to 1,507 ppm either with or without previous gestational/lactational exposure (WIL Research, 2003).

Inconsistent results were observed across rat studies using the same strain. In one study, no decrease in maternal body weight was noted following a total exposure period of 60 to 70 days to concentrations up

to 6,006 ppm (WIL Research, 2003). However, other studies reported maternal body weight reduction at 7,647 and 1,005 ppm, during shorter exposure periods (Battelle PNL, 1987a; Hazleton Labs, 1981a).

4.1.2.1.3 Mechanistic and Supporting Evidence

As described in Section 3.3, multiple studies have demonstrated species-specific differences in 1,3-butadiene metabolism, which may vary based on factors such as sex, dose, duration, and other variables. Nevertheless, the specific role of 1,3-butadiene metabolites in during gestation and early post-natal periods remains unclear. It is a reasonable hypothesis that 1,3-butadiene epoxide metabolites likely contribute to the observed maternal and developmental toxicity. However, there are key gaps in knowledge with respect to the available pharmacokinetic data in pregnant animals, fetuses, and early post-natal laboratory animals. Evidence does not support a role for any particular metabolite over another. Two studies in both mice and rats demonstrated that DEB is toxic to developing fetuses and embryos (Chi et al., 2002; Clerici et al., 1995). A proposed mechanism involves decreased progesterone and inhibition of placental pituitary adenylate cyclase-activating polypeptide expression and matrix metalloproteinase activity in rats (Chi et al., 2002). Mechanistic studies on parental 1,3-butadiene or other metabolites are not available. Therefore, there are not sufficient data available for EPA to make a determination as to species sensitivity as was performed for ovarian atrophy.

4.1.2.1.4 Summary and Conclusions

There is slight human evidence from a single study that identified an association between 1,3-butadiene exposure and autism risks. Robust animal evidence is based on studies that show and concordant and dose-responsive effects in mice that are relevant for human health risk assessment. The robustness of the animal evidence is supported by qualitatively similar findings in rats at higher doses. Mechanistic evidence is slight based on two studies demonstrating fetal and embryonic toxicity of DEB. See Table_Apx A-2 for the evidence integration table for this outcome.

Based on the weight of scientific evidence, evidence integration judgements, and available dose-response data for maternal and developmental toxicity, dose-response analysis is considered appropriate for assessing maternal and developmental toxicity following gestational exposures.

4.1.2.2 Male Reproductive System and Resulting Developmental Toxicity

4.1.2.2.1 Human Evidence

EPA did not identify any reasonably available information assessing effects of 1,3-butadiene exposure on the male reproductive system or associated developmental toxicity in humans.

4.1.2.2.2 Laboratory Animal Evidence

Male reproductive toxicity has been examined in several rodent studies across short-term, subchronic, and chronic exposures, including dominant lethal assays and other reproductive toxicity assessments, revealing a range of adverse effects on sperm and testicular function. Exposure for 5 days to concentrations up to 1,300 ppm for 6 hours/day led to reductions in testis weight (Xiao and Tates, 1995), with similar findings of reduced testis weight and immature spermatoid counts at 130 ppm (Pacchierotti et al., 1998). Subchronic exposure of mice to 980 ppm for 13 weeks resulted in pronounced reproductive effects, including testicular atrophy and reduced testis weight, with histopathological evaluations showing decreased cellularity of the seminiferous tubules (Bevan et al., 1996). Long-term studies further demonstrated the dose dependent reproductive effects of 1,3-butadiene. Mice exposed for 60 weeks to concentrations up to 1,236 ppm for 6 hours/week showed histopathological changes in male reproductive organs (NTP, 1984). In a 2-year study, exposure to concentrations 619 ppm for 6

hours/day, 5 days/week resulted in testicular atrophy, which became most pronounced after 2 years (NTP, 1993).

Dominant lethality, a marker of reproductive toxicity, was observed in several mouse studies in a dose- and duration-responsive manner. In dominant lethality studies, males were exposed to the 1,3-butadiene and then mated with unexposed females to assess and reproductive/developmental outcomes. Increased fetal deaths were observed at greater or equal to 65 ppm following 4 weeks of exposure (0, 12.5, 65, or 130 ppm for 6 hours/day, 5 days/week) (Anderson et al., 1998; BIBRA, 1996b) and at greater or equal to 12.5 ppm following 10 weeks of exposure (0, 12.5, or 125 ppm for 6 hours/day, 5 days/week) (Brinkworth et al., 1998; Anderson et al., 1996). External and skeletal abnormalities were also observed in these studies included exencephaly, hydrocephaly, and runt formations—with skeletal defects particularly affecting the skull, vertebra, ribs, and limbs. Ten weeks of exposure also resulted in decreased implantation (Anderson et al., 1996) and delayed time-to-coition (Brinkworth et al., 1998). Additionally, early fetal deaths were observed following only 5 days of exposure to 500 ppm (Adler et al., 1998) and 1,300 ppm (Adler et al., 1994). Interestingly, one study reported clear effects seen at 5 days of exposure to less than or equal to 1,000 ppm but weaker results at 5,000 ppm (Hackett et al., 1988b). The only acute study did not report any dominant lethality at 1,250 or 6,250 ppm, and reduced implantations were seen only at 1,250 ppm (Anderson et al., 1993), summarized in (Anderson et al., 1996).

In contrast to the findings in mice, studies in rats did not demonstrate significant male reproductive toxicity. A subchronic study conducted according to the OECD 421 guideline in rats, with exposure to concentrations up to 6,006 for 12 weeks, found no adverse effects on fertility, sperm parameters, or testicular histopathology (WIL Research, 2003). Additionally, no reproductive effects were observed in rats exposed to as high as 1,250 ppm for 4 weeks (Anderson et al., 1998) or 10 weeks (BIBRA, 1996a).

4.1.2.2.3 Mechanistic and Supporting Evidence

Various indications of genotoxicity have been observed in developing sperm and testicular cells from both 1,3-butadiene and metabolites, which provide mechanistic insight into the dominant lethal effects observed in several reproductive studies. Data from animal studies show that 1,3-butadiene causes micronuclei, chromosome aberrations, and DNA damage in sperm, testicular cells, and embryos from mice. The three common metabolites EB, DEB, and EBD all induce chromosomal damage in both mouse (U.S. EPA, 2002b; Xiao and Tates, 1995) and rat (U.S. EPA, 2002b; Lähdetie et al., 1997) spermatids. However, only DEB (but not EBD) induces genotoxicity in rat seminiferous tubule sections (U.S. EPA, 2002b; Sjoblom and Lahdetie, 1996), and mixed dominant lethality results in mice from exposure to DEB or EB suggest that developing sperm have stage-specific sensitivity (U.S. EPA, 2002b). The mechanistic evidence suggests a genotoxic mode of action, potentially mediated through 1,3-butadiene metabolites; however, definitive data linking a specific metabolite to the observed reproductive toxicity and dominant lethality remains inconclusive. Overall, there are not sufficient data available for EPA to make a determination as to species sensitivity as was performed for ovarian atrophy.

4.1.2.2.4 Summary and Conclusions

There is an absence of any relevant human data, moderate animal evidence showing both dominant lethality and associated male reproductive system toxicity but only in mice, and moderate mechanistic evidence indicating genotoxic effects of parental 1,3-butadiene in mice and genotoxicity of metabolites in both mice and rats. See Table_Apx A-3 for the evidence integration table for this outcome.

Based on the weight of scientific evidence, evidence integration judgements, and available dose-response data for dominant lethality, dose-response analysis is considered appropriate for male reproductive system and resulting developmental toxicity.

4.1.2.3 Hematological Effects

4.1.2.3.1 Human Evidence

Epidemiology data on hematological effects of 1,3 butadiene are limited by small population sizes and evaluation of few hematological parameters. Two studies suggested associations between 1,3-butadiene and hemoglobin levels. None of the studies reported exposure-related alterations in erythrocyte counts. A slight but statistically significant decrease in hemoglobin concentration was observed among petrochemical workers exposed to 1,3-butadiene, compared to an unexposed internal referent group (Tsai et al., 2005). After adjusting for confounders, a significant association was observed between 1,3-butadiene exposure level and increased mean corpuscular hemoglobin concentration in a health survey of styrene-butadiene workers (Checkoway and Williams, 1982). However, a low-quality cohort study with small numbers of participants found no association between erythrocyte count and 1,3-butadiene exposure (Hayes et al., 2000). In other human studies, no association between erythrocyte count and 1,3-butadiene exposure was observed in petrochemical workers (Tsai et al., 2005; Cowles et al., 1994) or styrene-butadiene workers (Checkoway and Williams, 1982). In another study, (Tsai et al., 2001), no association was identified for any hematological measure.

4.1.2.3.2 Laboratory Animal Evidence

The hematotoxicity of 1,3-butadiene has been extensively studied in animal models—particularly in mice—with multiple studies demonstrating its role in inducing anemia and associated hematological changes. One study investigated the myelotoxic effects of 1,3-butadiene exposing mice to 1,250 ppm for 6 hours/day, 5 days/week, over 6 weeks. Significant decreases in red blood cell counts, hemoglobin concentrations, and hematocrit, along with increases in mean cell volume (MCV) and circulating micronuclei were observed (Irons et al., 1986a, b). Another study evaluated mice exposed to 1,250 ppm for either 6 or 12 weeks and found no persistent effects on humoral or cell-mediated immunity. However, exposed mice exhibited a 20 percent reduction in relative spleen weight and a 29 percent decrease in spleen cellularity (Thurmond et al., 1986).

Long-term studies further demonstrated the dose-dependent hematological effects of 1,3-butadiene. In a 13-week subchronic study, exposure to 980 ppm led to hematological changes indicative of anemia—including decreases in erythrocyte counts, hemoglobin concentrations, and mean erythrocyte volume—along with an increase in Howell-Jolly bodies and MCV (Bevan et al., 1996). A 9-month study found that male mice exposed to 62.5 ppm and female mice exposed to 200 ppm had significant reductions in erythrocyte counts, hemoglobin concentrations, and packed cell volume, while MCV was elevated in male mice exposed to 625 ppm and female mice exposed to 200 ppm (NTP, 1993). These hematological changes persisted only at 625 ppm after 15 months of exposure, with increases in the percentage of erythrocytes with Howell-Jolly body inclusions and elevated mean cell hemoglobin (MCH) were observed at both 9 and 15 months.

In contrast, studies in rats did not demonstrate treatment related hematological changes. In a 13-week exposure to 980 ppm, no alterations in hematology or histopathology of the spleen and bone marrow were observed (Bevan et al., 1996). Similarly, even at concentrations as high as 8,000 ppm over a 2-year period, rats showed no significant hematological effects (Hazleton Labs, 1981b).

4.1.2.3.3 Mechanistic and Supporting Evidence

Inhalation exposure to 1,3-butadiene in mice resulted in significant alterations in key cytotoxicity and genotoxic parameters, including sister chromatid exchanges, chromosomal aberrations, micronuclei formation, all of which suggest potential damage to hematopoietic cells. (ATSDR, 2012; U.S. EPA, 2002b). The observed genotoxicity, particularly in bone marrow, may impair the production and function of red blood cells aligning with the development of anemia. Multiple studies have demonstrated genotoxic effects in the bone marrow and spleen of mice following exposure to 1,3-butadiene and its metabolites. However, studies in rats exposed to 1,3-butadiene by inhalation showed no increases in micronuclei or SCEs in bone marrow, suggesting species-specific difference in susceptibility (ATSDR, 2012; U.S. EPA, 2002b). Furthermore, an alternative mechanism involving the inhibition of stem cell differentiation has also been proposed, which could contribute to the bone marrow dysfunction and subsequent anemia observed in mice (Leiderman et al., 1986). This observed bone marrow dysfunction may also be linked to 1,3-butadiene induced lymphohematopoietic cancers in both mice and humans, further highlighting the role of bone marrow toxicity in the broader hematological effects, including anemia (ATSDR, 2012; U.S. EPA, 2002b; Leiderman et al., 1986). Overall, there are not sufficient data available for EPA to make a determination as to species sensitivity as was performed for ovarian atrophy.

4.1.2.3.4 Evidence Integration Summary and Conclusions

There is indeterminant evidence from human data, which provides conflicting data concerning hematological effects. However, moderate evidence from animal studies—particularly in mice—indicates that 1,3-butadine exposure leads to dose and duration responsive changes in hematology parameters, which are consistent with red blood cell anemia. Limited data also suggests potential effects on white blood cell count at high exposure concentration. Slight mechanistic evidence suggests that genotoxicity in bone marrow cells might contribute to the hematological effects observed in mice, but the presence of blood cancer in humans (Section 5.1.1.1) and alternative MOAs support relevance to humans. See Table_Apx A-4 for the evidence integration table for this outcome.

Based on the weight of scientific evidence, evidence integration judgements, and available dose-response data for hematological parameters, dose-response analysis is considered appropriate for hematological and immune effects.

4.2 Non-cancer Dose-Response Assessment

4.2.1 Selection of Studies and Endpoints for Non-cancer Toxicity Dose-Response Analysis

EPA considered studies and endpoints from the suite of epidemiological and animal toxicology studies for which the weight of scientific evidence supported adverse health outcomes following 1,3-butadiene exposure, as described in Section 4.1.2. These were the critical hazard domains of (1) developmental and maternal toxicity from exposure during gestation, (2) male reproductive system and resulting developmental toxicity, and (3) hematological and immune effects. When considering non-cancer PODs for estimating risks for acute, intermediate, and chronic exposure scenarios, EPA reviewed the available evidence and studies within these hazard domains.

The Agency selected specific studies and targeted endpoints within each hazard domain for dose-response analysis based on the following considerations:

- Overall quality determinations;
- Exposure duration;
- Dose range;

- Relevance (*e.g.*, what species was the effect in, was the study directly assessing the effect, is the endpoint the best marker for the tox outcome?);
- Uncertainties not captured by overall quality determination;
- Endpoint sensitivity;
- Total UF; and
- Uncertainty and sensitivity of BMR selection from BMD modeling.

4.2.1.1 Non-cancer Endpoints for Acute Exposures

Strong evidence supports no effect of acute 1,3-butadiene exposure on non-cancer mortality in humans under relevant exposure circumstances. Other health effects following single exposures in humans or animals were only observed at air concentrations in the thousands of ppm (Appendix D.1). The AEGL-1 value (for non-disabling discomfort) is extrapolated from the Carpenter et al human data (1944) showing mild eye irritation at 2,000 ppm, AEGL-2 (irreversible disabling effects) was based on the absence of effects observed at 8,000 ppm in (Carpenter et al., 1944), and AEGL-3 (life-threatening) is based on the rat mortality data from (Shugaev, 1969). With uncertainty factors to account for human variability, the most sensitive AEGL-1 value is 670 ppm at all durations for difficulty to focus (NAC/AEGL, 2009). AEGL-2 is 2,700 ppm at 8 hours and AEGL-3 is 6,800 ppm at 8 hours. There is uncertainty in use of the AEGL-1 as an acute POD because it was based on only two volunteers and no complaints were reported at higher concentrations. Meanwhile, the Occupational Safety and Health Administration (OSHA) enforces a 15-minute short-term exposure limit (STEL) of only 5 ppm, which is several orders of magnitude below the lowest concentration at which even these mild acute symptoms have been observed.

Fetal body weight was the basis of the acute reference concentration (RfC) in the 2002 EPA IRIS Assessment (U.S. EPA, 2002a). At this time, the Agency finds that a biologically relevant decrease in fetal weight or maternal weight gain (especially given the relatively mild magnitudes observed following repeated exposures to lower concentrations) are unlikely to result from a single exposure to 1,3-butadiene—especially not at a similar exposure level as which induced effects following repeated exposures. Fetal weight in mice was decreased only 5 percent at the lowest concentration repeated for 10 days and was observed in the absence of teratogenic effects (Battelle PNL, 1987b), further suggesting a progressive, repeated-dose effect.

In contrast with continuous measures such as body weight, teratogenic effects are usually binary and can be impacted by a single exposure. Various skeletal observations were reported in both mice and rats, including supernumerary ribs at similar concentrations as body weight changes and malformations such as abnormal sternebrae and fused ribs at higher concentrations (Section 4.1.2.1.2). It is unclear whether supernumerary ribs can develop following acute exposures since they are associated with reduced fetal body weight, and there is uncertainty as to their overall adversity (Desesso and Scialli, 2018). Therefore, supernumerary ribs are not considered applicable to acute exposures. The other skeletal malformations (*e.g.*, abnormal sternebrae including misaligned, scrambled, cleft, or fused sternebrae) have similar uncertainty as to their adversity in humans, as some observations may be considered more of a variation than a malformation. However, they could conservatively be considered relevant adverse effects that may arise from a single exposure albeit at higher concentrations than the more sensitive repeated-dose effects.

Observed damage to developing sperm and dominant lethality are likely downstream of germ cell genotoxicity (Section 4.1.2.2.3 and Table_Apx A-3), which can potentially result from single exposures. The data for male reproductive/developmental toxicity suggests that apical outcomes likely require

multiple days of exposure. While effects on spermatogenesis and sperm quality were observed in mice following as little as 5 days of exposure, these effects were either at 1,000 ppm or higher (Hackett et al., 1988a) or from a low-quality study (Pacchierotti et al., 1998). More importantly, dominant lethality studies demonstrated a clear relationship between dose sensitivity and exposure duration/frequency. Lethality was observed following 10 weeks of exposure to as low as 12.5 ppm and following 4 weeks of exposure to as low as 65 ppm, while a few studies observed lethality at 500 ppm or above following 5 days of exposure (Adler et al., 1998; Adler et al., 1994; Hackett et al., 1988b)—dependent on the timing of mating relative to specific stages of spermatogenesis. A single acute study (Anderson et al., 1993), summarized in (Anderson et al., 1996) did not observe any dominant lethality following 1 day of exposure to 1,250 or 6,250 ppm. Although the study did observe reduced implantations, this was only seen at the lower dose and is not corroborated by other developmental toxicity studies.

Overall, the evidence suggests that functional male reproductive and downstream developmental effects in 1,3-butadiene require more than a single day of exposure. Any potential impact on fertility would not be considered adverse as it would be for a very narrow window relative to mating and not at relevant exposure concentrations.

In considering all reasonably available information, EPA has determined that it is unlikely any adverse effects will result following a single exposure at concentrations relevant to human exposures. Therefore, the Agency has decided not to propose an acute non-cancer hazard value because any options would have low confidence while being less protective than the intermediate POD. Although EPA is not formally deriving an acute POD for use in risk estimation, options for potential acute PODs based on the endpoints described above are presented for comparison purposes in Appendix E.2. These are shown alongside the intermediate/chronic POD to be used for risk estimation (discussed in the sections that follow).

4.2.1.2 Non-cancer Endpoints for Intermediate and Chronic Exposures

As stated above, EPA determined that the weight of scientific evidence supports dose-response analysis for the three critical hazard domains from Section 4.1.2. As a first step, EPA identified the most appropriate studies and set of endpoints to undergo BMD modeling for comparison. All considered studies received either a medium or high overall quality determination (OQD) across relevant endpoints.

For developmental and maternal toxicity from gestational exposure, one mouse study (Battelle PNL, 1987b) and three rat studies (WIL Research, 2003; Battelle PNL, 1987a; Hazleton Labs, 1981a) were considered. Both mouse and rat data were considered relevant to humans due to the lack of any mechanistic data supporting any species-specific relevance (Section 4.1.2.1.4). Three were 10-day developmental toxicity studies (Battelle PNL, 1987a, b; Hazleton Labs, 1981a). The WIL Research study (2003), a reproductive screening study conducted according to the OECD 421 guideline in rats, contained several different exposure groups covering 60 to 70 days of exposures through pre-mating, gestation, and lactation, for one group through weaning, and for another group only during weaning. EPA performed dose-response analysis for decreased maternal weight gain, decreased fetal weight, and increased incidence of supernumerary ribs from the mouse study, (Battelle PNL, 1987b). Reduced fetal body weight and skeletal variations/malformations were inconsistently observed across the rat studies, so these results were not modeled. A dose-responsive decrease in maternal body weight gain was observed in (Hazleton Labs, 1981a) so this data set was also considered for dose-response assessment. These endpoints are relevant to both intermediate and chronic exposure because they were observed in a 10-day developmental toxicity study that exposed animals specifically during gestation.

For male reproductive system and resulting developmental toxicity, EPA determined that dominant lethality was the endpoint appropriate for dose-response modeling. Dominant lethality was observed in mice in a dose- and duration-responsive manner spanning orders of magnitude of exposure levels for a range of 5 days to 10 weeks. The two medium-quality, 10-week studies by Brinkworth et al. (1998) and Anderson et al. (1996) were considered equally relevant and used the same lowest dose. Therefore, using an approach consistent with the ORD IRIS assessment (U.S. EPA, 2002a), the data sets from these studies were combined to increase statistical power and the total number of dose groups examined. Dominant lethality is also relevant to both intermediate and chronic exposure because it results from exposure of male mice to 1,3-butadiene during a critical window of spermatogenesis followed by mating shortly thereafter.

Hematological and immune effects were only consistently observed in mice. The 1993 NTP study (NTP, 1993) is the most appropriate study for dose-response analysis of hematological and immune effects. An earlier NTP study (1984) used higher dose levels and other mouse studies only used single doses (Bevan et al., 1996; Thurmond et al., 1986). Therefore, results from (NTP, 1993) were used for dose-response modeling because it contained multiple dose groups less than 1,000 ppm. EPA performed dose-response analysis on three hematological parameters from (NTP, 1993) indicative of anemia: decreased erythrocytes, decreased hemoglobin, and decreased packed red blood cell volume (hematocrit). In that study, hematological outcomes were measured following 9 and 15 months of exposure and therefore are only considered applicable to chronic exposures.

4.2.2 Dose-Response Derivation for Non-cancer Hazard Values

As described in Section 4.2, EPA considered studies for 1,3-butadiene for quantitative dose-response analysis. The sections below describe the steps used to derive the hazard values used to calculate risks for 1,3-butadiene. Exposure via dermal or oral pathways are not expected for 1,3-butadiene (see *Draft Occupational Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024e) and *Draft General Population Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024d)). Therefore, only inhalation hazard values were derived.

4.2.2.1 Duration, Dosimetric, and Unit Adjustments for Inhalation Hazard Values

Dosimetric Adjustments

EPA considers 1,3-butadiene to be a category 3 gas for dosimetry of all systemic endpoints, in accordance with EPA's RfC guidance (U.S. EPA, 1994). Therefore, the relative blood:air partition coefficient between the test organism and humans is considered the driving factor underlying relative dosimetry. The estimated coefficient is greater for rodents than humans (Section 3.1), so in accordance with guidance (U.S. EPA, 2012a, 1994) EPA defaults to a relative ratio of 1, and therefore the internal dose is considered equivalent for rodents and humans. While limited evidence suggests that 1,3-butadiene may be mildly irritating to the respiratory system (Appendix D.2), this does not impact dosimetry for systemic effects.

EPA did not apply a DDEF for any of the critical hazard outcomes. EPA guidance on the use of DDEFs requires a strong understanding of the MOA for the endpoint of interest, with relevant quantitative data informative of specific key events underlying the endpoint. This understanding must also include knowledge of the toxicokinetic exposure-response associated with the endpoint (U.S. EPA, 2014). There is insufficient mechanistic information supporting any MOA for maternal and developmental outcomes following gestational exposure and it is unclear whether any particular metabolite is more or less important. Male reproductive/developmental and hematological effects have some evidence suggesting potential genotoxic mechanisms; however, this is unclear and the relative contribution of each

metabolite is unknown. Therefore, EPA relied on default dosimetric adjustments and did not establish a DDEF to derive HECs for these endpoints in accordance with agency guidance (U.S. EPA, 2014).

Duration Adjustments

The studies selected for dose-response assessment utilized differing exposure durations and frequencies. In order to better compare results across studies and exposure scenarios, administered doses/concentrations were linearly adjusted to continuous exposure (24 hr/day, 7 days/week) prior to POD derivation based on Haber's Law (Haber, 1924) using the following equation:

Equation 4-1. Adjusting Average Exposure Concentration or Inhalation POD for Differences in Days and Hours of Exposure across Scenarios

$$Concentration_{continuous} = Concentration_{study} \times (\frac{D_S}{7}) \times (\frac{H_S}{24})$$

Where:

$Concentration_{continuous}$	=	Adjusted air concentration/inhalation POD
$Concentration_{study}$	=	Air concentration/inhalation POD from study data set
D_s	=	Days per week/year exposure in study data set
H_s	=	Hours per day exposure in study data set

HECs were derived incorporating both dosimetric and duration adjustments, resulting in a lower value than the original study POD.

Unit Conversion

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all inhalation hazard values in Section 4.2.2.5 and Section 8 in both units. The following equation presents the conversion of the HEC from mg/m^3 to ppm.

Equation 4-2. Converting ppm to mg/m^3

HEC (mg/m^3) = HEC (ppm) × (Molecular Weight / 24.45[1])
HEC (mg/m^3) = HEC (ppm) × (54.0916 / 24.45[1])

4.2.2.2 Benchmark Concentration Analysis

EPA conducted BMD modeling in accordance with guidance (U.S. EPA, 2012b) to refine PODs for the endpoints and studies described in Section 4.2.1. EPA decisions on modeling of specific data sets and results are described below. See Table 4-1 for PODs for each hazard outcome and Appendix B for a summary of all BMD modeling results, including model selection and alternative endpoint options. See *Draft Benchmark Dose Modeling Results for 1,3-Butadiene* (U.S. EPA, 2024a) for full BMD modeling details including statistical tests, results from all models, and any associated graphs.

4.2.2.2.1 Exposure During Gestation: Maternal and Developmental Effects

Reduced Fetal Body Weight

For reduced fetal body weight from (Battelle PNL, 1987b), male data was selected as it was demonstrated a clearer dose-response and relative body weight change compared to females. The lowest

[1] The Ideal Gas Law can be used to convert between ppm and mg/m^3. At standard temperature and pressure (STP; 25 °C and 760 mm Hg), 1 mole of gas occupies 24.45 L. However, when conditions differ from STP, a different gas conversion factor can be calculated using the reported experimental temperature or pressure.

dose tested of 40 ppm was identified as a statistically significant LOAEL; however, the fetal weight was only reduced by 5 percent at this dose and a subsequent re-analysis determined that the result may not in fact be statistically significant (Green, 2003). EPA therefore BMD modeled the endpoint in order to refine the POD as mean fetal body weight in male fetuses/litter.

Although litter is the relevant unit of measurement for developmental outcomes, mean fetal body weight for males and for all fetuses combined were also modeled for comparison purposes (BMD modeling failed for these data sets). BMD modeling fetal weight as a continuous variable failed without dropping the top dose group. EPA selected a benchmark response (BMR) of 5 percent relative deviation (RD) for this endpoint, due to the sensitive developmental effect, and with the top dose dropped the selected the lower 95th percentile estimate of the BMD $(BMDL)_5$ was 10.7 ppm. EPA then used a different method for dose-response modeling, adapting one of the approaches used in (U.S. EPA, 2002a). EPA dichotomized the individual male fetal weight data in treatment groups based on whether it was below the 5th or 10th percentile of the distribution for the control group, essentially determining if a weight measurement was statistically different from controls. In a departure from (U.S. EPA, 2002a), EPA only modeled male data, in addition to the reasons stated above, there was concern that combining both sexes could skew the reliability of the percentile cutoffs due to potential bimodal weight distributions across sexes. The resulting dichotomized data was then nested (individual fetal data associated with the corresponding litter) and BMD modeled.

EPA determined that the 5th percentile significance threshold with a 5 percent extra risk (ER) BMR was the most appropriate modeling result for balancing statistical confidence and endpoint adversity. The dichotomized BMD modeling was successful, with the 5th percentile $BMDL_5$ equaling 2.5 ppm and equivalent to the 10th percentile $BMDL_{10}$. While there is some uncertainty because the associated BMD (5.5 ppm, see Table_Apx B-1) is below the lowest dose tested, it is less than 2-fold below the lowest concentration (adjusted to HEC), which was also determined to be a LOAEL in the study. With application of a lowest-observed-adverse-effect level (LOAEL) to no-observed-adverse-effect level (NOAEL) uncertainty factor the risk threshold would be either similar or lower than the $BMDL_5$. Additionally, a recent update to European Food Safety Authority guidance on the use of BMD modeling recommends alternatives to BMD modeling only when the BMD (and not the BMDL) is more than 10× below the lowest dose/concentration tested (EFSA Scientific Committee et al., 2022). Therefore, the $BMDL_5$ value is the most appropriate POD for reduced fetal body weight.

Reduced Maternal Body Weight Gain

EPA also performed BMD analysis on (Battelle PNL, 1987b) both absolute body weight gain from GD 11 to 16 and extra-gestational weight gain from GD 0 to 18. In accordance with BMD modeling guidance (U.S. EPA, 2012b) EPA used a default BMR of 1 standard deviation (SD) and both measurements were successfully modeled. The more sensitive $BMDL_{1SD}$ was 10.4 ppm, for GD 11 to 16 maternal absolute body weight gain. There is over a 5.5-fold range between the BMD (58.2 ppm) and BMDL (suggested range is under 3-fold), so this POD has some increased uncertainty.

The parallel rat data sets were also modeled for comparison from (Hazleton Labs, 1981a): absolute body weight gain from GD 6-15, extragestational weight at GD 20, and extragestational weight gain from GD 0 to 20. Absolute body weight gain was successfully modeled as the most sensitive rat POD with a $BMDL_{1SD}$ of 48.9, less than five times higher than the mouse POD. The corresponding BMD was 101.3 ppm, less than 2 times higher than the mouse BMD and with smaller modeling uncertainty. See Table_Apx B-1 for more modeling details.

Supernumerary Ribs

EPA also modeled the number of litters and fetuses with supernumerary ribs as well as the mean percentage of supernumerary ribs per litter. The data was also nested to account for inter- and intra-litter variation. The nested data was successfully modeled, with both 5 and 10 percent ER considered for BMR due to the questionable adversity/severity of the endpoint (Desesso and Scialli, 2018). The $BMDL_{10}$ was 6.1 ppm and the $BMDL_5$ was 2.9 ppm. The BMD for both PODs was only about 2 times higher, indicating low modeling uncertainty, and the BMD_{10} is above the lowest HEC tested (Table_Apx B-1).

4.2.2.2.2 Dominant Lethality

As mentioned in Section 4.2.1.2, dominant lethality was the endpoint selected for dose-response assessment of male reproductive system and resulting developmental toxicity. The combined data set from Brinkworth et al. (1998) and Anderson et al. (1996) was used for BMD modeling. The total incidence of all fetal deaths was modeled, and the associated litter for each fetus was not tracked due to paternal-specific exposure. Previous modeling in (U.S. EPA, 2002a) separated early and late deaths, but this distinction was not considered relevant/important. A 5 percent ER was selected as BMR due to the severe developmental effect (the large number of fetuses may have supported as low as a 1 percent ER), with $BMDL_5$ equal to 4.8 ppm. The BMD is above the lowest HEC tested and 2.7 times greater than the BMDL (Table_Apx B-2).

4.2.2.2.3 Anemia

All three hematological parameters indicative of anemia were BMD modeled as continuous parameters, and all three failed BMD modeling without dropping at least one dose. Reduced erythrocyte counts were successfully modeled with the highest concentration dropped, hemoglobin concentration required the dropping of two doses, and packed red cell volume required dropping of the highest dose. The default BMR of 1 SD was applied to all three data sets, with resulting $BMDL_{1SD}$ of 8.07, 7.95, and 3.91 ppm, respectively. The BMD for all 3 values was 10.8 (±0.1), within the range of the tested concentrations but suggesting that modeling uncertainty explains much of the variation across the endpoints (Table_Apx B-3).

4.2.2.3 POD Selection for Risk Estimation

All the modeled intermediate endpoints are related to developmental toxicity and only differ by a few fold across all endpoints and BMD results. Although all critical hazard outcomes categories were considered appropriate for dose-response analysis, maternal and developmental toxicity from gestational exposure are considered the most reliable for application to human risk characterization. These effects were observed to some extent in both rats and mice, underlying the “robust” judgement for the animal data that is being used for POD derivation. In contrast, dominant lethality was only observed in mice, resulting in a “moderate” judgement for animal evidence. Additionally, mechanistic evidence suggests some potential differential species sensitivity through genotoxicity of metabolites. Appendix A presents details on these weight of scientific evidence judgements.

Among the maternal and related developmental effects, fetal body weight reduction is usually associated with both reduced maternal weight (gain) and skeletal malformations (Desesso and Scialli, 2018). Reduced fetal body weight represents the least ambiguously adverse endpoint and presents the most sensitive POD among the hazard outcome via the nested dichotomous BMD modeling approach. EPA has high confidence in the resulting POD of 2.5 ppm because the result was identical within two significant figures from two combinations of percentile thresholds and BMRs. The POD for reduced fetal body weight is also protective of the POD for dominant lethality ($BMDL_{1SD}$ = 4.83 ppm). This POD for fetal body weight therefore covers all developmental toxicity endpoints and will thus be used

for risk estimation of intermediate exposures. The selection of fetal weight as the basis of the intermediate POD is in agreement with EPA IRIS (2002a), who used fetal weight for their subchronic (and acute) POD.

Similar to dominant lethality, chronic hematological and immune effects were only observed in mice. While there is evidence of a genotoxic contributing mechanism, genotoxicity in white blood cells and bone marrow have been observed in humans (Sections 4.1.2.3.3 and 5.2), suggesting that these effects are unlikely to be specific to mice only (Section 4.1.2.3.4). Nonetheless, the animal data was assigned a judgement of "moderate" (compared to "robust" for maternal developmental toxicity) and there is lower confidence in the POD estimates for anemia endpoints due to the failed BMD modeling with all doses. Additionally, the $BMDL_5$ for fetal weight (2.5 ppm) that was selected for risk estimates of intermediate exposure is protective of the most sensitive POD for anemia ($BMDL_5$ = 3.91 ppm), which can be considered co-critical. Therefore, the POD for fetal weight was also selected for risk estimation of chronic exposures. The selected POD and associated data set are **bolded** in Table 4-1, which also presents the other PODs that EPA considered across the critical hazard domains.

Table 4-1. Dose-Response Analysis of Selected Studies and Endpoints Considered for Deriving Intermediate and Chronic PODs

Reference and Study Details (mg/kg-day)	Study POD/ Type (ppm)	Effect/Data set	HEC (ppm)	UFs
Battelle PNL (1987b) **Pregnant CD-1 mice; inhalation; 0, 40, 200, 1,000 ppm; 6 hr/day; GD 6-15** **Toxicity following gestational exposure**	**LOAEL = 40** (NOAEL based on statistical reanalysis) **(HEC = 10)**	Male fetal body weight, continuously modeled	$BMDL_5$ = 10.7 (Highest concentration dropped)	UF_A = 3 UF_H = 10 *Total UF = 30*
		Nested model, male data dichotomized based on 5th percentile of control distribution	**$BMDL_5$ = 2.5**	
	NOAEL = 40 (HEC = 10)	Absolute maternal body weight gain, GD 11-16	$BMDL_{1SD}$ = 10.4	
	NOAEL = 40 (HEC = 10)	Incidence of supernumerary ribs, nested model	$BMDL_5$ = 2.9 $BMDL_{10}$ = 6.1	
Hazleton Labs (1981a) Pregnant SD rats; inhalation; 0, 200, 1,000, 8,000 ppm; 6 hr/day; GD 6-15 Toxicity following gestational exposure	NOAEL = 200 (HEC = 50)	Absolute body weight gain (GD 6–15)	$BMDL_{1SD}$ = 48.9	UF_A = 3 UF_H = 10 *Total UF = 30*
Brinkworth et al. (1998); Anderson et al. (1996) (combined) Male CD-1 mice; inhalation; 0, 12.5, 125 ppm, 1,250 ppm; 6h/day; 5 days/week; 10 weeks Dominant lethality	NOAEL = 12.5 (LOAEL for (Anderson et al., 1996) data set (HEC = 2.14)	Combined incidence of deaths across two data sets	$BMDL_5$ = 4.83	UF_A = 3 UF_H = 10 *Total UF = 30*
NTP (1993) Male B6C3F1 mice; inhalation; 0, 6.21, 19.8,	NOAEL = 19.8 (HEC = 3.54)	Absolute erythrocyte counts (10^6/µl) in males following 9 months of exposure	$BMDL_{1SD}$ = 8.07 (Highest concentration dropped)	UF_A = 3 UF_H = 10 *Total UF = 30*

Reference and Study Details (mg/kg-day)	Study POD/ Type (ppm)	Effect/Data set	HEC (ppm)	UFs
61.4, 199, or 619 ppm; 6h/day; 5 days/week; 40 weeks Anemia		↓ Hemoglobin concentration in males following 9 months of exposure	$BMDL_{1SD}$ = 7.95 (Two highest concentrations dropped)	
		↓ Packed red cell volume (hematocrit) in males following 9 months of exposure	$BMDL_{1SD}$ = 3.91 (Highest concentration dropped)	

4.2.2.4 Uncertainty Factors Used for Non-cancer Endpoints

As shown in Table 4-1, EPA used a total uncertainty factor (UF) of 30 for the benchmark MOEs for intermediate and chronic exposure durations based on BMDLs or NOAELs. Details on each UF are provided below. EPA guidance from (U.S. EPA, 1994), (2002c), and (2012a) further discuss considerations for application of UFs in human health hazard dose-response assessment. Other potential uncertainty factors not relevant to this assessment that EPA may consider are described in Appendix E.3.

1. **Interspecies Uncertainty Factor (UF_A) of 3**
   EPA used data from inhalation toxicity studies in animals to derive relevant HECs. As described in Section 4.2.2.1, interspecies toxicokinetic dosimetry for systemic endpoints utilized relative blood:air coefficient across species, which defaults to 1. This consideration is expected to account for interspecies toxicokinetic differences for the selected endpoints. Therefore, only toxicodynamic differences across species are not accounted for in the HEC derivation, and the standard 10x UF_A is reduced to 3.

2. **Intraspecies Uncertainty Factor (UF_H) of 10**
   EPA used a default UF_H of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human toxicokinetic and toxicodynamic variability may impact the disposition of or response to 1,3-butadiene.

4.2.2.5 Non-cancer Hazard Values Selected for Use in Risk Estimation

The POD for reduced fetal body weight from (Battelle PNL, 1987b) is being proposed for risk estimation of intermediate and chronic exposures. Table 4-2 presents this POD along with the UFs and basic study information for the endpoint.

Table 4-2. Non-cancer Points of Departure and Critical Endpoints Used for Risk Estimates of Each Exposure Scenario

Target Organ System	Species	Duration	Study POD/Type	Effect	HEC (ppm) [mg/m^3]	Uncertainty Factors (UFs)	Reference	Overall Quality Determination
Intermediate/chronic exposure scenarios								
Maternal/ Developmental	Mouse (Male)	10 days throughout gestation (GD 5–16)	LOAEL = 40 ppm	Reduced fetal body weight and other associated endpoints	$BMDL_5$ = 2.5 ppm (5.5 mg/m^3)	UF_A = 3; UF_H = 10; *Total UF = 30*	(Battelle PNL, 1987b)	Medium

5 CANCER HAZARD ASSESSMENT

The sections below outline human (Section 5.1), animal (Section 5.1.2), and mechanistic (Section 5.1.3) evidence for carcinogenicity. The cancer classification and summary of evidence integration conclusions is in Section 5.1.4. For complete details on the evidence for cancer, see the evidence profile tables organized by cancer type in Table_Apx A-5. Full details on all evaluated health outcomes from all key studies are in *Draft Data Extraction Information for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024b). Additional hazard information supporting evidence integration is presented in *Draft Further Filtering Results for Human Health Hazard Animal Toxicology and Epidemiology for 1,3-Butadiene* (U.S. EPA, 2024c).

5.1 Cancer Hazard Identification

5.1.1 Epidemiology Studies

According to the TSCA systematic review process (U.S. EPA, 2024h), the EPA systematic review process identified seventy-two epidemiological publications. Of the 72 epidemiological publications, EPA identified 35 publications that conducted dose-response association based on at least 2 exposure levels (plus a reference level) or continuous exposure data. Of these 35 epidemiological publications with dose-response analyses and cumulative exposure, 21 investigated leukemia, and 7 investigated bladder cancer.

5.1.1.1 Lymphohematopoietic cancers

Numerous retrospective occupational cohort publications of styrene-butadiene rubber workers, involving more than 22,000 men and women, have studied the health effects of 1,3-butadine (Valdez-Flores et al., 2022; Sathiakumar et al., 2021b; Sathiakumar et al., 2019; Sathiakumar et al., 2015; Sielken and Valdez-Flores, 2013, 2011; Graff et al., 2009; Sathiakumar and Delzell, 2009; Sielken, 2007; Delzell et al., 2006; Graff et al., 2005; Sathiakumar et al., 2005; Delzell et al., 2001; Sielken and Valdez-Flores, 2001; IISRP, 1999; Delzell et al., 1996; UAB, 1995; IISRP, 1986). Similarly, another retrospective occupational cohort study that used data from a part of the same cohort study focused specifically on 2,800 butadiene monomer workers (n = 2,800 men) (Divine and Hartman, 2001). Most of these occupational cohort studies or publications found a positive association between 1,3-butadiene exposure and leukemia.

Beyond occupational studies, several case-control studies have investigated the association between 1,3-butadiene exposure and childhood leukemia. A study investigated maternal exposure to 1,3-butadiene exposure during pregnancy and the risk of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) in children under 6 years old, using air monitoring data from the nearest station to the maternal address (Heck et al., 2014). Another study focused ALL in children under 5 years old, utilizing modeled air concentrations at the maternal address at birth (Symanski et al., 2016). An ecological study investigated leukemia, Hodgkin's disease, and non-Hodgkin lymphoma (NHL) in individuals under 20 years old, based on modeled air concentrations at the residence at diagnosis (Whitworth et al., 2008).

Governmental reviews of older epidemiology data concluded that occupational exposure to 1,3-butadiene was associated with increased mortality from leukemia and NHL (ATSDR, 2012). One semiquantitative study assessed relative levels of male hematopoietic cancer near hydrocarbon processing centers in Canada (Simpson et al., 2013). In a large cohort of styrene-butadiene rubber (SBR) workers, exposure to 1,3-butadiene was associated with an increased risk of mortality from leukemia in men and women. The risk increased with the magnitude and duration of exposure and remained elevated after control for covariates including styrene exposure, consideration of alternative exposure

assessments, and longer follow-up times (Valdez-Flores et al., 2022; Sathiakumar et al., 2021b; Sathiakumar et al., 2019; Sathiakumar et al., 2015; Sielken and Valdez-Flores, 2013, 2011; Graff et al., 2009; Cheng et al., 2007; Graff et al., 2005; Sathiakumar et al., 2005; Delzell et al., 2001; Sielken and Valdez-Flores, 2001; IISRP, 1999; Delzell et al., 1996; UAB, 1995; IISRP, 1986). The most recent analyses with the longest follow-up of this cohort reported an exposure-response trend for lymphoid leukemia but not myeloid leukemia, and trends for B-cell malignancies in some, but not all, analyses (Sathiakumar et al., 2021b). Consistent with the occupational cohort, in butadiene monomer workers, exposure to 1,3-butadiene was associated with increased mortality from lymphohematopoietic cancer (Divine and Hartman, 2001). Furthermore, in case-control studies of non-occupational populations, higher measured or modeled air concentration of 1,3-butadiene was associated with increased odds of leukemia, ALL, and/or AML (Symanski et al., 2016; Heck et al., 2014; Whitworth et al., 2008). Male hematopoietic cancers were elevated (no statistics provided) near a hydrocarbon processing center with high 1,3-butadiene levels, but the causal association between these cancers and 1,3-butadiene exposure cannot be confirmed due to the study design(Simpson et al., 2013). In butadiene monomer workers, the relative risk of leukemia death was not correlated with increasing 1,3-butadiene exposure (Divine and Hartman, 2001). The classification of lymphohematopoietic cancers is complex and has changed over time. Overall, extensive analyses of a large cohort of styrene-butadiene rubber workers reveal a clear association between occupational 1,3-butadiene exposure and elevated mortality from leukemia. Based on the human evidence, the overall judgment for the association between 1,3-butadiene exposure and leukemia and other lymphohematopoietic cancers is robust.

5.1.1.2 Bladder Cancer

Bladder cancer mortality has been linked to exposure to 1,3-butadiene in styrene-butadiene rubber workers. The most recent analysis with the most extended follow-up has demonstrated an increased risk of mortality from bladder cancer associated with 1,3-butadiene exposure, exhibiting a clear exposure - response trend (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019). However, this association may be confounded by smoking, as smoking data were unavailable for the cohort (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019). In contrast, no association between 1,3-butadiene exposure and bladder cancer was observed in a smaller cohort of butadiene monomer workers (Divine and Hartman, 2001). Overall, although an association between 1,3-butadiene exposure and exposure-related increase in bladder cancer mortality was observed in styrene-butadiene rubber workers, the absence of smoking data may limit the interpretation of these findings.

5.1.1.3 Central Nervous System Cancers

Central nervous system cancer has been studied in relation to 1,3-butadiene exposure, with varied results. An increased incidence rate ratio for astrocytomas other than juvenile pilocytic astrocytoma (JPA) were associated with modeled 1,3-butadiene concentrations in quartile 2 (Q2) and Q3, but not in Q4 (Danysh et al., 2015). Additionally, increased odds of primitive neuroectodermal tumors were associated with 1,3-butadiene in ambient air during pregnancy and first year of life (Von Ehrenstein et al., 2016). In the study conducted by Danysh, (2015), there may have been misclassification of exposure due to the use of census tract-level estimates to represent individual exposure. In addition, exposure estimates were assigned based on address at time of diagnosis. Furthermore, confounding factors are possible because exposure estimates were higher near major metropolitan areas but, urban/rural status was not evaluated as a potential confounder; and the modeled 1,3-butadiene concentration was highly correlated with modeled concentrations of other chemicals but confounding by co-exposures was not evaluated (Danysh et al., 2015). No association was observed between 1,3-butadiene exposure and astrocytomas in the study by (Von Ehrenstein et al., 2016), nor with central nervous system cancer and/or central nervous system cancer mortality in various occupational cohort publications (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; Divine and Hartman, 2001;

IISRP, 1986). Overall, an association between modeled 1,3-butadiene concentration and non-JPA astrocytomas in children was reported in an ecological study, but not in the highest quartile of exposure. The study was limited by its design as well as lack of adjustment for important confounders and co-exposures.

5.1.1.4 Other Cancer Types

Exposure to 1,3-butadiene has been investigated for its potential link to germ cell tumors. Increased odds of all germ cell tumors and yolk sac tumors associated with 1,3-butadiene concentration in ambient air during the second trimester (Hall et al., 2019). However, no associations were identified for germ cell tumors or yolk sac tumors with 1,3-butadiene concentration in ambient air during the first or third trimester (Hall et al., 2019). One known risk factor for germ cell tumors, cryptorchidism, was not accounted for in the study because data were not available for the study population. Overall, in a single study, an association was observed between 1,3-butadiene concentration in ambient air during pregnancy and all germ cell tumors and yolk sac tumors in children. No other studies of this endpoint were located.

Exposure to 1,3-butadiene has been studied in relation to lung cancer—particularly in occupational settings. In a large cohort of styrene-butadiene rubber workers, exposure to 1,3-butadiene was associated with increased mortality (standardized mortality ratio) from lung cancer among female workers (Sathiakumar et al., 2019; Sathiakumar et al., 2009; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB, 1995; IISRP, 1986). However, there was no exposure response trend observed, and the analysis was adjusted for smoking. The publication authors indicated that indirect adjustment for smoking partially explained the increase in mortality among female workers (Sathiakumar et al., 2019). In contrast, no association between 1,3-butadiene exposure and lung cancer was observed in male styrene-butadiene rubber workers (Sathiakumar et al., 2019; Sathiakumar et al., 2009; Sathiakumar et al., 2005; Divine and Hartman, 2001; UAB, 1995; IISRP, 1986). General population studies provided limited information on lung cancer due to ecological study design (Luo et al., 2011) or analysis limited to male smokers (Yuan et al., 2012). Overall, an association between 1,3 butadiene exposure and lung cancer mortality was observed in female styrene-butadiene rubber workers, but this association was not seen in male workers. The lack of a dose-response relationship and potential confounding by smoking complicate the interpretation of these findings.

Increased odds of retinoblastoma were associated with 1,3-butadiene concentration in ambient air during pregnancy (Heck et al., 2015). However, no association was observed between 1,3 butadiene exposure and mortality from ocular tumors in a large cohort of male styrene-butadiene rubber workers, tumors affecting vision are still adverse even if not fatal (IISRP, 1986). Overall, an association between 1,3-butadiene concentration in ambient air during pregnancy and retinoblastoma in children was observed in a single study.

Regarding other cancers, in a retrospective cohort study of a small group of butadiene monomer workers, employment in the rubber reserve unit for at least 2 years was associated with increased mortality from stomach cancer, although exposure levels were not quantified (Ward et al., 1996a; Ward et al., 1995). In contrast, larger retrospective cohort publications of styrene-butadiene rubber workers (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB, 1995; IISRP, 1986) and butadiene monomer workers (Divine and Hartman, 2001), found no association between 1,3-butadiene exposure and mortality from cancers of the gastrointestinal tract. Overall, available studies have also identified no association between 1,3 butadiene exposure and cancers of the breast, liver, ovaries, pancreas, skin, thyroid, or uterus. The weight of evidence from available studies also does not support an association with stomach cancer.

5.1.2 Laboratory Animal Studies

In laboratory animals, 1,3-butadiene consistently induced tumors at multiple sites in both mice and rats. A total of four studies were conducted, with four in mice and one rats. These studies assessed various tumor types, including lung, liver, mammary gland, as well as testicular tumors, across different exposure durations and concentrations. The majority of these studies, such as those conducted by NTP (NTP, 1993) and Hazleton labs (Hazleton Labs, 1981b) were guideline-like studies.

Regarding lymphohematopoietic system cancers, one study exposed mice to concentrations up to 1,250 ppm for 60 to 61 weeks (NTP, 1984), while another study exposed mice up to 619 ppm for 103 weeks (NTP, 1993). Additional studies included stop exposure experiments focusing on males (NTP, 1993) and a separate study where mice were exposed to up to 10,000 ppm for a single 2-hour exposure followed by a 2-year observation period (Bucher et al., 1993). The NTP 1993 study demonstrated significant dose related trends and pairwise comparison with concurrent controls for histiocytic sarcoma in both male and female mice, with significant increases persisting after survival adjustment (NTP, 1993). In male mice, all stop-exposure groups exhibited significantly elevated tumor incidence, including those exposed for shorter durations at higher concentrations, such as 625 ppm for 13 weeks. Furthermore, significant dose related trends were observed for malignant lymphoma/lymphatic lymphoma in both male and female mice across the studies in all groups of the stop-exposure experiment (NTP, 1993).

In the 103-week study, these increases remained significant even after survival adjustment. Malignant lymphomas, appearing as early as weeks 20 to 23, were identified as the primary cause of early deaths in exposed mice (NTP, 1993). Importantly, no increase in hematopoietic system tumors were observed in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b). Overall, exposure to 1,3 butadiene induced dose-related increased incidences of hematopoietic system cancers in male and female mice, which were the primary cause of early deaths in these studies.

Regarding heart hemangiosarcomas, two studies demonstrated significant dose-related increases in both male and female mice exposed to 1,3-butadiene. These increases remained significant even after adjusting for survival and were observed in all stop-exposure groups of male mice (NTP, 1993). Importantly, heart angiosarcomas are rare in B6C3F1 mice and were not observed in historical control (NTP, 1993). In the 103-week study, heart hemangiosarcomas were the second-most common cause of early death (NTP, 1993). In contrast, there was no increase in heart tumor incidence in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b). Overall, exposure to 1,3 butadiene induced dose-related increases in the incidences of heart hemangiosarcomas in male and female mice, and these cancers were the second-most common cause of early deaths in exposed mice in both studies. No increase in heart tumor incidence was observed in exposed rats.

Regarding gastrointestinal tumors, significant dose-related trends and/or pairwise comparisons with concurrent controls were observed for forestomach papilloma or carcinoma incidences in male and female mice in two studies (NTP, 1993, 1984). In the 103-week study, significant increases remained after adjustment for survival. Significantly increased incidences of forestomach papilloma or carcinoma were also seen in male mice in stop-exposure studies (NTP, 1993). Exposure to 1,3 butadiene induced increased incidences of forestomach papilloma or carcinoma in male and female mice, but no such increase was observed in exposed rats. Rats are obligate nasal breathers while mice can also breathe through their mouth, suggesting there could be oral swallowing of the 1,3-D in mice and dual route exposures.

Regarding Harderian gland tumors, mouse studies showed significant dose-related trends and pairwise comparisons with concurrent controls for Harderian gland adenoma or carcinoma in male mice, with exposure up to 619 ppm for 103 weeks (NTP, 1993). These significant increases remained after adjustment for survival and were noted in all stop-exposure groups, males only (NTP, 1993). Additionally, survival-adjusted incidences of Harderian gland adenoma or carcinoma were significantly increased (pairwise relative to concurrent control) in female mice (NTP, 1993). In contrast, rat studies involved exposure to up to 8,000 ppm for 105 to 111 weeks but showed no increase in Harderian gland tumor incidence, indicating a lack of consistency across species (Hazleton Labs, 1981b). The concurrent female mouse control incidence exceeded the upper limit of historical control incidence (NTP, 1993). Overall, exposure to 1,3-butadiene induced increased incidences of Harderian gland adenoma or carcinoma in male and female mice. However, no increase in Harderian gland tumor incidence was observed in exposed rats.

Significant dose-related trends and pairwise comparisons with concurrent controls were observed for hepatocellular adenoma and/or carcinoma in female mice in two studies (NTP, 1993, 1984). Survival-adjusted incidences were significantly increased in both male and female mice in the 103-week study (NTP, 1993). However, no increase in liver tumor incidence was observed in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b).

Significant dose-related trends and pairwise comparisons with concurrent controls were observed for alveolar/bronchiolar adenoma, adenocarcinoma, and/or carcinoma in male and female mice in two studies (NTP, 1993, 1984). In the 103-week study, while incidences exceeded the upper limit for historical control ranges, the most significant findings were observed in comparison to concurrent controls, with increases persisting even after adjustment of survival. Significantly increased incidences were also seen in male mice in all stop-exposure groups (NTP, 1993). In the 103-week study, the incidence of alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma in concurrent control males exceeded the upper limit for historical controls (NTP, 1993). However, no increase in lung tumor incidence in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b). Exposure to 1,3-butadiene induced increased incidences of lung tumors in male and female mice. No increase in lung tumor incidence was observed in exposed rats.

Significant dose-related trends and pairwise comparisons with concurrent control were observed for mammary gland acinar cell carcinoma in female mice (NTP, 1984) and for mammary gland adenoacanthoma, carcinoma, or malignant mixed tumor in female mice (NTP, 1993). In the 103-week study, significant increases in adenoacanthoma or carcinoma incidence remained after adjustment for survival. Furthermore, significant dose-related trends and pairwise comparisons with concurrent controls showed increased incidences of benign and total (benign + malignant) mammary gland tumors in female rats (Hazleton Labs, 1981b). However, historical control incidences were not reported for mice or rats. Overall, exposure to 1,3-butadiene induced increased incidences of mammary gland tumors in female mice and female rats.

A significant dose-related trend for the increased incidence of brain glial cell tumors was observed in male rats (Hazleton Labs, 1981b). Similarly, in a 60-week study, brain gliomas were identified in two male mice at 619 ppm and one male mouse at 1,260 ppm, while an ependymoma of the brain was observed in one male mouse at 619 ppm (NTP, 1984). Furthermore, in the 103-week study, a malignant glioma was observed in one male mouse at 199 ppm (NTP, 1993). In the stop-exposure studies at 619 ppm, malignant gliomas were found in two male mice after 13 weeks of exposure and in one male mouse after 26 weeks. Additionally, malignant neuroblastomas were identified in two male mice after 13 weeks (NTP, 1993). Gliomas and neuroblastomas are rare in B6C3F1 mice and were not seen in

historical controls according to (NTP, 1993). There were no statistically significant pair-wise comparisons with concurrent control group for male rats. No historical control data were reported (Hazleton Labs, 1981b). No statistically significant pair-wise comparisons with the concurrent control group for male rats and no historical control data were reported (Hazleton Labs, 1981b). No brain glial cell tumors were observed in female rats (Hazleton Labs, 1981b). Similarly, no gliomas, ependymomas, or neuroblastomas were observed in female mice (NTP, 1993), indicating a lack of consistency across sexes. Overall, brain glial cell tumors were observed in exposed male rats with dose-related trends and low incidences of gliomas, neuroblastomas, and ependymoma in exposed male B6C3F1 mice. These tumors are rare in B6C3F1 mice.

Ovarian atrophy was observed in female mice exposed to 1,3-butadiene (4.1.1). Significant dose-related trends and pairwise comparisons with concurrent control were observed for ovarian granulosa cell tumors in female mice in two studies (NTP, 1993, 1984). In the 103-week study, significant increases remained after adjustment for survival, and survival-adjusted rates exhibited monotonicity with exposure (NTP, 1993). Conversely, no increase in ovarian tumor incidence was observed in female rats, indicating a lack of consistency across species (Hazleton Labs, 1981b). Overall, exposure to 1,3-butadiene induced increased incidences of ovarian granulosa cell tumors in mice. No increase in ovarian tumor incidence was observed in exposed rats.

Pancreatic tumors showed a significant dose-related trend and pairwise comparison with concurrent control for the increased incidence of pancreatic exocrine adenomas in male rats (Hazleton Labs, 1981b). However, no increase in pancreatic tumor incidence was observed in mice (NTP, 1993, 1984), indicating a lack of consistency across species. Similarly, no increase in pancreatic tumor incidence was observed in female rats, suggesting a lack of consistency across sexes of rat (Hazleton Labs, 1981b). Historical control incidences were not reported (Hazleton Labs, 1981b). Overall, exposure to 1,3 butadiene induced increased incidences of pancreatic exocrine adenomas in male rats; no increase in pancreatic tumor incidence was observed in exposed female rats or in exposed male or female mice.

Significant pairwise comparisons with concurrent controls were observed for preputial gland adenoma or carcinoma in mice in the stop-exposure experiments with highest cumulative exposures (NTP, 1993). In the 103-week experiment, the survival-adjusted incidence for preputial gland carcinoma was significantly increased compared to concurrent controls. Importantly, preputial gland carcinomas are rare in B6C3F1 mice and were not observed in historical controls according to (NTP, 1993). Overall, increased incidences of preputial gland adenomas and/or carcinomas were observed in mice exposed to higher cumulative levels of 1,3-butadiene in a single study (NTP, 1993), with no corresponding data available for rats.

Subcutaneous skin tumors exhibited significant dose-related trends and pairwise comparisons with concurrent control for increased incidences of subcutaneous skin hemangiosarcoma and neurofibrosarcoma or sarcoma in female mice. Significantly, incidences in several groups exceeded the upper limits of the respective historical control ranges (NTP, 1993). In contrast, no increase in subcutaneous skin tumor incidence was observed in male mice across two studies (NTP, 1993, 1984), and similarly, no increase was found in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b). Overall, exposure to 1,3-butadiene resulted in increased incidences of subcutaneous skin tumors in female mice. However, no such increase was observed in exposed male mice or male or female rats.

Testicular tumors exhibited significant dose-related trends and pairwise comparisons with concurrent controls, indicating an increased incidence of testicular Leydig cell tumors in male rats (Hazleton Labs,

1981b). However, no increase in testicular tumor incidence was observed in male mice across two studies (NTP, 1993, 1984), indicating a lack of consistency across species. Overall, increased incidences of testicular Leydig cell tumors were observed in rats. In contrast, no similar increase in testicular tumor incidence was observed in male mice.

Studies showed significant dose-related trends and pairwise comparisons with concurrent control for increased incidence of thyroid follicular cell adenomas in female rats (Hazleton Labs, 1981b). However, no increase in thyroid tumor incidence was observed in male rats (Hazleton Labs, 1981b), indicating a lack of consistency across sexes. In addition, no increase in thyroid tumors was observed in mice (NTP, 1993, 1984), indicating a lack of consistency across species. Overall, Increased incidences of thyroid tumors were observed in female rats. No increase in thyroid tumor incidence was observed in exposed male rats or mice of either sex.

Significant dose-related trend for increased incidence of Zymbal gland carcinomas was observed in female rats (Hazleton Labs, 1981b). In contrast, low incidences of Zymbal gland adenomas and carcinomas were observed in male and/or female mice in all mouse studies including stop-exposure studies (NTP, 1993, 1984). Importantly, Zymbal gland tumors are rare in B6C3F1 mice and were not seen in historical controls according to (NTP, 1993). No significant pairwise comparisons for Zymbal gland carcinomas were found in female rats. No increase in tumor incidence in male rats. Historical control incidences were not reported (Hazleton Labs, 1981b). In addition, tumor incidences in mice were not significantly increased over concurrent controls at any exposure level, and there were no significant dose-related trends (NTP, 1993, 1984). Overall, Zymbal gland tumors were observed in female rats with dose-related trend and at low incidences in male and female B6C3F1 mice, where these tumors are rare.

Uterine tumors exhibited a significant dose-related trend for increased incidence of uterine sarcomas in female rats (Hazleton Labs, 1981b). However, no significant pairwise comparisons with concurrent control for uterine sarcomas were found in female rats and no historical control data were reported for uterine tumors in female rats (Hazleton Labs, 1981b). Additionally, there was no increase in uterine tumor incidence in female mice across two studies (NTP, 1993, 1984). This indicates a lack of consistency across species. Overall, although a dose-related trend for increased uterine tumors was observed in rats, the absence of significant pairwise comparison weakens the strength of these findings.

Across multiple studies, dose related increases in tumors were consistently observed in mice, particularly affecting lymphohematopoietic system, heart, gastrointestinal tract, lungs. These findings persisted even after survival adjustments and were noted in stop-exposure studies, confirming the carcinogenic potential of 1,3-butadiene. However, rats did not exhibit similar tumor profiles. For example, while malignant lymphomas were prevalent in mice, rats exposed to 1,3-butadiene did not show a corresponding increase in hematopoietic system cancers. Similarly, heart hemangiosarcoma's and gastrointestinal tumors were seen in mice, but not in rats, suggesting a species-specific response. Moreover, tumors observed at the lowest dose of 6.25 ppm in mice, particularly alveolar bronchiolar adenoma or carcinoma in females, whereas in rats, significant tumor development was only seen at 1,000 ppm and higher.

5.1.3 Mechanistic and Supporting Evidence

Mechanistic studies have provided substantial evidence regarding the mutagenic and carcinogenic properties of 1,3-butadiene. As outlined in Section 3.3, the bioactivation of 1,3-butadiene into DNA reactive metabolite is a critical step in its carcinogenic MOA, leading to DNA adduct formation, DNA damage, and mutations, as extensively discussed in Section 5.3.These effects have been observed in both human and rodent cells. The evidence shows that 1,3-butadiene exposure causes genotoxicity through

DNA adduct formation and mutations in cancer-related genes, correlating with species difference in metabolism.

5.1.4 Cancer Classification and Evidence Integration Conclusions

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,3-butadiene is considered "Carcinogenic to Humans" based on the adequate evidence demonstrating 1,3-butadiene's carcinogenic potential in both humans and animals across multiple tumor types.

Table 5-1 summarizes the evidence integration judgements for each evidence stream across all cancer types. Evidence for lymphohematopoietic cancer was robust across all three evidence streams. Bladder cancer had moderate human evidence, and all other cancers had indeterminate human evidence. See Appendix A for the full evidence integration table, organized by cancer type. For complete details on evidence integration judgements within and across evidence streams, see the evidence profile tables for data-rich organ systems in Appendix A. Evidence integration judgements were determined based on considerations described in Chapter 7 of the Draft Systematic Review Protocol (U.S. EPA, 2021). In short, strength of the evidence judgements (robust, moderate, slight, indeterminate, or compelling evidence of no effect) for individual evidence streams (*i.e.*, human, animal, mechanistic) were determined by expert judgement based on quality of the database, consistency, magnitude and precision, dose-response, and biological significance. For cancer, the overall cancer classification incorporates considerations across evidence streams for all cancers, consistent with (U.S. EPA, 2005a).

Table 5-1. Evidence Integration Judgements for Each Cancer Type

Cancer Type	Human	Animal	Mechanistic
Lymphohematopoietic	Robust	Robust	Robust
Bladder	Moderate	Indeterminate	Slight
Brain	Indeterminate	Slight	Slight
GI	Indeterminate	Moderate	Slight
Harderian	N/A	Moderate	Slight
Heart	Indeterminate	Robust	Slight
Liver	Indeterminate	Moderate	Slight
Lung	Indeterminate	Moderate	Slight
Mammary	Indeterminate	Moderate	Slight
Ovary	Indeterminate	Slight	Slight
Pancreas	Indeterminate	Slight	Slight
Preputial	Indeterminate	Slight	Slight
Skin	Indeterminate	Slight	Slight
Testes	Indeterminate	Slight	Slight
Thyroid	Indeterminate	Slight	Slight
Zymbal	N/A	Slight	Slight
CNS	Indeterminate	Indeterminate	Slight
Germ cell	Indeterminate	Indeterminate	Slight
Ocular	Indeterminate	Indeterminate	Slight
Uterus	Indeterminate	Indeterminate	Slight

5.2 Genotoxicity and Mutagenicity

Extensive evidence has demonstrated the genotoxic potential of 1,3-butadiene across various biological systems. Briefly, 1,3-butadiene has been found to induce genotoxic effects in a wide range of *in vitro* and *in vivo* test systems. 1,3-Butadiene genotoxicity is attributed to its metabolic activation into DNA-reactive epoxide intermediates, primarily the epoxide metabolites EB, DEB, and EBD (ATSDR, 2012; U.S. EPA, 2002a).

Studies have shown that these epoxide metabolites cause various types of genetic damage, including sister chromatid exchanges (SCEs), micronuclei, and DNA adducts (ATSDR, 2012; U.S. EPA, 2002a). Moreover, this genotoxic effect has been consistently observed in various experimental models, including bacterial mutagenicity assays, mammalian cell cultures, and *in vivo* studies (Albertini et al., 2010). Studies on the genotoxicity of 1,3-butadiene in bacteria show variable results, with positive findings consistently observed only in the presence of liver S9 metabolic activation system (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002a). Numerous inhalation studies have consistently demonstrated the genotoxic effects of 1,3-butadiene in rodents, including the increased formation of micronuclei in erythrocytes, spermatocytes, and bone marrow cells, as well as increased sister chromatid exchanges in mice (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002a). In both mice and rats, an increased HPRT locus mutation was observed in splenic T cells (ATSDR, 2012).

Limited studies in rats suggest that exposure to 1,3-butadiene at tested doses does not increase micronuclei or sister chromatid exchanges in bone marrow (Autio et al., 1994; Cunningham et al., 1986). The decreased genotoxicity observed in rats, potentially linked to the reduced formation DNA reactive metabolites compared to mice, may also play a role in the lower incidence of 1,3-butadiene induced cancers in rats.

In rodent models, DEB is recognized as the most genotoxic metabolite due to its ability to form DNA interstrand cross-links and is considered the primary carcinogenic metabolite (Swenberg et al., 2011; Cochrane and Skopek, 1994). Furthermore, quantitative genotoxicity studies in mice have revealed that DEB is 40-fold more genotoxic than EB and 100-fold genotoxic than EBD (Cochrane and Skopek, 1994). In contrast, humans predominantly metabolize 1,3-butadiene into EBD, as evidenced by higher levels of EBD-derived hemoglobin adducts compared to other metabolites (Boysen et al., 2012; Albertini et al., 2003). A recent study has revealed that EBD and analogs have the potential to induce DNA damage at a similar rate to DEB in cells deficient in Fanconi anemia genes (FANC) (Nakamura et al., 2021). This is significant due to the high bioavailability of EBD in humans, with bone marrow being the primary target of 1,3-butadiene toxicity (Tice et al., 1987). However, the precise contribution of these metabolites to 1,3-butadiene-induced carcinogenicity in humans remains to be fully elucidated.

Several occupational exposure cohorts have investigated 1,3-butadiene's genotoxicity with variable results. Some studies, particularly in Texas, have reported HPRT gene mutations significantly elevated in BD-exposed workers while studies in China and the Czech Republic did not find such elevations, possibly due to differences in exposure levels and methodologies (ATSDR, 2012). However, several studies using micronucleus assay on exposed humans have consistently shown that occupational 1,3-butadiene exposure induces chromosome damage (Federico et al., 2019; Xiang et al., 2015; Cheng et al., 2013; Xiang et al., 2012; Wang et al., 2010). Despite some variability in human studies, the overall weight of scientific evidence strongly suggests that 1,3-butadiene poses a significant genotoxic and mutagenic risk.

Results from mutagenicity or chromosome/cytogenetic damage assays are summarized together in Table 5-2. This table includes all data summarized in EPA IRIS (2002a), ATSDR (2012), and IARC (2008b). Positive studies are bolded. A formal evaluation of mutagenicity as the primary mode of action for carcinogenicity follows in Section 5.3.

Table 5-2. Summary of Mutagenicity and Chromosome Damage Studies from KE3

Assay Type	Test System (Species/ Strain/ Sex)	Metabolic Activation	Results	Reference
Gene mutations – *in vitro*				
Bacterial reverse mutation assay	*S. typhimurium* TA100	With S9 fraction activation	**Positive**	Araki et al. (1994)
Bacterial reverse mutation assay	*S. typhimurium* TA1535	With S9 fraction activation	**Positive**	De Meester et al. (1980)
Bacterial reverse mutation assay	*S. typhimurium* TA1535	With S9 fraction activation	**Positive**	Arce et al. (1990)
Bacterial reverse mutation assay	*S. typhimurium* TA1535	With S9 fraction activation	**Positive**	Araki et al. (1994)
Bacterial reverse mutation assay	*S. typhimurium* TA1535	With S9 fraction activation	**Positive**	Madhusree et al. (2002)
Bacterial reverse mutation assay	*S. typhimurium* TA1537	With and without activation	Negative	Araki et al. (1994)
Bacterial reverse mutation assay	*S. typhimurium TA98*	With and without activation	Negative	Arce et al. (1990)
Bacterial reverse mutation assay	*S. typhimurium* TA98	With and without activation	Negative	Araki et al. (1994)
Bacterial reverse mutation assay	*S. typhimurium* TA97	With and without activation	Negative	Arce et al. (1990)
Bacterial reverse mutation assay	*E. coli WP2 uvrA*	With and without activation	Negative	Araki et al. (1994)
Bacterial reverse mutation assay	*S. typhimurium* TA100	With and without activation	Negative	Victorin and Ståhlberg (1988)
Bacterial reverse mutation assay	*S. typhimurium* TA100	With and without activation	Negative	Arce et al. (1990)
Gene mutations – rodents *in vivo*				
Mice data				
hprt locus in T lymphocytes	CD1 mice	Not applicable	Negative	Tates et al. (1998)
hprt locus in T lymphocytes	B6C3F1 mice	Not applicable	**Positive**	Tates et al. (1994)
hprt locus in T lymphocytes	(102 × C3H)F1 mice	Not applicable	**Positive**	Tates et al. (1998)
hprt locus in T lymphocytes	B6C3F1 mice	Not applicable	**Positive**	Meng et al. (1999); Meng et al. (1998)
hprt locus in T lymphocytes (high dose)	B6C3F1 mice	Not applicable	**Positive**	Meng et al. (1998)
hprt locus in T lymphocytes (low dose)	B6C3F1 mice	Not applicable	**Positive**	Meng et al. (1999)

Assay Type	Test System (Species/ Strain/ Sex)	Metabolic Activation	Results	Reference
hprt loci mutations in splenic T lymphocytes	B6C3F1 mice	Not applicable	**Positive**	Cochrane and Skopek (1994)
Spot test	T-stock female mice	Not applicable	**Positive**	Adler et al. (1994)
lacI locus in bone marrow (*i.e.*, Big Blue)	B6C3F1 mice	Not applicable	**Positive**	Recio et al. (1996); Sisk et al. (1994)
lacI locus in spleen (*i.e.*, Big Blue)	B6C3F1 mice	Not applicable	**Positive**	Recio et al. (1998)
lacZ mutant frequency in lung	B6C3F1 mice	Not applicable	**Positive**	Recio et al. (1992)
lacZ-mutant frequency in liver and bone marrow	B6C3F1 mice	Not applicable	Negative	Recio et al. (1992)
Rat data				
hprt locus in T lymphocytes (high dose)	F344 rats	Not applicable	**Positive**	Meng et al. (1998)
hprt locus in T lymphocytes (lower dose)	F344 rats	Not applicable	**Positive**	Meng et al. (1999)
Gene mutations – humans				
Hprt loci in peripheral lymphocytes	Humans	Not applicable	Negative	Hayes et al. (2001); Hayes et al. (2000); Hayes et al. (1996)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	Negative	Tates et al. (1996)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	Negative	Albertini et al. (2007); Albertini et al. (2001)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	Negative	Liu et al. (2008)
Hprt exon deletion	Humans	Not applicable	**Positive**	Liu et al. (2008)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	**Positive**	Ward et al. (1994)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	**Positive**	Abdel-Rahman et al. (2005); (Abdel-Rahman et al., 2003); Abdel-Rahman et al. (2001); Ammenheuser et al. (2001)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	**Positive**	Ma et al. (2000)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	**Positive**	Ward et al. (2001); Ward et al. (1996b)
Hprt loci in peripheral lymphocytes	Humans	Not applicable	**Positive**	Wickliffe et al. (2009)

Assay Type	Test System (Species/ Strain/ Sex)	Metabolic Activation	Results	Reference
Somatic cytogenetic effects				
Rodent studies				
Micronuclei and sister chromatid exchange	B6C3F1 mice	Not applicable	**Positive**	Cunningham et al. (1986)
Micronuclei in bone marrow and peripheral blood	(102 x C3H)F1 mice	Not applicable	**Positive**	Adler et al. (1994)
Micronuclei in spleen and peripheral blood	(102 x C3H)F1 mice	Not applicable	**Positive**	Stephanou et al. (1998)
Chromosomal aberration, sister chromatid exchange, Average generation time, mitotic index	B6C3F1 mice	Not applicable	**Positive**	Tice et al. (1987)
Micronuclei	B6C3F1 mice	Not applicable	**Positive**	Autio et al. (1994)
Micronuclei	Swiss mice	Not applicable	**Positive**	Irons et al. (1987)
Micronuclei	B6C3F1 mice	Not applicable	**Positive**	Jauhar et al. (1988)
Micronuclei	NMRI mice	Not applicable	**Positive**	Vodicka et al. (2006)
Micronuclei, Chromosomal aberration, sister chromatid exchange	B6C3F1 mice	Not applicable	**Positive**	Tice (1988)
Chromosomal aberration, sister chromatid exchange	C57B1/6 mice	Not applicable	**Positive**	Sharief et al. (1986)
Micronuclei	Wistar rats	Not applicable	Negative	Autio et al. (1994)
Micronuclei and sister chromatid exchange	SD rats	Not applicable	Negative	Cunningham et al. (1986)
Human studies				
Chromosome aberrations in peripheral blood	Humans	Not applicable	Negative	Au et al. (1995)
Chromosomal aberration, Sister chromatid exchange	Humans	Not applicable	Negative	Lovreglio et al. (2006)
Chromosomal aberration, Sister chromatid exchange	Humans	Not applicable	**Positive**	Šrám et al. (1998)
Micronuclei	Humans	Not applicable	**Positive**	Wang et al. (2010)
Micronuclei	Humans	Not applicable	**Positive**	Xiang et al. (2012)
Micronuclei	Humans	Not applicable	**Positive**	Cheng et al. (2013)
Micronuclei	Humans	Not applicable	**Positive**	Xiang et al. (2015)
Micronuclei	Humans	Not applicable	**Positive**	Federico et al. (2019)
Positive results are **bolded** for easier scoring.				

5.3 Mutagenic Mode of Action Analysis

1,3-butadiene is a potent multi-organ carcinogen in laboratory animals, notably inducing lymphomas in mice and exhibiting greater carcinogenic potential in mice than rats (NTP, 1993; Hazleton Labs, 1981b). Epidemiological evidence consistently links occupational 1,3-butadiene exposure to increased mortality from lymphatic and hematopoietic cancers (ATSDR, 2012; U.S. EPA, 2002a). As an alkylating agent, 1,3-butadiene induces genotoxic effects across various biological systems (Albertini et al., 2010). 1,3-Butadiene is an indirect carcinogen, requiring biotransformation into electrophilic metabolites to exert mutagenicity and carcinogenicity (Kirman et al., 2010a). The MOA underlying the development of cancer in humans and tumors in rodents is hypothesized to be associated with the mutagenic potential of one or more of 1,3-butadiene metabolites. While 1,3-butadiene's genotoxic and carcinogenic potential is clearly linked to its DNA-reactive metabolites, the specific metabolites responsible for its multi-organ carcinogenicity remain to be fully elucidated. EPA evaluated the potential for 1,3-butadiene to exhibit a mutagenic MOA (Albertini et al., 2010; Kirman et al., 2010a; Preston, 2007) and the mutagenic analysis previously presented by EPA (U.S. EPA, 1985). Evidence for each key event (KE) through which a mutagenic MOA might be instrumental in 1,3-butadiene induced hematopoietic cancers is presented in the following section. This analysis was performed in accordance with EPA guidelines for carcinogen risk assessment (U.S. EPA, 2005a) and the draft framework for determining a mutagenic mode of action for carcinogenicity (U.S. EPA, 2007).

The mutagenic MOA involves the following sequence of key events (KEs):

- KE1: Bioactivation of 1,3-butadiene to DNA-reactive metabolites.
- KE2: Formation of DNA adducts and DNA damage by 1,3-butadiene metabolites in target cells.
- KE3: Chromosomal aberrations and/or mutations arising from 1,3-butadiene-induced DNA damage.
- KE4: Development of cancer from 1,3-butadiene-induced mutations.

5.3.1 Key Event 1: Bioactivation of 1,3-Butadiene to DNA-Reactive Metabolites

Metabolism plays a crucial role in determining 1,3-butadiene's carcinogenicity. Specifically, as detailed in Section 3.3, 1,3-butadiene undergoes metabolic activation primarily in the liver by cytochrome P450 enzymes. This process converts 1,3-butadiene into electrophilic intermediates, including EB, EBD, and DEB. In brief, cytochrome P450 initially transforms 1,3-butadiene into EB, which can then be further metabolized into DEB or B-Diol through epoxide hydrolase. Subsequently, B-Diol can be converted into EBD, which can undergo further bioactivation to form a bifunctional epoxy aldehyde. Additionally, DEB can also be converted into EBD via epoxide hydrolase. These three epoxides—EB, DEB, and EBD—are highly reactive with nucleophilic sites in DNA, forming adducts that are genotoxic and mutagenic (Albertini et al., 2010).

The substantial interspecies variation in cancer susceptibility to 1,3-butadiene, with mice exhibiting markedly higher sensitivity than rats, is consistent with documented differences in 1,3-butadiene metabolism and resulting genotoxicity (Albertini et al., 2010; Kirman et al., 2010a; Himmelstein et al., 1997). Specifically, mice exhibit faster rates of metabolism to DNA reactive metabolites and slower rates of hydrolysis compared to other species, resulting in higher DEB blood levels (Kirman et al., 2010b). Importantly, both species, as well as humans, metabolize 1,3-butadiene into reactive intermediates capable of DNA interaction, thereby presenting a potential carcinogenic hazard. Human enzyme kinetics result in greater EBD compared to rats and mice. This point is evident from the higher levels of EBD-derived hemoglobin adducts detected in humans compared to other metabolites (Boysen et al., 2012; Albertini et al., 2003). In addition to these well- characterized 1,3-butadiene metabolites, recent studies have identified alternative pathways leading to the formation of additional bifunctional

metabolites. These include chlorinated metabolites formed via myeloperoxidase and hypochlorous acid (Wu et al., 2019; Wang et al., 2018; Elfarra and Zhang, 2012), as well as ketone/aldehyde metabolites of EBD formed via alcohol dehydrogenase (Nakamura et al., 2021). These bifunctional metabolites are particularly significant because of their unique ability to induce complex DNA damage, such as DNA-protein cross links or DNA interstrand cross links. These complex lesions are more difficult for DNA repair mechanisms to resolve, thereby increasing the risk of mutations and contributing to cellular toxicity. Although the potential contribution of these additional bifunctional metabolites to 1,3-butadiene induced mutagenicity and carcinogenicity remains to be fully elucidated, they may play a critical role in human carcinogenicity. Given the high bioavailability of EBD in humans and the presence of myeloperoxidase in neutrophils and monocytes, these alternative pathways may exist in the bone marrow and blood, potentially leading to the production of leukemia, with bone marrow being a primary target of 1,3-butadiene (Tice et al., 1987). See Section 3.3 for more details.

5.3.2 Key Event 2: Formation of DNA Adducts and DNA Damage by 1,3-Butadiene Metabolites in Target Cells

The formation of DNA adducts by 1,3-butadiene is a crucial step in initiating its carcinogenic process. These adducts arise from the covalent binding of 1,3-butadiene metabolites to DNA, leading to mutations that ultimately contribute to cancer development. 1,3-Butadiene's electrophilic metabolites, specifically EB, DEB, and EBD, are highly reactive with nucleophilic sites in DNA, forming a variety of adducts and playing a central role in mediating the genotoxic and mutagenic effects (ATSDR, 2012; U.S. EPA, 2002a). These adducts have been detected *in vitro* and *in vivo*, as well as in occupationally exposed workers (ATSDR, 2012). *In vitro* studies across bacterial, mammalian, and human cell lines demonstrate the DNA damaging potential of 1,3-butadiene metabolites (IARC, 2008b). These electrophilic metabolites have been shown to form DNA adducts, induce DNA strand breaks, stimulate unscheduled and DNA excision repair (Albertini et al., 2010). In addition to being observed in *in vitro*, DNA adducts are detected *in vivo* across multiple tissues in 1,3-butadiene exposed mice, including within tissues identified as targets of carcinogenesis (ATSDR, 2012). The specific types of adducts identified in mice include N7-(2-hydroxy-3-butenyl)guanine and N6-adenine, which are primarily derived from the 1,3-butadiene metabolite DEB (Goggin et al., 2009). 1,3-Butadiene exposure has been linked to increased formation of N-7-(2,3,4 trihydroxybutyl) guanine adducts in liver DNA across various mouse strains (Koturbash et al., 2011b; Koturbash et al., 2011a). N7-(2,3,4-trihydroxybutyl)guanine adducts, formed from B-diol, were also found in the liver DNA of the animals (Walker and Meng, 2000).

Evidence from human studies supports the link between 1,3-butadiene exposure and DNA damage. The diepoxide, DEB, which is detected in human blood at significantly lower amounts compared to rodents (ATSDR, 2012; Swenberg et al., 2011), is considered the most potent genotoxic moiety among 1,3-butadiene metabolites (see Section 5.2 and (ATSDR, 2012; U.S. EPA, 2002a)). However, it has also been hypothesized that EBD, rather than DEB, is the primary metabolite responsible for human DNA adducts and resulting carcinogenicity (Nakamura et al., 2021; Boogaard et al., 2001). In a DNA repair assay on repair-deficient chicken DT40 B lymphocyte and human TK6 lymphoblastoid cells, EBD and analogs were similarly genotoxic to DEB, and authors propose that EBD may bioactivate into a bifunctional moiety through alcohol dehydrogenase (Nakamura et al., 2021). Several studies have also shown a positive correlation between occupational exposure to 1,3-butadiene and levels of DNA adducts in peripheral blood lymphocytes (ATSDR, 2012; U.S. EPA, 2002a). One study found that workers exposed to 1,3-butadiene had significantly higher levels of DNA adducts than control groups (Zhao et al., 2000). Another study found that the level of DNA adducts, specifically N-1-(2,3,4-trihydroxybutyl)adenine (N-1-THB-Ade) formed from EBD, increased with increasing levels of exposure to 1,3-butadiene (Zhao et al., 2001). Furthermore, 1,3-butadiene exposure might hinder DNA

repair mechanisms due to the potential formation of cross-links between DNA and proteins by its bifunctional metabolites (Albertini et al., 2010). Ultimately, these DNA adducts can induce mispairing during DNA replication, leading to point mutations, deletions, chromosome damage and other forms of genetic damage that contribute to tumor initiation and progression (Goggin et al., 2011; Albertini et al., 2010; Kirman et al., 2010a).

5.3.3 Key Event 3: Chromosomal Damage and/or Mutations Arising from 1,3-Butadiene-Induced DNA Damage

A critical mechanism underlying 1,3-butadiene induced carcinogenicity is its ability to induce chromosomal aberrations and mutations across various biological systems, from prokaryotes to humans. The mutagenic potential of 1,3-butadiene is well supported by extensive evidence, including numerous positive results from both *in vivo* and *in vitro* mutation assays conducted on human and rodent cells (ATSDR, 2012; U.S. EPA, 2002a). Studies have shown that 1,3-butadiene's mutagenic activity primarily arises from its metabolites, particularly the epoxides DEB, EB, and EBD, as well as potentially novel bifunctional metabolites such as chlorinated and ketone/aldehyde derivates. Studies have demonstrated the mutagenic activity of 1,3-butadiene in bacterial systems, specifically inducing gene mutations in *Salmonella typhimurium* strains TA100 and TA1535, but this activity was observed only in the presence of metabolic activation with an S9 fraction (Madhusree et al., 2002; Araki et al., 1994; Arce et al., 1990; De Meester et al., 1980).

In animals, even brief exposure (10 days) can significantly increase the frequency of sister chromatid exchange and chromosomal aberrations in blood cells, even at the lowest concentration tested in mice (Tice et al., 1987; Cunningham et al., 1986). Moreover, exposure to inhaled 1,3-butadiene has been shown to elevate micronucleus induction in erythrocytes, spermatocytes and bone marrow cells in mice (Vodicka et al., 2006; Tommasi et al., 1998; Xiao and Tates, 1995; Autio et al., 1994; Jauhar et al., 1988; Irons et al., 1987; Tice et al., 1987). However, results from a limited number of rat studies indicate that exposure to 1,3-butadiene does not result in increased induction of micronuclei (Autio et al., 1994) or sister-chromatid exchanges (Cunningham et al., 1986) in the bone marrow at the doses tested. This interspecies difference in response may contribute to the lower incidence of 1,3-butadiene induced cancer in rats, which is potentially attributable to reduced genotoxic metabolite formation and more efficient detoxification via epoxide hydrolase. In addition, studies have shown that 1,3-butadiene induces specific mutations in critical genes involved in cancer development, including oncogenes (*e.g.*, K-ras) and tumor suppressor genes (*e.g.*, TP53), as well as those in the Wnt signaling pathway (ATSDR, 2012; U.S. EPA, 2002a).

In vivo studies have also examined gene mutations at marker genes (lacI and lacZ) in the tissues of transgenic mice. Specifically, transgenic B6C3F1 mice exposed to 1,3-butadiene exhibited an elevated lacZ-mutant frequency in lung tissue (Recio et al., 1992). Similarly, inhalation exposure to 1,3-butadiene significantly increased the mutant frequency of lacI transgene (*i.e.*, Big Blue assay) in bone marrow of B6C3F1 transgenic mice, with a predominance of point mutations occurring at A base pairs (Sisk et al., 1994). This increase in mutation frequency was consistent across both short-term (5 days) and extended (4 weeks) exposures, indicating even brief inhalation exposure of 1,3-butadiene can induce significant mutagenic effect in mouse bone marrow (Recio et al., 1996) and spleen (Recio et al., 1998). Furthermore, increased percentages of H-ras and K-ras proto-oncogene mutations were observed in forestomach neoplasms of mice following chronic inhalation exposure to 1,3-butadiene (Sills et al., 2001). Similarly, mice and rats exhibited increased hprt locus mutations in splenic T cells (Meng et al., 2007; Meng et al., 2004; Meng et al., 2000; Meng et al., 1999; Cochrane and Skopek, 1994). Studies on exposed male mice (Adler et al., 1998; Anderson et al., 1998; Brinkworth et al., 1998; Anderson et al., 1996; BIBRA, 1996b; Adler et al., 1995; Xiao and Tates, 1995) but not rats (Anderson et al., 1998;

BIBRA, 1996a) have also consistently observed germ cell-specific cytogenetic damage and resulting dominant lethality following mating with unexposed females.

In contrast, epidemiological studies have produced mixed but still mostly positive (6 of 10 studies) findings regarding the association between 1,3-butadiene exposure and genetic damage in workers using HPRT and SCE assays (ATSDR, 2012). Some studies involving Texas workers found a potential association between elevated 1,3-butadiene exposure and increased frequencies of HPRT variants in lymphocytes (Wickliffe et al., 2009; Abdel-Rahman et al., 2005; Abdel-Rahman et al., 2003; Abdel-Rahman et al., 2001; Ammenheuser et al., 2001; Ward et al., 2001; Ma et al., 2000; Ward et al., 1996b). However, other studies found no significant increase in chromosome aberrations of HPRT mutations among workers exposed to 1,3-butadiene, such as those in the Czech Republic and China (Albertini et al., 2007; Albertini et al., 2001; Hayes et al., 2001; Hayes et al., 2000; Hayes et al., 1996; Tates et al., 1996). Despite these mixed results, more recent studies using micronucleus assay have consistently demonstrated that occupational exposure to 1,3-butadiene causes chromosomal damage in humans.

One study on highly exposed Chinese workers reported a positive association between 1,3-butadiene exposure and micronuclei induction in peripheral blood lymphocytes (Wang et al., 2010). Similarly, workers exposed to high levels of 1,3-butadiene in a poly-butadiene latex plant exhibited significantly higher micronucleus frequencies (Xiang et al., 2012). A follow-up study in a rubber factory confirmed this trend, demonstrating elevated micronucleus frequencies even at lower 1,3-butadiene exposure, although no significant changes in sister chromatid exchange were observed (Cheng et al., 2013). These studies also highlighted the influence of genetic polymorphisms on micronucleus frequency. In another study, the workers at a petrochemical factory exposed to high levels of 1,3-butadiene exhibited elevated micronucleus and nucleoplasmic bridge frequencies, with gene polymorphisms influencing these outcomes (Xiang et al., 2015).

An Italian study also observed increased micronuclei frequency in petroleum refinery workers and nearby residents, although 1,3-butadiene concentrations were not measured (Federico et al., 2019). While earlier studies using HPRT and SCE assay showed mixed results due to variations in exposure assessments (active vs. passive sampling) and mutation analysis methodologies (autoradiography vs. cloning hprt assays), recent studies employing the micronucleus assay consistently demonstrate that occupational 1,3-butadiene exposure induces chromosome damage. Furthermore, chronic myeloid leukemia (CML) has been reported with increased incidence among worker populations exposed to 1,3-butadiene (Delzell et al., 2006), and CML requires a specific t(9:22) translocation that can only arise via mutagenicity. A relatively recent study found that DEB does *not* induce t(9:22) translocations in a cultured leukemia cell line (Walker et al., 2019), supporting evidence from (Nakamura et al., 2021) and (Boogaard et al., 2001) suggesting that metabolites other than DEB may lead to lymphohematopoietic carcinogenesis, especially in humans. Additionally, EBD is positive for hprt mutations or micronuclei formation in four of five studies summarized by (U.S. EPA, 2002a), including human cells *in vitro*, with the only negative study from rats. For comparison, DEB is consistently positive for cytogenetic damage in all studies and species but mixed for gene mutations in mice and rats while EB is mostly positive for cytogenetic damage but mixed for mutations (U.S. EPA, 2002a). Table 5-2 summarizes results from mutagenicity and chromosome/cytogenetic damage assays relevant to this key event.

5.3.4 Key Event 4: Development of Cancer from 1,3-Butadiene-Induced Mutations

Following the induction of mutations as described previously, uncontrolled cell proliferation emerges as the final key event in 1,3-butadiene induced carcinogenesis. This arises from the cumulative effect of genetic damage, including mutations and chromosome aberrations. In rodent studies, chronic exposure to 1,3-butadiene leads to the development of tumors in various organs, including the hematopoietic

system (NTP, 1993; Hazleton Labs, 1981b). Supporting these findings, numerous epidemiological studies have established a strong correlation between occupational exposure to 1,3-butadiene and increased mortality due to hematological malignancies in humans (Sathiakumar et al., 2021b; Delzell et al., 2006; Delzell et al., 1996).

5.3.5 Mutagenic MOA: Weight of Evidence Analysis

In accordance with the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), this analysis of a mutagenic MOA follows the Bradford Hill criteria (or considerations) developed for evaluating epidemiological studies (Hill, 1965). Hill considerations are indicated in italics in the following discussion.

Strength and Consistency

The association between 1,3-butadiene exposure and the mutagenic outcomes is well established. As described above, numerous studies have demonstrated the formation of reactive metabolites along with statistically significant increases in DNA adducts, gene mutations, chromosome aberrations, and micronuclei formation following exposure to 1,3-butadiene (ATSDR, 2012). Although the bifunctional epoxide metabolite DEB (considered the most genotoxic moiety) is formed at low levels in humans (Section 3), evidence from Hb adducts indicates that EBD levels are the same or higher in humans compared to mice and significantly higher compared to rats (Boysen et al., 2012; Swenberg et al., 2011). A recent study suggests that EBD may be genotoxic as DEB through a novel bioactivation mechanism (Nakamura et al., 2021). Moreover, this association is relatively consistent across epidemiological and animal studies, which have consistently reported genetic damage and associated mutations due to 1,3-butadiene exposure with few exceptions; the only two studies in rats did not demonstrate genotoxicity in bone marrow at the dose tested. There is also some variability in results on peripheral lymphocytes across human occupational studies, but this may be explained by differences in polymorphism rates across populations (Section 7.2) or study methodologies. Additionally, the presence of chromosomal damage (which is difficult to repair and is consistent with the induction of CML) is observed consistently across exposed occupational cohorts. Overall, the weight of scientific evidence, including the consistent demonstration of DNA damage and resulting mutations across species and assay types, strongly supports the association between 1,3-butadiene exposure and mutagenic outcomes.

Specificity

Specificity is not required or even necessarily expected for a multisite mutagen and carcinogen such as 1,3-butadiene (U.S. EPA, 2005a). Nonetheless, mutations have been commonly observed in immune cells (including from transgenic mice assays)(ATSDR, 2012), corresponding to the blood cancers observed in both mice and humans as well as spleen and bone marrow (ATSDR, 2012). Among leukemia cases identified in the Alabama cohort, the strongest association in one study (Delzell et al., 2006) was identified for chronic myelogenous leukemia (CML), cancer that requires a specific activating t(9:22) genomic translocation. Increased incidence of a cancer for which a mutation is both necessary and sufficient strongly supports the mutagenic MOA human evidence of carcinogenicity in humans.

Temporality

A clear temporal relationship is evident, with genetic damage (Jauhar et al., 1988; Tice et al., 1987) and transgene mutations (Recio et al., 1996) observed shortly (*e.g.*, within days) after the exposure in various acute and subchronic studies. Lymphocytic lymphoma also developed very quickly in mice, appearing as early as 23 weeks into exposure (NTP, 1993), indicative of mutagen-induced carcinogenesis. Similarly, a minimal lag time of as little as 10 years was identified for human leukemia cases in (Sathiakumar et al., 2021b) as described in Section 5.4.3.

Dose-Response

Animal studies demonstrate a clear *dose-response* relationship between 1,3-butadiene exposure and genetic damage. Higher exposure levels consistently correlate with increased frequencies of DNA adducts, gene mutations, chromosome aberrations, and micronuclei formation (ATSDR, 2012). Some studies have observed genetic damage to peripheral blood in mice (Tice et al., 1987) (in the absence of cytotoxicity) at the same dose, resulting in blood cancer development (NTP, 1993) (lowest dose tested). This suggests that even relatively low exposure levels can induce genetic alterations sufficient for tumorigenesis. Furthermore, a parallel increase in both the types and magnitude of tumors and mutations are observed at increasing dose, including in bone marrow from transgenic mice *in vivo* (ATSDR, 2012).

Biological Plausibility

The biological plausibility of 1,3-butadiene's carcinogenicity is strongly supported by its ability to form mutagenic metabolites that directly interact with DNA and cause mutations both in mice and humans. DNA damage and mutations, which are known to cause cancer, are observed in bone marrow and blood cells, the primary targets of leukemia. CML is associated with 1,3-butadiene exposure in humans (Delzell et al., 2006) and requires a specific activating genomic translocation [(t9;22)].

Coherence

Experimental evidence from animal studies aligns with epidemiological data, demonstrating tumor formation in various tissues following chronic exposure. Genotoxicity and mutagenicity data on parental 1,3-butadiene also agrees with data on metabolites, with primarily positive results from *in vivo* mammalian/human studies and metabolic activation required for prokaryotes. Observed non-cancer blood effects such as anemia (Section 4.1.2.3) may be related to bone marrow dysfunction either upstream or downstream of carcinogenesis. Evidence of dominant lethality due to genotoxicity of male germ cells (Section 4.1.2.2.3) further supports mutagenicity as an important mode of action for 1,3-butadiene toxicity. The carcinogenicity of 1,3-butadiene and its metabolite aligns with the broader observation that other epoxide and reactive metabolites are known carcinogens, further strengthening the evidence of its carcinogenic potential.

Uncertainties and Alternative Modes of Action

Although the weight of evidence sufficiently supports a mutagenic MOA for the carcinogenicity of 1,3-butadiene, the possibility of alternative or additional MOAs cannot be excluded. Alternative modes of action have not been definitively identified or supported by the existing data. One study observed delayed differentiation and reduced maturation of bone marrow stem cells in mice following 6 weeks of exposure to 1,250 ppm 1,3-butadiene (Leiderman et al., 1986). However, EPA did not identify any subsequent supporting evidence for this mechanism. Additionally, this study applied an elevated dose well above that which causes blood cancer in mice, and genotoxicity cannot be ruled out as the initial key event for any impacts on stem cell differentiation.

Is the Hypothesized MOA Sufficiently Supported in Test Animals?

As detailed above, the weight of evidence strongly supports a mutagenic MOA for 1,3-butadiene in laboratory animals.

Is the Hypothesized MOA Relevant to Humans?

The evidence discussed above demonstrates that 1,3-butadiene is a mutagen in test animals as well as in humans. There is compelling evidence that 1,3-butadiene induces lymphohematopoietic cancer in humans and mice, which correlates with the observed genotoxicity and mutation data from blood and bone marrow. Additionally, no information has been identified to suggest that the interactions between

1,3-butadiene reactive metabolites and DNA are unique to any particular species. Therefore, the proposed mutagenic MOA is relevant to humans.

Which Populations or Life Stages Can Be Particularly Susceptible to the Hypothesized MOA?
1,3-Butadiene exhibits a mutagenic MOA, which is generally considered to pose a risk across all life stages and populations. According to the *EPA's Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), there may be increased susceptibility to early-life exposures to carcinogens with a mutagenic MOA. Therefore, given that the weight of evidence supports a mutagenic mode of action for 1,3-butadiene's carcinogenicity and no chemical-specific data on susceptibility differences, increased early-life susceptibility should be assumed. If early-life exposure occurs, age-dependent adjustment factors should be applied in accordance with the aforementioned guidance (U.S. EPA, 2005b). In conclusion, the weight of evidence supports a mutagenic MOA for 1,3-butadiene lymphohematopoietic carcinogenicity and the application of age-dependent adjustment factors (ADAFs) to address assumed early-life susceptibility.

5.3.6 Summary and Conclusions

The weight of scientific evidence strongly supports a mutagenic MOA for 1,3-butadiene in the development of lymphohematopoietic malignancies in both rodents and humans, in agreement with previous analyses (Kirman et al., 2010a; Preston, 2007; U.S. EPA, 1985). Other authoritative assessments reached the same conclusion (NTP, 2021a; IARC, 2008a), stating that 1,3-butadiene is carcinogenic through metabolism into direct-acting mutagens, likely resulting in modified function of oncogenes or tumor suppressors. However, there is insufficient evidence to determine a MOA for other cancer types.

The primary driver of 1,3-butadiene's mutagenic MOA is the formation of electrophilic metabolites (KE1), which readily react with DNA, causing adduct formation and other types of DNA damage (KE2). If not repaired, this persistent damage can lead to mutations, particularly in oncogenes and tumor suppressor genes, driving the process of carcinogenesis. Ultimately, the accumulation of mutations in critical genes results in uncontrolled cell proliferation and cancer development (KE3). The variability in 1,3-butadiene's mutagenic and carcinogenic potential across species and cancer types may be attributed to differences in its metabolism, resulting in varying levels and types of DNA damaging electrophilic metabolites. The extent and nature of this DNA damage ultimately determines the carcinogenic outcome in different biological contexts.

Given that a mutagenic MOA for 1,3-butadiene is sufficiently supported based on evidence from both laboratory animals and humans, a linear cancer assessment approach with the incorporation of ADAFs is used to calculate an inhalation unit risk (IUR) for lymphohematopoietic cancer in accordance with considerations of the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). There is uncertainty whether the mutagenic MOA (MMOA) also applies to other cancer types due to limited genotoxic data on other tissues.

5.4 Cancer Dose-Response Assessment

5.4.1 Selection of Studies and Endpoint Derivation for Carcinogenic Dose-Response Assessment

The selection of representative cancer studies and locations of tumors/tumor types for dose-response analysis is described below based on the following considerations:

- Overall quality determinations;
- Dose range and sufficiency of dose-response information;
- Strength of the evidence supporting the associated tumor type;
- MOA conclusions;
- Relevance (*e.g.*, what species was the effect in, was the study directly assessing the effect, is the endpoint the best marker for the tox outcome?);
- Uncertainties not captured by overall quality determinations;
- Endpoint sensitivity; and
- Uncertainty and sensitivity of BMR selection from BMD modeling.

According to the TSCA systematic review process (U.S. EPA, 2024h), the EPA systematic review process identified 72 epidemiological publications. Of the 72 epidemiological publications, EPA identified 35 publications that conducted dose-response association based on at least 2 exposure levels (plus a reference level) or continuous exposure data. Of these 35 epidemiological publications with dose-response analyses and cumulative exposure, 21 investigated leukemia and 7 investigated bladder cancer. Based on the evidence from Section 5.1.1, EPA concluded that the human evidence for increased risks of leukemia and other lymphohematopoietic cancers was robust and that of bladder cancer was moderate (summarized in Table 5-1) but strong enough to support the derivation of unit risk estimates. Due to the availability of substantial epidemiological dose-response information and uncertainties surrounding the most relevant rodent species for human cancer risk, animal data was not considered for cancer dose-response analysis.

The most recent hazard assessment by the International Agency for Research on Cancer (IARC) recognized sufficient evidence of carcinogenicity only for cancers of the hematolymphatic system (IARC, 2012). Similarly, the earlier listing of butadiene as a known human carcinogen in the National Toxicology Program (NTP's) *Report of Carcinogens, Fifteenth Edition* cites only evidence of an increased risk of leukemia (NTP, 2021b). Additionally, the evidence integration judgement for lymphohematopoietic cancer was robust for human, animal, and mechanistic evidence (Table_Apx A-5). Therefore, leukemia is considered as the most critical cancer outcome caused by the 1,3-butadiene exposure and was the focus of EPA's dose-response analysis.

Of the 21 leukemia epidemiological publications providing dose-response results, 18 publications used data from the U.S.-Canadian styrene-butadiene rubber (SBR) worker cohort study, 2 used data from the Texas Cancer Registry, and 1 used data from California Cancer Registry (Table 5-3). The exposure pathway of all 21 leukemia publications is inhalation.

Table 5-3. Summary of 31 Leukemia Epidemiological Studies Providing Dose-Response Association Based on at Least Two Exposure Levels (Plus a Reference Level) or Continuous Exposure Levels

Data Source	Reference	Study Period or Follow-up	Exposure Range in the Dose-Response Model	Health Outcomes	Statistically Significant Result?	Systematic Review Score
SBR Cohort	UAB (1995)	1950–1992	0 to >200 ppm-years	Leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute myelogenous or monocytic leukemia mortality	Significant positive associations	Medium
SBR Cohort	Delzell et al. (1996)	1943–1992	0 to 200+ ppm-years	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	IISRP (1999)	1944–1991	0 to >635.9 ppm-years	Leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute myelogenous or monocytic leukemia mortality	Significant positive associations	Medium
SBR Cohort	Delzell et al. (2001)	1944–1991	0 to >362.2 ppm-years	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	Sielken and Valdez-Flores (2001)	1943–1992	0–1,776 ppm-years	Leukemia mortality	Not reported, no confidence interval	Low
SBR Cohort	Graff et al. (2005)	1943–1998	0 to >124.7 ppm-years	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	Sathiakumar et al. (2005)	1944-1998	No quantitative data reported	Leukemia (Hodgkin's disease, multiple myeloma, all leukemia, non-Hodgkin's lymphoma) mortality	No significant associations	Medium
SBR Cohort	Cheng et al. (2007)	1944–1998	Average BD intensity ppm: mean (SD) leukemia cases = 35.5 (71.4), non-cases = 24.0 (54.8)	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	UAB (2007)	1943–2003	0 to >56.3 ppm-years	Leukemia mortality	No significant associations	Medium
SBR Cohort	Sielken (2007)	1943–1998	No quantitative data reported	Leukemia mortality, chronic myelogenous leukemia [CML] mortality, chronic lymphocytic leukemia [CLL] mortality, acute myelogenous or monocytic leukemia [ALM] mortality, all lymphoid neoplasms mortality, and all myeloid neoplasms mortality	Significant positive associations	Low

Data Source	Reference	Study Period or Follow-up	Exposure Range in the Dose-Response Model	Health Outcomes	Statistically Significant Result?	Systematic Review Score
SBR Cohort	Sathiakumar and Delzell (2009)	1943–2003	No quantitative data reported	Leukemia mortality, non-Hodgkin's lymphoma mortality	No significant associations	Medium
SBR Cohort	Graff et al. (2009)	1943–1998	0 to >425.0 ppm-years	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	Sielken and Valdez-Flores (2011)	1943–1998	0–1,338 ppm-years	Leukemia mortality, chronic myelogenous leukemia [CML] mortality, chronic lymphocytic leukemia [CLL] mortality, acute myelogenous or monocytic leukemia [ALM] mortality, all lymphoid neoplasms mortality, and all myeloid neoplasms mortality	Significant positive associations	Medium
SBR Cohort	Sielken and Valdez-Flores (2013)	1943–1998	0–1,338 ppm-years	Leukemia mortality, chronic myelogenous leukemia [CML] mortality, chronic lymphocytic leukemia [CLL] mortality, acute myelogenous or monocytic leukemia [ALM] mortality, all lymphoid neoplasms mortality, and all myeloid neoplasms mortality	Significant positive associations	Low
SBR Cohort	Sathiakumar et al. (2015)	1943–2009	0 to >908.35 ppm-years	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	Sathiakumar et al. (2019)	1943–2009	No quantitative data reported	Leukemia mortality	Significant positive associations	Medium
SBR Cohort	Sathiakumar et al. (2021b)	1943–2009	0–7,741 ppm-years	Leukemia mortality, lymphoid leukemia mortality	Significant positive associations	Medium
SBR Cohort	Valdez-Flores et al. (2022)	1943–2009	0 to 7,743 ppm-years	Leukemia, lymphoid leukemia, Myeloid leukemia, multiple myeloma, or non-Hodgkins' lymphoma mortality	Significant positive associations	Low
Texas Cancer Registry	Whitworth et al. (2008)	1995–2004	No quantitative data reported	Leukemia, acute lymphocytic leukemia	Significant positive associations	Medium
Texas Cancer Registry	Symanski et al. (2016)	1995–2011	No quantitative data reported	Acute Lymphocytic Leukemia	Significant positive associations	Medium

Data Source	Reference	Study Period or Follow-up	Exposure Range in the Dose-Response Model	Health Outcomes	Statistically Significant Result?	Systematic Review Score
California Cancer Registry (Air Pollution and Childhood Cancer Studies)	Heck et al. (2014)	1990–2007	No quantitative data reported	acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML)	Significant positive associations	Medium

5.4.1.1 Analysis of 18 Studies from the SBR Cohort

Eighteen research publications used data from the original U.S.-Canadian styrene-butadiene rubber (SBR) worker cohort study (Delzell et al., 1996). This occupational cohort study was conducted in eight plants from 1943 to 2009 by a research group at The University of Alabama (UAB) (Delzell et al., 1996). The SBR cohort is a 66-year cohort study. All study participants were male and female adults. The cohort started recruiting male workers in 1943, originally followed up until 1991, then extended to 1998. It was expanded further to recruit female workers until 2002, and lastly, the follow-up was extended through 2009 (Table 5-4). As summarized in Table 5-4, the SBR cohort recruited the large male and female study populations (16,579 men and 4,508 women), had 20 years of follow-up period, and collected long-term 1,3-butadiene exposure data. The 18 publications on the SBR cohort listed in Table 5-3 were evaluated using several criteria, including study populations, exposure assessment, associated exposure concentrations, statistical analysis, confounder adjustments, and estimates of population risk as follows.

5.4.1.1.1 Study Population

Of the 18 SBR cohort publications, 14 publications showed a statistically significant relationship between 1,3-butadiene exposure and leukemia (Table 5-3). These 14 publications include either male-only or both male and female participants. Besides the 14 publications, 1 (Sathiakumar et al., 2005) that investigated the male population showed no significant association. Two publications (Sathiakumar and Delzell, 2009; UAB, 2007) that investigated only female-only populations did not show a statistically significant relationship between 1,3-butadiene exposure and leukemia. These results show gender differences in the positive association.

Table 5-4. Updates and Description of Recruitment, Follow-Up, and Expansion of the SBR Cohort Study

Historical Changes in the SBR Cohort	Period of Recruitment and Follow-Up	Gender of Participant Recruitment	Number of Workers	Number of Deaths
Original study plan	1944–1991	Male	17,964	4,665
Extended follow-up for male workers	1944–1998	Male	17,924	6,237
Expanded recruitment for female workers	1943–2002	Female	4,861	1,198
Extended follow-up for male and female workers	1943–2009	Male and Female	21,087 (16,579 men and 4,508 women)	9,665 (8,214 men and 1,451 women)

5.4.1.1.2 Exposure Assessment and Concentration

During the follow-up period of the SBR cohort, Macaluso et al. (2004) revised the exposure estimates for 1,3-butadiene that incorporated additional information, including historical industrial hygiene surveys by NIOSH. The revised exposure assessment (Macaluso et al., 2004) identified tasks and jobs involving exposure, identified factors influencing historical changes, and utilized mathematical models to compute job- and time-period-specific exposure. It is better than the original exposure assessment (Delzell et al., 1996) due to several improvements: (1) the exposure scenarios were more specific than the previously grouped tasks; (2) the verification for the parameters' values in the exposure models through published materials; (3) plant personnel provided feedback on the exposure scenarios, which validated the assumptions for computing estimates; (4) the measured air velocities at selected locations replaced arbitrary assumptions in the original estimates with empirical data; (4) providing uncertainty ranges for the exposure parameters improved estimates and sensitivity analyses; and (5) peak exposure was further characterized.

The authors concluded that their original estimates were low and noted that the revised estimates for 1,3-butadiene exposure were up to an order of magnitude higher—particularly for the period of the 1940s to 1960s. However, the estimated number of 1,3-butadiene peaks declined following the revision (Macaluso et al., 2004). Overall, the pattern of the updated 1,3-butadiene exposure is high exposure prevalence and intensity during the 1940s to 1960s, with time-weighted averages (TWAs) around 10 ppm during those decades, sharply decreased in the 1970s and a lesser reduction in the 1980s. Median cumulative butadiene exposure was 71 ppm-years for all employees and 209 ppm-years for leukemia decedents. To compare the slope coefficients and rate ratios estimated from the revised exposure data in Cheng et al., Sathiakumar et al., and Valdez-Flores et al. publications (Valdez-Flores et al., 2022; Sathiakumar et al., 2021b; Cheng et al., 2007), the slope coefficients and rate ratios are lower by about an order of magnitude.

Most publications used the cumulative 1,3-butadiene (ppm-years) exposure to estimate the dose-response association in statistical models. Table 5-3 shows that the cumulative exposures ranged widely, from 0 to 7,743 ppm-years.

5.4.1.1.3 Statistical Analysis and Confounding Adjustment

Table 5-5 compares estimates of slope parameters and rate ratios for the association of leukemia mortality with cumulative butadiene exposure from log-linear relative risk models with quantitative exposure variables in successive analyses by the UAB researchers, Environment Canada (2000) and Valdez-Flores et al. (2022). Although analyses of various other cancer outcomes, exposure metrics, and model forms have been reported, only the relationship shown in the table has been reported consistently across studies.

Table 5-5. Comparison of Estimated Slope Coefficients and Relative Risks (RRs) For Leukemia from Comparable Log-Linear Relative Risk Models in Analyses of the U.S.-Canada SBR Cohort, 0-Year Lag

Reference	Cohort[a]	Coefficient (SE)	RR per 100 ppm-years (95% CI)	Model	Adjustments
Delzell et al. (1996)	1991; men original exposure	0.0041 (0.0019)	1.507 (1.038-2.187)	Grouped Poisson	Age, period, time since hire, race, STY
EC (2000)	1991; men original exposure	0.0029 (0.0014)	1.336 (1.016-1.758)	Grouped Poisson	Age, period, time since hire, race, STY
Cheng et al. (2007)	1991; men, revised exposure	0.0003 (0.0001)	1.029 (1.009-1.050)	Proportional hazards	Age, birth year, time since hire, race, plant, DMDTC
Sathiakumar et al. (2021b)	2009; men and women; revised exposure	0.00026 (0.0001)	1.026 (1.006-1.047)	Proportional hazards	Age, age at hire, year of hire, sex, race, plant, hourly status
Valdez-Flores et al. (2022)	2009; men and women; revised exposure	0.00028 (0.0001)	1.028 (1.009-1.049)	Proportional hazards	Age
Valdez-Flores et al. (2022)	2009; men and women; revised exposure	0.00013 (0.0001)	1.013 (0.997-1.029)	Proportional hazards	Age, peak exposure

STY = styrene; DMDTC = dimethyldithiocarbamate; peak exposure = cumulative number of tasks with estimated butadiene concentration ≥100 ppm
[a] Last year of follow-up, inclusion, original or revised exposure estimates

The most notable difference among the estimates shown in Table 5-5 is a 10-fold reduction in slope estimates that occurs with the 2007 publication of Cheng et al. (2007). That paper was based on the same cohort as earlier analyses but incorporated new exposure estimates that were revised upward by as much as an order of magnitude (Macaluso et al., 2004). Cheng et al. (2007) also used proportional hazards regression, rather than the grouped Poisson regression models used previously. However, while decisions in grouping and assigning exposure scores in grouped Poisson regression can induce bias in exposure-response estimates, as discussed above, the bias is unlikely to be as large as the order-of-magnitude difference between the results of Cheng et al. (2007) and Environment Canada (2000).

Valdez-Flores et al. (2022) used the data from the Styrene-Butadiene rubber (SBR) worker cohort and the Cox proportional hazards statistical model to address the exposure-response association between 1,3-butadiene and leukemia. In this Cox model, cumulative exposure to 1,3-butadiene (ppm-years) was used as the dose metric, and the number of leukemia decedents was used as the response in exposure-response modeling. To improve the likelihood of this Cox model, Valdez-Flores et al. tested various covariates and selected the covariate effects (*i.e.*, cumulative number of 1,3-butadiene high-intensity tasks) that significantly improved the likelihood of the Cox model. A covariate is a variable that can influence the outcome but is not the main variable being investigated or controlled (*e.g.*, age would be a covariate when investigating the relationship between physical activity and blood pressure. Age is not the variable of interest but impacts physical activity). Likelihood describes how well a statistical model explains the observed data. Afterward, as is standard practice, select covariates were added to the Cox model in a process called the "adjustment for the covariates in the model." However, EPA

epidemiologists do not agree with the covariate selected in Valdez-Flores et al. (2022). The flaw of Valdez-Flores et al. (2022) proposed IUR is the inclusion of 1,3-butadiene high-intensity tasks (*i.e.*, tasks with exposure ≥100 ppm 1,3-butadiene) as a covariate, called "peak exposure," to adjust for the relationship between cumulative 1,3-butadiene exposure and leukemia mortality.

Macaluso et al. (2004) reported that peak exposure accounted for a large portion of cumulative 1,3-butadiene exposure in the SBR worker cohort. Exposure, peak or otherwise, is the main variable in the exposure-response relationship for 1,3-butadiene and leukemia mortality. By adjusting for peak exposure as a covariate, the effect of exposure to 1,3-butadiene on leukemia mortality is reduced. Valdez-Flores et al. (2022) showed that an adjustment for peak 1,3-butadiene exposure in their Cox model reduced the coefficient of cumulative 1,3-butadiene exposure from 0.0002808 (no adjustment for $_{\text{peak exposure}}$) to 0.0001316, a notable change in the slope parameter. This results in a 53 percent reduction in the slope of the line that describes the relationship between cumulative 1,3-butadiene exposure and leukemia mortality, representing less cancer potency. EPA believes that peak exposure is an inappropriate adjustment for the statistical model describing the impact of cumulative 1,3-butadiene exposure on leukemia mortality.

Other differences in input data and analytical methods are unlikely to have had major effects on the findings. The addition of women to the cohort and extension of follow-up in a subsequent analysis by Sathiakumar et al. (2021b) did not result in a notable change in the slope parameter. Adjustments for multiple occupational and demographic covariates does not appear to have had notable effects on the estimated slope parameter, either. Cheng et al. (Cheng et al., 2007) reported that results were similar with adjustment for age alone and for the full suite of covariates; Sathiakumar et al. (2021b) also reported similar results for full models with all covariates and reduced models. Valdez-Flores et al. (2022) obtained similar results to those of Sathiakumar et al. (2021b) from models adjusted only for age, but not for butadiene peaks.

It, therefore, appears that when comparable exposure-response models are used, differences in key parameter estimates are due primarily to changes in exposure estimates for the SBR cohort and, to a lesser extent, to adjustment for peak exposures in the analysis by Valdez-Flores et al. (2022). Although exposure-response models of similar forms with comparable adjustments for covariates provide parameter estimates that vary by about a factor of 10, as shown above, the risk estimates of EPA (U.S. EPA, 2002a) and Valdez-Flores (Valdez-Flores et al., 2022) are based on models of different forms. Health Canada's analysis using four model forms, including the linear model ultimately used by EPA (U.S. EPA, 2002a) and the log-linear model used by Valdez-Flores et al. (2022) illustrates the effect of model form on the estimated relative risk.

5.4.1.2 Analysis of One Ecological and Two Case-Control Studies

Three studies (Symanski et al., 2016; Heck et al., 2014; Whitworth et al., 2008) did not use SRB cohort data to estimate dose-response associations between 1,3-butadiene exposure and leukemia and showed a statistically positive association. Whitworth et al. (2008) used data from the Texas Cancer Registry to conduct an ecological study. Symanski et al. (2016) and Heck et al. (2014) use data from the Texas Cancer Registry and California Cancer Registry to conduct case-control studies, respectively.

An ecological study (Whitworth et al., 2008) assessed hazardous air pollutant (HAP) levels in Texas against lymphohematopoietic cancer incidence in children per census tract (953 cases). The Whitworth et al. (2008) assessment is considered a quality study, aside from a limitation in exposure assessment: the study correlates cancer incidence with only 1 year of HAP data that is during the period of diagnoses (1999 vs. 1994–2004) and may not have been etiologically relevant exposure for some if not all, cancer

incidences. Additionally, the study was limited by the modeled exposure and the fact that 1,3-butadiene and benzene exposures were closely correlated and could not be assessed individually. Even though the study observed significantly increased rates of all leukemia in tracts with the highest levels of 1,3-butadiene (RR = 1.40), this study result is not appropriate to be considered as a dose-response relationship.

Symanski et al. (2016) conducted a case-control study (1,248 cases; 12,172 controls) analyzing the relationship between estimated ambient outdoor exposure to 1,3-butadiene and acute lymphocytic leukemia (ALL) diagnosed in children aged younger than 5 years old. Cases in the Texas Cancer Registry diagnosed in 1995 to 2011 were matched to controls identified from Texas birth certificates by birth year and month. Children included were born between 1991 and 2011. Exposure during pregnancy was estimated based on maternal address at delivery and census tract EPA National-Scale Air Toxics Assessment (NATA) estimates available for 1996, 1999, 2002, and 2005. Estimates available for 1,3 butadiene were available for very few years, and misclassification of personal exposure is a potentially important concern. In adjusted single pollutant models, the authors reported an odds ratio of 1.28 (95% CI 1.08–1.52) for the association between the highest vs. lowest quartile of 1,3-butadiene and childhood ALL. Exposure model validity for 1,3-butadiene was not discussed. Sources of error include using spatial variation (*e.g.*, use of census tract level modeling as an estimate of personal exposure) as well as temporal variation (data were available for limited years, seasonal variation was not discussed). In co-pollutant models, after adjusting for benzene, although not after adjusting for polycyclic organic matter (POM), associations with 1,3-butadiene remained significant. Data analysis used exposure variables defined using quartiles for each year of NATA data; there were substantial changes in levels of exposure over time. Another potential concern is that quantitative differences in levels of exposure within these quartiles were not taken into account: effect estimates appear to pool associations with exposure ranked as low, medium, medium-high, and high, regardless of temporal shifts. Even though no evidence of bias would differentially misclassify exposure, the mentioned concerns in exposure assessment and misclassification may cause uncertainties in the effect estimate.

The Air Pollution and Childhood Cancer Study (APCC) (Heck et al., 2014) is a case-control study that used the California Cancer Registry to examine the association between 1,3-butadiene levels in ambient air and two forms of leukemia among children under the age of 6 in California. The 1,3-butadiene exposure during the 3rd trimester and across the entire pregnancy was associated with increased odds of acute lymphoblastic leukemia (3rd trimester OR [95% CI]: 1.54 [1.19, 1.99], entire pregnancy (OR [95% CI]: 1.76 [1.09, 2.86]). 1,3-Butadiene exposure during the child's first year of life was associated with increased odds of acute myeloid leukemia (OR [95% CI]: 2.35 [1.02, 5.39]). Concerns include the potential for exposure misclassification due to exposure assignment based on birth address, which only estimated exposure throughout pregnancy and into infancy, and limited information on some aspects of the analysis (*e.g.*, missing data) and study aspects related to sensitivity (*e.g.*, no information provided on the exposure distribution in this subset of the overall study population).

5.4.1.3 Comparison of SBR Cohort Studies and Other Ecological and Case-Control Studies

EPA compares the SBR cohort, ecological, and case-control studies based on the study design, statistical power, and beta value, regression coefficient, as described below.

Study Design

The ecological study design cannot investigate the causal relationship between 1,3-butadiene exposure and leukemia. Thus, Whitworth et al., (2008) study results are not appropriate for IUR derivation. Between cohort and case-control studies, both the case-control and cohort designs have unique strengths

and limitations. Some of the major advantages of cohort studies over case-control studies are (1) the ability to study multiple outcomes that can be associated with a single exposure or multiple exposures in a single study; (2) well suited for assessing the effects of rare exposures, especially those in occupational settings; and (3) the proportions of exposed persons among a group of individuals with the disease would be far too small to permit meaningful comparisons of risk. The SBR cohort had 65 years of health outcome and long-term exposure data, a long follow-up period (20 years), and very large male and female study participants (16,579 men and 4,508 women), so SBR cohort studies are more suitable than the case-control studies for deriving leukemia IUR.

Regression Coefficient

These two case-control studies did not provide beta values from regression models, so their study results cannot be used for IUR. On the other hand, the dose-response analyses by Sathiakumar et al. and Valdez-Flores et al. (Valdez-Flores et al., 2022; Sathiakumar et al., 2021b) include beta values from regression models, which are essential to derive an IUR.

Statistical Power

Compared to Symanski et al. and Heck et al. studies (2016; 2014), the SBR cohort has very large male and female study participants (16,579 men and 4,508 women) and long-term exposure data. The eighteen studies that used SBR cohort data have higher statistical power than the other three studies.

Based on these advantages of cohort study design, regression coefficients, and study power in the dose-response models from SBR cohort publications, and a thorough systematic review of the scientific literature in the TSCA SR process, EPA concluded that epidemiology publications using SBR cohort data would be appropriate to derive 1,3-butadiene IUR and evaluate human cancer risk.

5.4.1.4 Study Selection for IUR Derivation

The follow-up period of the SBR cohort ended in 2009 (Table 5-4). Thus, only publications after 2009 and including all male and female participants (16,579 men and 4,508 women) are considered for the next step of IUR derivation because they had the most complete leukemia and exposure data and longest follow-up period. To ensure an acceptable quality of study for IUR derivation, if a study is rated low in the systematic review, it is excluded from the study selection process. After considering all these factors, only the dose-response relationship in two publications—(Sathiakumar et al., 2021b; Sathiakumar et al., 2019)—are considered in the study selection for IUR derivation.

EPA's standard approach for deriving an IUR estimate using results from epidemiology studies involves using a regression coefficient that describes the relationship between increases in cancer risk and increases in cumulative exposure and estimating an upper-bound lifetime extra risk-per-unit exposure concentration through a lifetable analysis. The results of the statistical models of Sathiakumar et al. publication (Sathiakumar et al., 2021b) supported the classification of butadiene as a human carcinogen, confirmed a positive exposure-response relationship between butadiene and all leukemia, provided the regression coefficients, β, that described the relationship between increases in cancer risk and increases in cumulative exposure, which can be used for the lifetable analysis. *As a result, the Sathiakumar et al.* (2021b) *publication was selected to derive leukemia IUR, which is described in Section 5.4.3.*

According to the evidence integration in Section 5.1.1.2 and Table_Apx A-5, the evidence integration judgment for human evidence of bladder cancer from 1,3-butadiene exposure is moderate. After excluding publications with low or uninformative data quality scores, two out of seven publications showed positive dose-response relationships. However, in these publications with positive dose-response relationships, two concerns were raised: (1) bladder cancer case numbers were small, and (2) smoking

was not adjusted for the dose-response association in statistical models. Given the judgment of evidence integration and two concerns about study data, there are uncertainties in the 1,3-butadiene IUR for bladder cancer. Thus, EPA has determined that more evidence may be needed to support the causation between 1,3-butadiene exposure and bladder cancer and to calculate bladder cancer risk. The IUR derivation process and results of 1,3-butadiene dose-response analysis for bladder cancer are described in Appendix C.

5.4.2 Duration, Dosimetric and Unit Adjustments

Dosimetric Adjustments

As described in Section 5.4.1.4 and Appendix C.1, dose-response data in Sathiakumar et al. studies were selected to derive IUR for leukemia (Sathiakumar et al., 2021b) and bladder cancer (Sathiakumar et al., 2021a), respectively. Because both studies are occupational epidemiology studies, the occupational exposure was converted to continuous exposures in the lifetable analysis and adjusting for the total amount of 1,3-butadiene in air inhaled per day (20/10 m^3). Based on the EPA methods for the derivation of inhalation reference concentrations and application of inhalation dosimetry (U.S. EPA, 1994) 10 m^3 is the default occupational ventilation volume for an 8-hour work shift, and 20 m^3 is the default 24-hour ambient ventilation volume.

Duration Adjustments

The studies selected for dose-response assessment utilized differing exposure durations and frequencies. In order to better compare results across studies and exposure scenarios, administered doses/ concentrations were linearly adjusted to continuous exposure (24 hours/day, 7 days/week) prior to POD derivation based on Haber's Law (Haber, 1924) using the following equation:

Equation 5-1. Adjusting Average Exposure Concentration or Inhalation POD for Differences in Days and Hours of Exposure across Scenarios

$$Concentration_{continuous} = Concentration_{study} \times (\frac{D_S}{7}) \times (\frac{H_S}{24})$$

Where:

$Concentration_{continuous}$	=	Adjusted air concentration/inhalation POD
$Concentration_{study}$	=	Air concentration/inhalation POD from study data set
D_s	=	Days per week/year exposure in study data set
H_s	=	Hours per day exposure in study data set

IURs were derived incorporating both dosimetric and duration adjustments, resulting in a lower value than the original study POD.

Unit Conversions

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all inhalation hazard values in Section 8 in both units. The following equation presents the conversion of the HEC from mg/m^3 to ppm.

Equation 5-2. Converting risk per ppm to risk per mg/m^3

IUR (per mg/m^3) = IUR (ppm) × (24.45^1 / molecular weight)

IUR (per mg/m^3) = IUR (ppm) × (24.45^1)/ 54.0916)

5.4.3 Cancer IUR and UR for Leukemia from Lifetime Exposures

Based on the dose-response analysis in Section 5.4.1, the Sathiakumar et al. (2021b) publication was ultimately chosen as the best available science to derive unit risks for two reasons. First, the relationship between 1,3-butadiene and leukemia in this publication is consistent with those reported earlier by other researchers in supporting a positive association in the synthetic rubber polymer industry. Second, it includes male and female workers, the revised exposure assessment from Macaluso et al. (Macaluso et al., 2004) that is described in above paragraph, long follow-up period until 2009 (additional 20 years of follow-up), updated analytical framework using proportional hazards models, and reasonable confounder adjustment. Adding women to the cohort provides essential data for population risk assessment, and the additional 20 years of follow-up, which added 418,546 person-years of observation and 5,000 deaths, enhance statistical power to assess the association between 1,3-butadiene exposure and leukemia. The vital features of this SBR worker cohort study are summarized in Table 5-6.

Table 5-6. Characteristics of the SBR Cohort (Sathiakumar et al., 2021b)

Descriptor	Overview of the Study and Cohort Data
Cohort period (years)	1943–2009
Health outcomes	Hematopoietic cancers, including Leukemia, in all published studies using data from the SBR worker cohort study; bladder cancer in few published studies.
Number of all workers in the cohort	21,087 workers (16,579 men; 4,508 women)
Number of workers exposed to butadiene	14,004
Number of male workers exposed to butadiene	12814 (77% of butadiene-exposed workers)
Number of female workers exposed to butadiene	1190 (26% of butadiene-exposed workers)
Number of all Leukemia decedent	132
Number of all Leukemia decedent exposed to butadiene	103

5.4.3.1 Selection for Statistical Model and Data

Sathiakumar et al. (2021b) showed that their analyses of exposure-response relations in the SBR cohort by UAB researchers improved and extended their previous analyses, including those that informed the IRIS assessment. The cohort had been expanded and updated over 66 years. The analytical framework in Sathiakumar et al. (2021b) was also updated by replacing classical grouped Poisson regression models with proportional hazards models, which can allow analysts to avoid bias from grouping and assigning exposure values.

Table 5-7. Summary of Crucial Cox Regression Models to Analyze Exposure-Response Relations in (Sathiakumar et al., 2021b)

Statistical Model	Lag Time (years)	β (Beta Coefficient)	Upper 95% Confidence Bound on β	Trend P Value
1. All person-time (untrimmed, including unexposed)	0	2.55E−04	4.57E−04	0.014
2. All person-time (untrimmed, including unexposed)	10	2.58E−04	4.78E−04	0.022
3. All person-time (untrimmed, including unexposed)	20	2.63E−04	5.31E−04	0.055
4. Exposed person-time (exclude unexposed)	0	2.50 E−04	4.73E−04	0.028
5. Exposure person time ≤95th percentile: Restricted cubic spline (RCS) Cox regression model (trim to restrict data)	0	9.94E−04	18E−04	0.016

Table 5-7 shows that Sathiakumar et al. (2021b) used various models to estimate the association between butadiene exposure and leukemia. The first three models in Table 5-7 include the unexposed and exposed populations. Since the purpose of 1,3-butadiene IUR derivation is for butadiene exposure and leukemia, the first three models that include the unexposed population are not under our consideration. More than 90 percent of the leukemia cases died 20 years or more after hire. Based on very close beta-coefficients in the first three models with lag times 0, 10, and 20 years, lagging exposure had little effect on results for leukemia. As exposure diminished over calendar time, lagging exposure was not a concern when updating the IUR process. In addition, the CDC (2015) concluded that the minimum latency of leukemia is 0.4 years. Since β values in the first three models are not significantly different for lag time 0, 10, and 20 years, and the minimum latency of leukemia is 0.4 years, 0 years is chosen as the lag time in the lifetable analysis to update the IUR.

Between the fourth and fifth models, the beta-coefficients of the fifth model (restricted cubic spline (RCS) Cox regression model) are selected to conduct lifetable analysis because of (1) previous study results before Sathiakumar et al. (2021b); (2) better model fitting of Sathiakumar et al. (2021b); and (3) advantages of RCS Cox regression model, which are described below.

A. Evidence from Previous Study Results before the Sathiakumar et al. Study

Sathiakumar et al. (2021b) cited several previous study results to describe statistical analysis and provided the rationale to determine the exposure data input for the fifth model (RCS Cox regression model), "Cohort studies in other industry settings have reported that exposure-response curves tend to diminish at higher exposure levels. Two of our earlier studies of male synthetic rubber polymer workers found stronger exposure-response trends for butadiene and leukemia in analyses that excluded exposures above the 95th percentile or categorized butadiene into deciles. Both of the latter procedures can reduce the impact of exposure outliers. In addition, an investigation at the largest study plant to validate our butadiene exposure estimates found greater misclassification for jobs entailing higher exposures than for jobs with lower exposures." These previous study results support the use of exposure person time less than or equal to 95 percent in three aspects below:

1. At high exposure levels: (i) Excluding greater than 95 percent exposure person time can reduce the impact of exposure outliers (Sathiakumar et al., 2015; Cheng et al., 2007), and (ii) greater

misclassification for jobs entailing higher exposures than for jobs with lower exposures according to the validation investigation at the largest study plant (Sathiakumar et al., 2007).

2. At low exposure levels: Exposure-response curves tend to dimmish at higher exposure levels. IUR represents a lower exposure range (Stayner et al., 2003), so the concern about high-exposure workers is not as relevant to IUR derivation.
3. Model fitting performance: Cheng et al. (Cheng et al., 2007) showed stronger exposure-response trends for butadiene and leukemia in analyses while excluding exposures above the 95th percentile.

B. Model Fitting of Sathiakumar et al. Study

Sathiakumar et al. (2021b) showed more robust model fitting using the RCS Cox regression model than other models and stated, "Trimming to restrict data to ppm-years greater than 0 and less than or equal to the 95th percentile (1,144 ppm-years) of all leukemia decedents yielded a somewhat stronger exposure-response trend for butadiene (ß= 9.94×10^{-4}, (95% CI 1.88 to 18.00)$\times10^{-4}$, trend p=0.016)."

C. Advantage of the RCS Cox Regression Model

The analyses incorporating smoothing splines are a useful adjunct that allows the exposure-response function to be visualized. The analyses reported by Sathiakumar et al. (2021b) follow current state-of-the-art practices and do not raise any significant concerns about methodology or interpretation.

In summary, according to the described study results above, the fifth model results are acceptable for conducting lifetable analysis.

5.4.3.2 Lifetable Analysis

To be consistent with EPA IRIS' method, the Agency adopted the same method of lifetable analysis to derive 1,3-Butadiene IUR (U.S. EPA, 2002a). The mathematical methodology was established by BEIR (Biological Effect of Ionizing Radiation) Committee (BEIR, 1988), and the EPA IRIS started implementing it in the IRIS 1,3-butadiene assessment (U.S. EPA, 2002a). Lifetables are an actuarial procedure to account for the dose-respondent effects of exposure over the lifetimes of a population in the presence of competing causes of death. The steps to conduct lifetable analysis are as follows.

A. Data Input

Three kinds of inputs are essential to be used in the lifetable analysis:

1. Population statistics including U.S. age-specific all-cause mortality and cause-specific incidence/mortality: U.S. age-specific all-cause mortality rates for deaths in 2019 among all race and gender groups combined are retrieved from the Multiple Cause of Death (final) database of the Wide-ranging ONline Data for Epidemiologic Research (WONDER) database from the Centers for Disease Control and Prevention (CDC) (CDC, 2024). For 1,3-butadiene lifetable analysis, the leukemia-specific incidence was obtained from the Surveillance, Epidemiology, and End Results (SEER) 22 from the National Cancer Institute (NCI), National Institutes of Health (NIH, 2024). Both the U.S. all-cause mortality and leukemia incidence are age-specific, but rates above the age of 85 years are not included because leukemia-specific incidence did not list for those ages. Therefore, EPA assumed 84.99 years of exposure for the lifetable analysis.
2. Epidemiological studies with cumulative exposure model from the linear or log-linear model for exposure: It is required to use epidemiological studies that provide exposure-response analyses so that β and upper 95 percent confidence bound (CB) on β can best characterize the hazard and be incorporated into the lifetable analysis. The beta is the estimate of the increase in the outcome (*e.g.,* leukemia) that results from an increase of one unit of exposure to 1,3-butadiene. Depending

on cancer outcomes, if the dose-response had an exposure lag (*e.g.*, 0 or 10 years), that must be included in the lifetable analysis. Table 5-7 shows that varying lag time has no significantly different impact on β. In addition, appropriate adjustments from occupational or general population studies in the lifetable analysis can also enhance the accuracy of the IUR.

3. Selection of Benchmark Response (BMR): BMR is usually 1 percent for cancer data (U.S. EPA, 2012b), but other BMR values are possible for rare outcomes (*e.g.*, 0.1%). Since the selected health outcome is leukemia, EPA used 1 percent for BMR.

B. Data Output

The lifetable analysis aims to find the 95 percent lower confidence limit of the exposure concentration (LEC_{BMR}) that results in leukemia's extra risk (ER) after exposure to 1,3 butadiene. ER is a calculation of the risk of adverse effects, which adjusts for background incidence rates of the same effect by estimating risk at dose only among the fraction of the population not expected to respond to the background causes (U.S. EPA, 2024i). The target extra risk in this lifetable analysis is set as 0.01. LEC_{BMR} is the cumulative lifetime exposure levels that yield extra risk as 0.01 by interpolating the exposure level corresponding to the 95 percent upper bound on β. Through an iterative process that evaluates the risk levels resulting from selected exposure levels, the exposure expected to result in a specified level of excess risk (*e.g.*, 1%) can be determined.

5.4.3.3 IUR and UR Calculation

IRIS defines Inhalation Unit Risk as "The upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 μg/m^3 in air." (U.S. EPA, 2024j). For 1,3-butadiene IUR, excess lifetime cancer risk means the additional or extra risk of developing leukemia due to exposure to a 1,3-butadiene over the lifetime of an individual (U.S. EPA, 2024i). LEC_{BMR}, the 95 percent lower confidence limit of the exposure concentration associated with a 1 percent increased risk, is used to calculate the UR at 95% upper bound estimate using the equation below:

UR at 95% upper bound estimate = BMR/LEC_{BMR} per unit of exposure

BMR is the benchmark response of an adverse effect and is used to define a benchmark dose. The change in response rate over the background of the BMR is usually in the range of 5 to 10 percent, which is the limit of responses typically observed in well-conducted animal experiments. EPA used epidemiologic data in the 1,3-butadiene IUR derivation because of the rich and good-quality data collected from a 66-year SBR worker cohort study. As the EPA *Benchmark Dose Technical Guidance* indicated, based on biological and statistical considerations, BMR is set as 1 percent for most cancers, except for rare cancers (U.S. EPA, 2012b). Therefore, the 1 percent value is referred to as an extra risk for the BMR for leukemia. As a result, the equation can be expressed as follows:

UR at 95% upper bound estimate = BMR_{01}/LEC_{01} = 0.01 / LEC_{01}.

EPA has determined that 1,3-butadiene is "Carcinogenic to Humans" and exhibits a mutagenic mode of action in the Section 5.3. In accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens*, the following ADAFs were applied to the adult unit risk: 10 for children ages less than 2 years; 3 for children ages 2–15; and 1 for persons aged 16–78 (Barton et al., 2005). The weighted sum of these three partial unit risks is the ADAF-adjusted lifetime IUR (Barton et al., 2005). This lifetime IUR is used for estimating general population risk for leukemia based on lifetime exposure (0-78 years). The UR at 95% upper bound is used for the occupational risk estimate and defined as the chronic occupational UR.

5.4.3.4 IUR and UR Results

Based on the above computation, the LEC_{01} calculated by lifetable analysis is 1.62 ppm, and the UR at 95% upper bound based on ages from less than 1 to 84.9 years old is 0.0062 per ppm (2.8×10^{-6} per µg/m^3) (Table 5-8). The chronic occupational UR is 0.0062 per ppm (2.8×10^{-6} per µg/m^3) (Table 8-2). Due to the carcinogenic mode of action of 1,3-butadiene, the age-dependent adjustment factor (ADAF) is applied to the UR at 95% upper bound to yield the IUR. After applying the ADAF to the UR, the IUR is computed to be 0.0098 per ppm (4.4×10^{-6} per µg/m^3) (Table 8-3). The interpretation of the IUR (4.4×10^{-6} per µg/m^3) is that 4.4 excess leukemia cases (as the upper bound estimate) are expected to develop per 1,000,000 people if exposed daily for a lifetime to 1 µg of 1,3-butadiene per m^3 of air. Compared with the current IRIS IUR (3×10^{-5} per µg/m^3) published in 2002 (**U.S. EPA, 2002a**), this updated IUR (4.4×10^{-6} per µg/m^3) is approximately 7-fold lower. This updated IUR (4.4×10^{-6} per µg/m^3) derived from the 95 percent upper-bound confidence interval on β will be used for lifetime risk evaluation for general population. The main factors contributing to the lower, updated IUR are the revised exposure assessment and the statistical model used to assess the relationship between 1,3-butadiene exposure and leukemia (Table 5-9).

Table 5-8. Calculation of Cancer Unit Risk Estimate

Model of the Beta-Coefficient (β), Reference	β		Exposure Concentration Associated with BMR (1% Extra Risk) Starting Exposure at Age <1 Years (µg/m^3)		Unit Risk[d]	
	MLE[a]	95% UB[b]	EC_{01} MLE	LEC_{01} 5% LB[c]	MLE	95% UB[b]
Cox regression model Sathiakumar et al. (2021b)	9.94E−04	0.0018	2.9 ppm	1.62 ppm	0.0034 per ppm	**0.0062 per ppm**

[a] MLE means Maximum Likelihood Estimate, a statistical method for estimating a population parameter most likely to have produced the sample observations.
[b] UB means the upper bound estimate.
[c] LB means the lower bound estimate.
[d] The unit risk was corrected as described in *1,3-Butadiene: Corrected lifetable analyses for leukemia and bladder cancer* (U. S. EPA, 2024a). The corrected unit risk (78 years) = 0.0062 per ppm (Appendix F). The corrected chronic occupational unit risk (62 years) = 0.0049 per ppm (2.2×10^{-6} per µg/m^3)

Table 5-9. Potential Effects of Certain Characteristics on the Estimated IURs between EPA IRIS (2002) and OPPT (2024)

Characteristic	EPA IRIS	EPA OPPT	Effect on Estimated IUR
Cohort			
Inclusion	15,649 men	21,087 men and women	Negligible
Follow-up	1944–1991	1944–2009	Negligible
Exposure Assessment	Original	Revised	10x lower
Exposure-response analysis			
Model type	Grouped Poisson	Proportional hazards	Unknown, probable range 0 to +/– 10%
Response function form	Linear	Cox regression	Varies with exposure level
Adjustment for demographic/occupational covariates	Age, race, calendar period, time since hire, styrene	Age, age at hire, year of hire, sex, race, plant, hourly status	Minimal
Adjustment for peak exposure	No	No	Negligible
Lifetable, associated exposure, and potency estimation			
Lifetable age span (years)	0–85	0–85	Negligible
Post-estimation adjustment – total	Yes	Yes	Negligible
Incidence	Yes	Yes	Negligible
Sex and multiple tumor sites	Yes	Yes	Negligible

6 WEIGHT OF SCIENTIFIC EVIDENCE CONCLUSIONS FOR HUMAN HEALTH HAZARD

EPA considered evidence integration conclusions and dose-response considerations from Sections 4 through 7 and additional factors to choose overall hazard confidence levels based on the following characteristics:

- evidence integration/weight of scientific evidence judgements (see Appendix A);
- selection of most critical endpoint and study;
- relevance to exposure scenario;
- dose-response considerations; and
- PESS sensitivity.

The following section summarizes the strengths and limitations from summary table of confidence for each hazard endpoint and exposure duration, drawing upon information from previous sections and additional considerations as needed. Appendix E.1 presents the overall rankings for the above characteristics.

6.1 Strengths, Limitations, Assumptions, and Key Sources of Uncertainty for the Hazard Identification and Selection of PODs for Human Health Hazard Assessment

6.1.1 Acute Non-cancer

EPA did not derive a POD for acute exposures (Section 4.2.1.1). Based on comparison of results from short-term studies with intermediate-duration studies, EPA has only indeterminate to slight confidence in any potential health effects following a single exposure at relevant human exposure levels. Intermediate PODs are expected to be protective of acute exposures.

6.1.2 Intermediate/Chronic Non-cancer

Hazard ID Conclusions and Evidence Integration Judgements

All three critical health effect domains were supported by the weight of scientific evidence and considered appropriate for dose-response analysis. For all three domains there were multiple endpoints Some differences exist in the relative evidence integration confidence across health effects. Developmental effects following gestational exposure were observed across multiple studies and in both mice and rats with additional support from a single neurodevelopmental epidemiological study. Male reproductive effects were observed in a dose-responsive manner at varying durations, however only in mice in the absence of any relevant epidemiological studies. Hematological/immune effects (especially anemia) was also only observed in mice with conflicting epidemiological data.

Selection of Most Critical Endpoint and Study

EPA has the strongest confidence in the selection of study and endpoints representing maternal and developmental toxicity. Although there was only a single developmental mouse study (which was selected for POD derivation), maternal weight gain, fetal body weight, and skeletal effects were all associated with each other in that study. Additionally, there were at least indications of these effects in multiple rat studies as well, with fetal body weight the most unambiguously adverse endpoint that can account for the other associated effects.

Relevance to Exposure Scenarios

All endpoints were overall highly relevant to the assigned exposure duration scenario. The studies used for POD derivation were intermediate duration, and intermediate duration is the most relevant category for gestation or male germ cell development. These health effects are similarly applicable to chronic exposures because gestation and male germ cell development are cyclical. Exposure to offspring is only occurring during pregnancy/lactation and mechanistic data for male reproductive toxicity demonstrates a stage-specificity, suggesting that chronic human exposure would have the same impact as intermediate exposure during critical windows. Although it is possible that chronic exposure could sensitize the reproductive system to adverse effects during critical windows, there is no evidence to support a concern for chronic reproductive effects impacting these developmental outcomes. There is some uncertainty in whether any of these intermediate effects could be applicable to acute exposures, especially at higher doses. EPA performed a sensitivity analysis comparing potential acute PODs to the most sensitive POD of reduced fetal weight in Appendix E.2.

Hematological effects were observed in a chronic study and apply to chronic exposure scenarios; however, consistent statistically significant effects were only observed at the 9-month time period. There appeared to be an adaptive response at 15 months of exposure, indicating some lower confidence in the continued applicability of the endpoint and POD over decades of life.

Dose-Response Considerations

EPA has strong confidence in dose-response considerations for maternal/developmental effects, especially the most sensitive and robust endpoint of reduced fetal body weight. LOAEL/NOAEL values and BMD modeling via multiple approaches resulted in PODs that were all within a few fold of each other across both approaches and endpoints. Additionally, this health outcome was observed in both mice and rats, with only about a 5-fold difference in PODs across species. This is consistent with the absence of evidence for any mechanism that would suggest significantly differential sensitivity across species. EPA therefore has very high confidence in the applicability of the selected POD for humans.

Male reproductive/developmental and hematological effects were both scored moderate (++). All derived PODs can be considered co-critical as they were within only a few-fold of each other, and close to the PODs for maternal/developmental effects. Additionally, PODs were derived from studies with clear dose-response relationships and large sample sizes. Hematological data could not be BMD modeled without dropping at least one dose, but this concern is mitigated because the resulting PODs are all within about 2-fold.

PESS Sensitivity

Laboratory inbred animal strains were used for examination of all key endpoints and limited human evidence was available for non-cancer endpoints. Therefore, EPA was unable to quantify considerations from unique sensitivities. The Agency did identify quantifiable differences across species; however, and EPA selected the most sensitive sex (male) for dose-response modeling of all endpoints. Additionally, the maternal and male reproductive/developmental effects account for sensitive lifestages. See Section 7.2 for more details on how EPA considered PESS in the human health hazard assessment.

Overall Confidence

Based on the above factors, EPA has robust overall confidence for the evidence integration, study/endpoint selection, exposure scenario applicability, dose-response, PESS sensitivity of the conclusions, PODs for maternal/developmental toxicity, and the most sensitive endpoint of reduced fetal body weight**.** EPA has moderate overall confidence for the other critical hazard outcomes with PODs at very similar levels that further support the POD to be used for risk estimation.

6.1.3 Cancer: Leukemia

EPA determined that 1,3-butadiene is carcinogenic to humans, and the evidence supporting lymphohematopoietic cancer is robust based on human, animal, and mechanistic data.

Selection of Most Critical Endpoint and Study

Human epidemiological data from the UAB occupational cohort was selected for dose-response analysis. The use of human data precludes the complexities of species extrapolation from rodents, and the UAB cohort covered more than 60 years of follow-up with multiple updates over time. Leukemia was identified as the most reliable and sensitive cancer type identified in this population, supported by the robust evidence integration judgements. Among this cohort, EPA utilized (Sathiakumar et al., 2021b) for IUR derivation. Sathiakumar et al (2021b) was determined to be the best publication among all recent cohort updates that incorporated both sexes and refined exposure estimates. Based on the highly relevant occupational cohort, large sample size, decades of follow-up, and the reliability of the statistical adjustments made in the study, EPA has high confidence for this consideration.

Relevance to Exposure Scenarios

As discussed above, EPA utilized human data covering over 60 years of follow-up for derivation of lifetime inhalation unit risk. This approaches the 78-year lifespan assumed for lifetime exposure estimates. Therefore, the cancer assessment and parameters incorporated into the derived IUR are highly relevant to the lifetime exposure scenario.

Dose-Response Considerations

EPA derived a novel IUR based on data from (Sathiakumar et al., 2021b), which covers 418,546 person-years of observation and 5,000 deaths. This IUR derivation involved consideration of the most appropriate exposure-response model and development of a lifetable to account for the dose-respondent effects of exposure over the lifetimes of a population in the presence of competing causes of death. EPA also accounted for background population cancer risk rates and potential lag time.

PESS Sensitivity

The IUR for leukemia incorporated data on the most highly exposed population (both men and women of the SBR cohort) and utilized the lower 95th percentile modeling estimate assuming a linear response at low doses. Additionally, an ADAF was applied based on the mutagenic MOA to account for increased susceptibility. EPA was unable to quantitatively incorporate other considerations such as considerations lifestyle activities (*e.g.*, smoking), sociodemographic status, or nutrition.

Overall Confidence

There is robust human, animal, and mechanistic evidence associating leukemia and other lymphohematopoietic cancers with 1,3-butadiene exposure. An IUR for leukemia was derived from a study incorporating years of updates to a large occupational cohort covering more than 60 years of follow-up and a novel lifetable analysis was performed to account for extra risk relative to background population rates. Both men and women were included in the analysis, and an ADAF was applied to incorporate elevated childhood susceptibility. Based on the above factors, EPA has robust overall confidence in the hazard assessment and dose-response analysis for leukemia.

EPA did not combine cancer risks from leukemia and bladder due to inconsistent results across publications and concern for smoking as a confounder in the association between bladder cancer and 1,3-butadiene exposure (see Appendix C); however, total risk may be underestimated without

incorporating other tumor sites. EPA will solicit further input from the Science Advisory Committee on Chemicals.

7 CONSIDERATION OF PESS AND AGGREGATE EXPOSURE

7.1 Hazard Considerations for Aggregate Exposure

Human exposure is only expected to occur via inhalation (see *Draft Occupational Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024e) and *Draft General Population Exposure Assessment for 1,3-Butadiene* (U.S. EPA, 2024d)) and consumer sources of 1,3-butadiene exposure are not expected (see *Draft Risk Evaluation for 1,3-Butadiene* (U.S. EPA, 2024g)). Therefore, aggregating exposures across routes or environmental pathways is not necessary.

7.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations expected to be more susceptible to 1,3-butadiene exposure than other populations. Table 7-1 presents the data sources that were used in the PESS analysis that evaluated susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the risk evaluation of 1,3-butadiene.

EPA examined sources of biological susceptibility for each of the susceptibility factors in the below table. The Agency quantitatively incorporated these considerations into hazard values and subsequent risk estimates when possible; however, for many factors EPA did not identify any reasonably available information to support quantitative adjustment of hazard/risk values. For these other factors, the Agency acknowledges either direct or indirect information suggesting additional susceptibility of certain subpopulations.

EPA was able to directly incorporate lifestage susceptibility into hazard values for both cancer and non-cancer endpoints. Two of the three health categories that underwent non-cancer, dose-response analysis represent developmental outcomes following exposure to either pregnant females or males of reproductive age. A 10× UF_H factor was applied to account for human toxicokinetic and toxicodynamic variability, which is expected to account for considerations such as genetic polymorphisms and existing disease states. For the cancer health endpoint, EPA used an occupational epidemiological cohort, comprised of both male and female workers, with more than 50 years of follow-up and subsequent exposure estimate updates to derive inhalation hazard values for leukemia applicable to general population and occupational exposures. Due to an identified mutagenic mode of action for cancer, EPA applied an ADAF for the general population to account for elevated childhood susceptibility. The combination of using the most sensitive endpoint protective of the pregnant worker (decreased fetal weight), robust evidence from a large, highly exposed occupational human cohort tracked over many decades along with the application of an ADAF, allows the derived hazard values used for non-cancer and cancer risk characterizations to fully account for potentially exposed or susceptible subpopulations.

2921 **Table 7-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations**

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to 1,3-Butadiene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to 1,3-Butadiene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	1,3-butadiene *in utero* exposure likely results in decreased fetal weight with associated skeletal rib effects.	Battelle PNL (1987b); Hazleton Labs (1981b)			The most protective and best supported non-cancer POD is based on reduced fetal weight.
	Pregnancy/ lactating status	1,3-butadiene causes decreased weight gain in pregnant dams.	Battelle PNL (1987b); Hazleton Labs (1981b)	Pregnant women have a higher risk for anemia	Le (2016)	Reduced maternal weight gain was BMD modeled and is protected for by the reduced fetal weight POD.
	Males of reproductive age	1,3-butadiene likely causes male reproductive effects, including dominant lethality through genotoxicity to developing sperm.	Anderson et al. (1998); Anderson et al. (1996); BIBRA (1996b); Hackett et al. (1988a)			Dominant lethality was BMD modeled and is protected for by the reduced fetal weight POD.
	Children	Younger lifestages are more susceptible than adults to mutagenic carcinogens. EPA identified a mutagenic MOA for 1,3-butadiene.	U.S. EPA (2005b) and Section 5.3			EPA applied ADAFs to the IUR for in general population risk characterization (U.S. EPA, 2005b).
	Elderly			Elderly people have a higher risk for anemia; however, they should be less susceptible to cancer and reproductive issues than other lifestages.	Le (2016)	This susceptibility is expected to be covered by the 10× UF_H.
Pre-existing disease or disorder	Health outcome/ target organs			Any pre-existing condition affecting a target organ will increase susceptibility to 1,3-butadiene-toxicity in that organ.		This susceptibility is expected to be covered by the 10× UF_H.
	Toxicokinetics			Higher metabolism of reactive metabolites would increase susceptibility		Conservatively applied most animal PODs to humans with 10× UF_H despite indications that humans may produce less toxic metabolites.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to 1,3-Butadiene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to 1,3-Butadiene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestyle activities	Smoking			Heavy smoking and other tobacco usage may increase susceptibility for reproductive outcomes and cancer.	CDC (2023a, 2023b)	
	Alcohol consumption			Alcohol consumption increases risk for several types of cancer, although it is not associated with leukemia or bladder cancer	CDC (2023b)	Qualitative discussion in this section and table only. No direct evidence available.
	Physical activity			Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	CDC (2023a, 2023b, 2022)	Qualitative discussion in this section and table only. No direct evidence available.
Sociodemo-graphic status	Race/ethnicity	Black workers at a styrene/1,3-butadiene manufacturing facility demonstrated significantly elevated standardized mortality ratio (SMR) for leukemia and other lymphatic neoplasms as well as heart disease compared to white workers.	Matanoski et al. (1990)	Blacks and Hispanics have a higher risk for anemia.	Le (2016)	Qualitative discussion in this section and table only. No quantifiable data available to support dose-response analysis for heart disease and dose-response analysis could not be performed on this data set.
	Socioeconomic status			Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	ODPHP (2023b)	Qualitative discussion in this section and table only. This factor may also inform increased exposure.
	Sex/gender	Male mice demonstrated a more sensitive dose-response relationship for reduced fetal body weight and anemia. Indirect data on biomarkers suggests human males may produce higher concentrations of reactive metabolites, and a statistically significant association for leukemia was identified only for exposed male workers.	NTP (1993); Battelle PNL (1987b). Boysen et al. (2022); Boysen et al. (2012); Vacek et al. (2010); Albertini et al. (2007); Albertini et al. (2003) Sathiakumar et al. (2021b); Delzell et al. (1996)			The most sensitive sex from rodent assays were used for non-cancer dose-response modeling.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to 1,3-Butadiene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to 1,3-Butadiene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Nutrition	Diet			Obesity can increase susceptibility to cancer, although this is not established for leukemia or bladder cancer	CDC (2023a)	Qualitative discussion in this section and table only. No direct evidence available.
	Malnutrition			Micronutrient malnutrition can lead to multiple conditions that include birth defects, maternal and infant deaths, preterm birth, low birth weight, poor fetal growth, childhood blindness, undeveloped cognitive ability.	CDC (2023c)	Qualitative discussion in this section and table only. No direct evidence available.
Genetics/ epigenetics	Health outcome/ target organs	Epigenetic variation (histone modifications and DNA methylation) across mouse strains is associated with differential levels of 1,3-butadiene-induced DNA damage .	Lewis et al. (2019)	Deficient DNA repair would increase susceptibility to cancer.		Application of a linear low-dose cancer dose-response model should account for varying susceptibility across populations.
	Toxicokinetics	GSTM1 and GST11 mutations are associated with 30–60% higher sister chromatid exchange from 1,3-butadiene metabolites. Polymorphisms for microsomal epoxide hydrolase resulted in 3x greater mutation frequencies among exposed workers. CYP2E1 and microsomal epoxide hydrolase polymorphisms were associated with greater genotoxicity.	ATSDR (2012); U.S. EPA (2002a) Xiang et al. (2012)	Genetic variation across populations may explain differences in relative mutation and genotoxicity rates seen across Texas, Chinese, and Czech cohorts		EPA used the positive mutation data from Texas cohorts in supporting a mutagenic MOA and application of a linear low-dose cancer dose-response model should account for varying susceptibility across populations.

Susceptibility Factor	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to 1,3-Butadiene		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to 1,3-Butadiene		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Other chemical and nonchemical stressors	Built environment			Poor-quality housing is associated with a variety of negative health outcomes.	ODPHP (2023a)	Qualitative discussion in this section and table only. This category is primarily relevant to increased exposure.
	Social environment			Social isolation and other social determinants (*e.g.*, decreased social capital, stress) can lead to negative health outcomes.	ODPHP (2023c)	Qualitative discussion in this section and table only. No direct or quantifiable evidence available.
	Chemical co-exposures	1,3-butadiene can degrade in the environment into other toxic chemicals, including formaldehyde. It is also often released alongside other hazardous air pollutants	U.S. EPA (2024f) EPA Final Rule Final Rule to Strengthen Standards for Synthetic Organic Chemical Plants and Polymers and Resins Plants			Qualitative discussion in this section and table only. No direct evidence available.

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8 HUMAN HEALTH HAZARD ASSESSMENT CONCLUSIONS

In this human health assessment of 1,3-butadiene, EPA determined that reduced fetal weight based on a developmental mouse study was the most sensitive and robust endpoint for risk characterization of intermediate and chronic exposures. The Agency additionally derived PODs for male reproductive/ developmental toxicity and hematological effects that can be considered supportive and part of the weight of evidence for selecting the fetal body weight effect. The weight of scientific evidence and dose-response considerations based on the reasonably available information did not support derivation of a POD for acute exposures. For cancer, EPA derived an IUR for the general population and chronic occupational UR for adults for leukemia based on robust data from an occupational human cohort. An ADAF is applied to this value based on a proposed mutagenic mode of action. An IUR for bladder cancer (from the same occupational cohort) was also separately derived for comparison purposes.

Table 8-1 lists the studies and corresponding HECs and UFs that EPA is using for risk characterization following intermediate and chronic exposure. Table 8-2 provides the IUR for evaluating lifetime exposure to workers—all of which are assumed to be either adults or adolescents of at least 16 years old and therefore ADAFs do not apply. Based on the mutagenic MOA for cancer concluded in Section 5.3, EPA also applied ADAFs to the adult-based IUR to account for childhood exposures in the general population (Table 8-3).

For consistency, all HECs and the IUR are based on daily, continuous exposure (24 hours/day) to consistent concentrations. HECs from animal studies assume an individual at resting breathing rate, while the IUR derived from an occupational cohort assumes worker breathing rate. Adjustments to exposure durations, frequencies, and breathing rates are made in the exposure estimates used to calculate risks for individual exposure scenarios.

Table 8-1. Non-cancer Points of Departure and Critical Endpoints Used for Risk Estimates of Each Exposure Scenario

Target Organ System	Species	Duration	Study POD/Type	Effect	HEC (ppm) [mg/m^3]	Uncertainty Factors (UFs)	Reference	Overall Quality Determination
Intermediate/chronic exposure scenarios								
Maternal/ Developmental	Mouse (Male)	10 days throughout gestation (GD 5–16)	LOAEL = 40 ppm	Reduced fetal body weight and other associated endpoints	$BMDL_5$ = 2.5 ppm (5.5 mg/m^3)	UF_A= 3; UF_H=10 *Total UF=30*	(Battelle PNL, 1987b)	Medium

Table 8-2. Cancer Hazard Values for Occupational Cancer Risk Estimation

Chronic Occupational Unit Risk[a]	Reference	Overall Quality Determination
0.0062 per ppm[b] (2.8E−03 per mg/m^3) (2.8E−06 per µg/m^3)	(Sathiakumar et al., 2021b)	Medium

[a] EPA considers a range of extra cancer risk from 1E−04 to 1E−06 to be relevant benchmarks for risk assessment (U.S. EPA, 2017); however, these are not considered bright lines for unreasonable risk determination.

[b] The occupational unit risk was corrected as described in *1,3-Butadiene: Corrected lifetable analyses for leukemia and bladder cancer* (U. S. EPA, 2024a). The corrected occupational unit risk = 0.0049 per ppm (2.2×10^{-6} per µg/m^3)

Table 8-3. Incorporation of Age-Dependent Adjustment Factors for General Population Risk Estimation

Age	ADAF Adjustment[a]	Adjusted Partial Life and General Population IUR
0 to <2	10×	0.0062 × 10 × (2/78) = 0.0016
2 to <16	3×	0.0062 × 3 × (14/78) = 0.0033
≥16[b]	1×	0.0062 × 1 × (62/78) = 0.0049
0 to 78		**0.0098 per ppm (4.4E−06 per µg/m^3)**

[a] ADAFs are applied based on the determination of a mutagenic MOA (Section 5.3) and in accordance with (U.S. EPA, 2005b).
[b] Adjusted IUR value is based on an assumption of 78 years lifetime (U.S. EPA, 2011).
[c] The unit risk was corrected as described in *1,3-Butadiene: Corrected lifetable analyses for leukemia and bladder cancer* (U. S. EPA, 2024a). The corrected Adult-exposure-only (62 years) unit risk = 0.0049 per ppm (2.2E−06 per µg/m^3). However, the correction did not change the general population (78 years) IUR value which remains 0.0098 per ppm (4.4E−06 per µg/m^3) (Appendix F).

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APPENDICES

Appendix A EVIDENCE INTEGRATION TABLES

These evidence integration tables are presented for hazard outcomes with substantial evidence that underwent a more detailed evidence integration process in consideration of the weight of scientific evidence. The format and process for determination of the within-stream and evidence integration judgements are described in the Draft Systematic Review Protocol (U.S. EPA, 2021). This process and format was adapted from the EPA ORD staff handbook for developing IRIS assessments (U.S. EPA, 2022), particularly the consideration of human and animal evidence streams. The TSCA draft systematic review protocol formally adds an additional evidence stream for mechanistic that incorporates both qualitative and quantitative considerations of human relevance and plausibility. The hazard identification and evidence integration for additional health outcomes with limited data are described in Appendix D.

Table_Apx A-1. Evidence Integration for Ovarian Atrophy and Associated Female Reproductive System Toxicity

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence in studies of exposed humans considered for deriving toxicity values				
No human studies were identified that examined female reproductive toxicity	None	None	*Key findings:* None *Overall judgement for female reproductive toxicity based on human evidence*: • Indeterminate	*Overall judgement for female reproductive toxicity (ovarian atrophy) based on integration of information across evidence streams:* **Evidence suggests but is not sufficient to conclude that 1,3-butadiene exposure causes ovarian toxicity in humans under relevant exposure circumstances.**
Evidence from *in vivo* mammalian animal studies considered for deriving toxicity values				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Mouse studies *Subchronic studies* • Exposed 15 days or 14 weeks to ≤8,000 ppm for 6 hours/day, 5 days/week (NTP, 1984). Evaluated histopathology and functional observations of female reproductive organs. OQD=Uninformative. • Exposed for 13 weeks to 980 ppm for 5 hours/day, 5 days/week (Bevan et al., 1996). Evaluated histopathology and functional observations of female reproductive organs. OQD=Medium. *Chronic studies* • Exposed 40 weeks to 2 years to ≤200 ppm for 6 hours/day, 5 days/week (NTP, 1993). OQD=High • Exposed 61 weeks to ≤1,250 ppm for 6 hours/day, 5 days/week (NTP, 1984). OQD=High. • Exposed 62 weeks to < 1,250 ppm for 5 hours/day, 6 hours/day, 5 days/week (Battelle PNL, 1982). Evaluated histopathology of female reproductive organs. OQD=Uninformative. Rat studies *Chronic studies*	• Ovarian atrophy observed in all acceptable studies at 13 weeks, 40 weeks, and 2 years of exposure in mice. • Ovarian atrophy severity was dose-responsive and observed following as low as 6.25 ppm exposure for 2 years in mice. • Ovarian atrophy in mice was accompanied by an absence of oocytes, follicles, and corpora lutea along with angiectasis and uterine involution.	• No histopathological changes observed in 15-day mouse study, however this provides minimal weight due to the sub-acute exposure duration and uninformative OQD. • No ovarian effects observed at any dose or duration in rats up to 8,000 ppm and for as long as 2 years exposure indicating a lack of consistency across species.	*Key findings:* Severe ovarian toxicity is observed in mice in a dose-responsive and duration-responsive manner in both medium and high quality studies. However, no signs of ovarian toxicity are observed in rats exposed for 2 years to a high dose. *Overall judgement for female reproductive toxicity based on animal evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Exposed for 2 years to ≤8,000 ppm for 6 hours/day, 5 days/week (Hazleton Labs, 1981b). Evaluated histopathology and functional observations of female reproductive organs. OQD = Medium.				
Evidence in mechanistic studies and supplemental information				
<u>Metabolism differences</u> • Multiple studies demonstrate differences in metabolism across species, although estimates vary based on sex, dose, duration, and other factors <u>Metabolite and species-specific potencies</u> • Studies on metabolites of both DEB and analogs demonstrate differences in ovarian sensitivity between rats and mice or between mono and di-epoxides <u>Mechanism of action</u> • The di-epoxide form of an analog appear to cause ovotoxicity through induction of apoptosis in follicles (Hoyer and Sipes, 2007; Hu et al., 2001) and DEB activates apoptotic signaling in lymphoblasts (Yadavilli and Muganda, 2004).	• Mono-epoxides are capable of inducing ovotoxicity in mice (Hoyer and Sipes, 2007; Doerr et al., 1996). • DEB does form in humans, albeit orders of magnitude lower than in rodents (Motwani and Törnqvist, 2014; Swenberg et al., 2011). • c-kit receptor and kit ligand have been detected in human ovaries and therefore the proposed MOA is plausible in humans (Tuck et al., 2015).	• Levels of the metabolite DEB are 100–300× ore more higher in mice compared to humans, 40-100× higher in mice compared to rats (Motwani and Törnqvist, 2014; Swenberg et al., 2011). Estimates of human metabolite levels are very variable however and typically use only male subjects. • EB (mono-epoxide) caused ovotoxicity only in mice not rats; DEB caused effects in both, but mice were several-fold more sensitive (Doerr et al., 1996). • The diepoxide form of vinylcyclohexene is 2–3× more active than the monoepoxide, and 2–3× more active in mice vs rats (Hoyer and Sipes, 2007)	*Key findings*: Studies on metabolites of both 1,3-butadiene and analog 4-vinylcyclohexene suggest that mice are both toxicokinetically and toxicodynamically more sensitive than rats, and likely humans. Humans exhibit the same metabolites and signaling pathways as mice and rats however, so ovarian toxicity in humans is qualitatively plausible, albeit likely requiring much higher exposures due to significantly reduced metabolism of DEB relative to mice. The precise quantification of metabolite levels is uncertain due to variability across experimental conditions and the use of hemoglobin adducts as a surrogate measure. *Overall judgement for female reproductive toxicity based on mechanistic evidence*:	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• DEB induces chromosome damage in oocytes (Tiveron et al., 1997), and a di-epoxide analog induces oocyte apoptosis through blocking the c-kit signaling pathway (Kappeler and Hoyer, 2012).			• Indeterminate	

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3860 **Table_Apx A-2. Evidence Integration for Maternal and Related Developmental Toxicity**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence in studies of exposed humans considered for deriving toxicity values				*Overall judgement for maternal/developmental toxicity based on integration of information across evidence streams:* **Evidence indicates that 1,3-butadiene exposure is likely to cause maternal and related developmental toxicity in humans under relevant exposure circumstances.**
• A cohort study examined risks for autism in children associated with location relative to an air monitor (von Ehrenstein et al., 2014). OQD=Medium.	• *In utero* exposure to 1,3-butadiene was positively associated with autism. Higher risks were associated with closer distance to the air monitor.	• Exposure was not directly quantified in the sole developmental toxicity study. • No epidemiological studies measured similar outcomes to what was observed in animal studies.	*Key findings:* None *Overall judgement for maternal/developmental toxicity based on human evidence*: • Slight	
Evidence from *in vivo* mammalian animal studies considered for deriving toxicity values				
Mouse studies *Female gestational exposure* • Females exposed for 10 days (gestation days [GD] 6-15) to ≤1,000 ppm for 6 hours/day (Battelle PNL, 1987b). Evaluated maternal and developmental toxicity. OQD=Medium. Rat studies	• In female mice exposed during gestation, maternal toxicity was observed, including three mortalities with signs of dehydration at 1,000 ppm and decreased maternal weight gain at ≥199.8 ppm (Battelle PNL, 1987b). • In offspring of female mice exposed during gestation, decreased fetal body weight	• Inconsistent results were observed for developmental outcomes among rat studies using the same strain (Battelle PNL, 1987a; Hazleton Labs, 1981a). • Maternal body weight was not decreased following a total exposure period of 60-70 days) to ≤6006 ppm (WIL Research, 2003).	*Key findings:* In mice exposed during gestation, decreased maternal body weight gain, decreased fetal body weight, and increased fetal malformations were observed in a dose-responsive and dose-concordant manner in medium-quality studies.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Female gestational exposure • Females exposed for 10 days (GD 6–15) to ≤7,647 ppm for 6 hours/day (Hazleton Labs, 1981a). Evaluated maternal and developmental toxicity. OQD=Medium. • Females exposed for 10 days (GD 6–15) to ≤1,005 ppm for 6 hours/day (Battelle PNL, 1987a). Evaluated maternal and developmental toxicity. OQD=High (maternal effects), Medium (developmental effects). *Male and female exposure* • Males were exposed for 83–84 consecutive days and females were exposed for 60–70 days (15 exposures prior to breeding, through GD 20, and from lactation day 5 until the day prior to euthanasia, total exposure period of 60–70 days). One group of F1 pups was sacrificed at weaning; a second was exposed for 7 days (from PND 21–27) at the same concentrations as their dams; and a group of control (unexposed during gestation and lactation); a third group of F1 pups were exposed for 7 days (from PND 28–34). Exposures were ≤6,006 ppm for 6 hours/day (WIL Research, 2003). Evaluated maternal and developmental toxicity. OQD=Medium.	was observed at ≥39.9 ppm in males and at ≥200 ppm in females (Battelle PNL, 1987b). • In offspring of female mice exposed during gestation increased supernumerary ribs was observed at ≥200 ppm and decreased ossification and abnormal sternebrae was observed at 1,000 ppm (Battelle PNL, 1987b). • In female rats exposed during gestation, decreased maternal body weight gain during exposure was observed at ≥202 ppm in one study (Hazleton Labs, 1981a) and at 1005 ppm in another study (Battelle PNL, 1987a). • In offspring of female rats exposed during gestation, statistically significant decreased fetal body weight and crown-rump length were observed at 7647 ppm; increased (no statistics were performed) dose-responsive incidences of major skeletal defects were observed at ≥990 ppm with other major fetal defects observed at 7,647 ppm; litter incidences were not reported (Hazleton Labs, 1981a). • In female rats exposed before and during mating and throughout gestation and lactation, clinical signs of		Reduced maternal weight gain was also observed in two of three rat studies (both on the same strain) and decreased female pup weight was observed following neonatal exposures, with other fetal outcomes inconsistently observed and only at high doses. *Overall judgement for maternal/developmental toxicity based on animal evidence*: • Robust	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
	toxicity (chromodacryorrhea, chromorhinorrhea, and salivation) were observed in the 1 hour after exposure at ≥1,507 ppm (WIL Research, 2003). • Body weight was statistically significantly reduced in female F1 pups (males had a biologically but not statistically significant reduction) exposed for 7 days to ≥1,507 ppm either with or without previous gestational/lactational exposure(WIL Research, 2003).			
Evidence in mechanistic studies and supplemental information				
Metabolism differences • Multiple studies demonstrate differences in metabolism across species, although estimates vary based on sex, dose, duration, and other factors. Metabolite studies • Female rats were administered DEB i.p. for 4 days during GD 5–8 to 0.25–0.40 mmol (Chi et al., 2002). Evaluated fetal growth and viability along with placental hormones and enzymes activity. • DEB was administered to early mouse embryos (1–5 µm) or pregnant dams (10 µm via injection) (Clerici et al., 1995). Evaluated embryo developmental.	• DEB negatively impacted embryonic development in mice in a dose-responsive manner. • DEB negatively impacted fetal growth and viability in rats in a duration and dose-responsive manner.	• There are no available mechanistic studies investigating parental 1,3-butadiene or other metabolites for comparison with these results.	*Key findings:* The 1,3-butadiene metabolite DEB disrupts embryonic and fetal development in both mice and rats. A proposed mechanism in rats involves decreased progesterone and placental enzyme activity. *Overall judgement for maternal/ developmental toxicity based on mechanistic evidence*: • Slight	

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3862 **Table_Apx A-3. Evidence Integration for Male Reproductive System and Resulting Developmental Toxicity**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence in studies of exposed humans considered for deriving toxicity values				
No human studies were identified that examined male reproductive toxicity	None	None	*Key findings:* None *Overall judgement for male reproductive toxicity based on human evidence*: • Indeterminate	
Evidence from *in vivo* mammalian animal studies considered for deriving toxicity values				
Sperm and testicular effects				
Mouse studies *Subacute studies* • Exposed for 5 days to ≤1,300 ppm for 6 hours/day (Xiao and Tates, 1995). Evaluated testis weight. OQD=Low. • Exposed for 5 days to ≤1,300 ppm for 6 hours/day (Pacchierotti et al., 1998). Evaluated testis weight and spermatid count. OQD=Low. • Exposed for 5 days to ≤5,000 ppm for 6 hours/day (Hackett et al., 1988a). Evaluated sperm morphology. OQD=Medium. *Subchronic studies* • Exposed for 13 weeks to 980 ppm for 6 hours/day, 5 days/week (Bevan et al., 1996). Evaluated testes weight and histopathology. OQD=Medium. *Chronic studies* • Exposed for 60 weeks to ≤1236 ppm for 6 hours/day, 5 days/week	• Sperm-head abnormalities observed in a dose-responsive manner at ≥1,000 ppm following 5 days of exposure in mice (Hackett et al., 1988a). • Reduced immature spermatid count observed in mice at ≥130 ppm for 5 days (Pacchierotti et al., 1998) in a low-quality study. • Testicular atrophy observed at 980 ppm for 13 weeks (Bevan et al., 1996) and at ≥619 ppm for at least 9 months of exposure in mice (NTP, 1993, 1984). Severity at 619 ppm was greatest at 2 years of exposure and was characterized by a "uniform minimal to mild decrease in cellularity of the seminiferous tubules" (NTP, 1993).	• No sperm or testicular effects observed in rats up to 6006 ppm for 12 weeks indicating a lack of consistency across species (WIL Research, 2003).	*Key findings:* Sperm and testicular effects in mice are observed in a dose- and duration-responsive manner in both medium- and high-quality studies. However, no sperm or testicular effects are observed in rats exposed for 12 weeks to a high dose, suggesting a species dependency. *Overall judgement for male reproductive toxicity based on animal evidence*: • Moderate	*Overall judgement for male reproductive toxicity (sperm and testicular effects and dominant lethality) based on integration of information across evidence streams:* **Evidence indicates that 1,3-butadiene exposure is likely to cause male reproductive and resulting developmental toxicity in humans under relevant exposure circumstances.**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
(NTP, 1984). Evaluated histopathology of male reproductive organs. OQD=High. • Exposed for 2 years to ≤619 ppm for 6 hours/day, 5 days/week (NTP, 1993). Evaluated histopathology of male reproductive organs. OQD=High. Rat studies *Subchronic studies* • Exposed for 12 weeks to ≤6,006 ppm for 6 hours/day, 7 days/week (WIL Research, 2003). Evaluated male reproductive performance, histopathology, and sperm parameters. OQD=Medium.	• Reduced testis weight observed in mice at ≥130 ppm for 5 days in two low-quality studies (Pacchierotti et al., 1998; Xiao and Tates, 1995) and at 980 ppm for 13 weeks (Bevan et al., 1996).			
Dominant lethal assays				
Mouse studies *Acute study* • Exposed one day for 6 hours to 1,250 or 6,250 ppm (Anderson et al., 1996; Anderson et al., 1993). OQD = Uninformative for 1996 study, not determined for 1993 study *Short-term studies* • Exposed for 5 days to ≤500 ppm for 6 hours/day (Adler et al., 1998). OQD=Medium. • Exposed for 5 days to 1,300 ppm for 6 hours/day (Adler et al., 1994). OQD=not determined. • Exposed for 5 days to ≤5,000 ppm for 6 hours/day (Hackett et al., 1988b). OQD=Medium. *Subchronic studies*	In dominant lethal assays, the following effects were observed in mice: • Increased early fetal deaths at 500 ppm (Adler et al., 1998) and 1,300 ppm (Adler et al., 1994)at 5 days, at ≥65 ppm at 4 weeks (Anderson et al., 1998; BIBRA, 1996b), and at >125 ppm at 10 weeks (Brinkworth et al., 1998; Anderson et al., 1996); • Decreased implantation at 1,250 ppm at 10 weeks (Anderson et al., 1996); • Delayed time-to-coition at 125 ppm at 10 weeks (Brinkworth et al., 1998); and	• Reverse dose-response seen at higher doses in acute/short-term studies (Anderson et al., 1993; Hackett et al., 1988b). • No effects seen in dominant lethality studies in rats.	*Key findings:* Dominant lethal effects in mice are observed in a dose- and duration- responsive manner in medium-quality studies. However, no signs of dominant lethality are observed in rats exposed for 4 or 12 weeks to a high dose, suggesting a species-dependency. *Overall judgement for dominant lethality based on animal evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Exposed for 4 weeks to ≤130 ppm for 6 hours/day, 5 days/week (Anderson et al., 1998; BIBRA, 1996b). OQD=Medium. • Exposed for 10 weeks to 12.5 or 125 ppm for 6 hours/day, 5 days/week (Brinkworth et al., 1998). OQD=Medium. • Exposed for 10 weeks to 12.5 or 1,250 ppm for 6 hours/day, 5 days/week (Anderson et al., 1996). OQD=Medium. Rat studies *Subchronic studies* • Exposed for 4 weeks to ≤1,250 ppm for 6 hours/day, 5 days/week (Anderson et al., 1998). OQD=Medium. • Exposed for 10 weeks to ≤1,250 ppm for 6 hours/day, 5 days/week (BIBRA, 1996a). OQD=Medium.	• Increased late fetal deaths including dead fetuses and abnormal fetuses at ≥12.5 ppm at 10 weeks (Anderson et al., 1996). • An increased percentage of abnormal fetuses was observed at ≥12.5 ppm. External and skeletal abnormalities were reported (however, only a subset of fetuses was processed for skeletal examination) (Anderson et al., 1998; Anderson et al., 1996; BIBRA, 1996b).			
Evidence in mechanistic studies and supplemental information				
Mechanism of toxicity • Genotoxicity testing of 1,3-butadiene (BD) and its metabolites (diepoxybutane [DEB], epoxybutane [EB], and epoxybutanediol [EBD]) in germ cells includes evaluation of micronuclei formation, chromosome aberrations, DNA damage, and heritable translocation (U.S. EPA, 2002b)	• Micronuclei were increased in early-stage spermatids from mice exposed to 1,3-butadiene or its metabolites (EB, DEB, and EBD) (U.S. EPA, 2002b; Xiao and Tates, 1995). • Chromosome aberrations were increased in first cleavage embryos derived from 1,3-butadiene- and EBD-exposed male mice (U.S. EPA, 2002b; Pacchierotti et al., 1998). • DNA damage was reported in haploid and polyploid cells	• Dominant lethality was not observed following i.p. injection of EB or DEB in mice; however, these results may be confounded by cytotoxicity leading to decreased implantation rate (U.S. EPA, 2002b). • Only DEB but not EBD or EB induced genotoxicity in cultured rat seminiferous tubule sections (U.S. EPA, 2002b; Sjoblom and Lahdetie, 1996).	*Key findings:* Dominant lethal effects appear to result from cytogenetic damage in male germ cells, especially late spermatids and spermatogonia (U.S. EPA, 2002b), and metabolites demonstrate *in vivo* germ cell genotoxicity in both mice and rats. No mechanistic data were available to evaluate testicular effects.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
	from the testis of male mice exposed to 1,3-butadiene (U.S. EPA, 2002b). • Heritable translocation studies demonstrate that cytogenetic effects are transmissible across generations (U.S. EPA, 2002b; Adler et al., 1998). • All three major metabolites (EB, DEB, EBD) induced clastogenicity in rat spermatids following i.p. injection (U.S. EPA, 2002b; Lähdetie et al., 1997).	• Mixed dominant lethality results on administered DEB and EB in mice suggest that developing sperm have stage-specific sensitivity (U.S. EPA, 2002b).	*Overall judgement for male reproductive toxicity based on mechanistic evidence*: • Moderate	

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3864 **Table_Apx A-4. Evidence Integration for Hematological and Immune Effects**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence in studies of exposed humans considered for deriving toxicity values				*Overall judgement for hematologic effects based on integration of information across evidence streams:* **Evidence indicates that 1,3-butadiene exposure is likely to cause hematologic changes consistent with anemia in humans under relevant exposure circumstances.**
• Cohort study comparing butadiene polymer workers (n=41) and internal comparison group (n=38). Exposure evaluated by occupational history, air measurements, and hemoglobin adducts. Endpoints included erythrocyte count, leukocyte parameters, and platelet count (Hayes et al., 2000). OQD=Low. *Other human studies* • Cohort studies of petrochemical workers and styrene-butadiene synthetic rubber manufacturing workers (Tsai et al., 2005; Tsai et al., 2001; Cowles et al., 1994; Checkoway and Williams, 1982).	• A slight, but statistically significant, lower hemoglobin concentration was observed in exposed petrochemical workers compared to an unexposed internal referent group (Tsai et al., 2005). • After adjustment for confounders, a significant association was observed between 1,3-butadiene exposure level and increased mean corpuscular hemoglobin concentration in a health survey of styrene-butadiene workers (Checkoway and Williams, 1982).	• In a low-quality cohort study with small numbers of participants, no was identified association between erythrocyte count and 1,3-butadiene exposure was observed (Hayes et al., 2000). • No association between erythrocyte count and 1,3-butadiene exposure was identified in other studies of petrochemical workers (Tsai et al., 2005; Cowles et al., 1994) or styrene-butadiene workers (Checkoway and Williams, 1982). In another study, (Tsai et al., 2001), no association was identified for any hematological measure.	*Key findings:* Epidemiology data on hematological effects of 1,3-butadiene are limited by small population sizes and evaluation of few hematological parameters. Two studies suggested associations between 1,3-butadiene and hemoglobin levels. None of the studies reported exposure-related alterations in erythrocyte counts. *Overall judgement for hematologic effects based on human evidence*: • Indeterminate	
Evidence from *in vivo* mammalian animal studies considered for deriving toxicity values				
Mouse studies[2] • Exposed for 6, 12, or 24 weeks to 0 or 1,250 ppm (Thurmond et al., 1986). Evaluated spleen weights and histopathology of spleen and bone marrow. OQD=Low (6- and	• Hematologic changes consistent with anemia[3] observed in mice at 980 ppm for 13 weeks (Bevan et al., 1996) and ≥61.4 ppm (males) or ≥199 ppm (females) for 9 months (NTP, 1993). After 15	• In the 103-week mouse study, survival was decreased at ≥19.8 ppm, and no females at ≥199 ppm or males at 619 ppm survived to the end of exposure due to tumors (NTP, 1993),	*Key findings:* 1,3-Butadiene produced dose- and duration-responsive effects on hematology parameters consistent with anemia in mice with supporting histopathological changes in the	

[2] In all studies, animals were exposed for 6 hours/day, 5 days/week.
[3] Hematology changes consistent with anemia included decreased erythrocyte counts, hemoglobin concentration, and mean erythrocyte volume and increased Howell-Jolly bodies and mean cell volume.

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
12-week). OQD=Uninformative (24-week). • Exposed 13 weeks to 0 or 980 ppm (Bevan et al., 1996). Evaluated hematology, spleen weight, and histopathology of spleen and bone marrow. OQD=Medium. • Exposed for 60–61 weeks to ≤1,236 ppm (NTP, 1984). Evaluated histopathology of spleen and bone marrow. OQD=High. • Exposed for up to 103 weeks to ≤619 ppm (NTP, 1993). Evaluated hematology (after 9 and 15 months), spleen weights, and histopathology of spleen and bone marrow. OQD=High. • Stop exposure experiments on relationship of cancer to product of concentration and duration: exposed to 199 ppm for 40 weeks, 312 ppm for 52 weeks, or 619 ppm for 13 or 26 weeks, and then monitored untreated until sacrifice at 103 weeks (NTP, 1993). Evaluated histopathology of spleen and bone marrow. OQD=Medium. Rat studies[2] • Exposed for 13 weeks to 0 or 980 ppm (Bevan et al., 1996). Evaluated hematology, spleen weights, and histopathology of spleen and bone marrow. OQD=Medium.	months, anemia was observed only at 619 ppm (NTP, 1993), possibly reflecting compensatory changes. • Decreased spleen weights were observed in male and/or female mice at 1,250 ppm for 6 weeks or 980 ppm for 13 weeks (Bevan et al., 1996; Thurmond et al., 1986) and in females at ≥199 ppm for 9 months (NTP, 1993). After 15 months, spleen weights were increased among survivors at ≥199 ppm (females) or 619 ppm (males) (NTP, 1993). • Histopathology changes in the spleen (atrophy, decreased cellularity, extramedullary hematopoiesis, erythroid hyperplasia) and/or bone marrow (atrophy, decreased cellularity) consistent with poorly regenerative macrocytic anemia were observed in mice exposed for short-term and subchronic durations (Bevan et al., 1996; NTP, 1993; Thurmond et al., 1986). • In other studies of mice exposed by inhalation, macrocytic-megaloblastic anemia was observed (Irons et al., 1986a, b). • Increased relative (but not absolute) spleen weight was observed among surviving male rats exposed to 8,000 ppm	limiting interpretation of histopathology findings at 2-year sacrifice. • No treatment-related changes in hematology or spleen or bone marrow histopathology were observed in rats exposed to 980 ppm for 13 weeks (Bevan et al., 1996) or up to 8,000 ppm for up to 2 years (Hazleton Labs, 1981b).	spleen and bone marrow. Exposed rats exhibited little evidence of hematological effects. *Overall judgement for hematologic effects based on animal evidence*: • Moderate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Exposed for 13 weeks to ≤8,000 ppm (Crouch et al., 1979). Evaluated hematology, spleen weights, and histopathology of spleen. OQD=Uninformative. • Exposed for 111 weeks (males) or 105 weeks (females) to 0, 1,000, or 8,000 ppm (Hazleton Labs, 1981b). Evaluated hematology (after 3, 6, 12, and 18 months), spleen weights, and histopathology of spleen and bone marrow. OQD=Medium. *Other animal studies* • Mice exposed for 6 weeks to 0 or 1,250 ppm (Irons et al., 1986a, b).	for 2 years (Hazleton Labs, 1981b).			

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence in mechanistic studies and supplemental information				
Metabolism differences • Multiple studies demonstrate differences in metabolism across species, although estimates vary based on sex, dose, duration, and other factors. Mechanism of action • Bone marrow genotoxicity and cytotoxicity were investigated in many studies of mice and two studies of rats exposed to 1,3-butadiene by inhalation (ATSDR, 2012; U.S. EPA, 2002b). • Several studies evaluated genotoxicity in bone marrow or spleen of mice, rats, or hamsters exposed to metabolites of 1,3-butadiene by inhalation or i.p. injection (U.S. EPA, 2002b). • Study of bone marrow stem cells exposed *in vitro* (Leiderman et al., 1986). • Anemia may be associated with lymphohematopoietic cancers through bone marrow dysfunction.	• In mice exposed by inhalation, 1,3-butadiene exposure induced significant cytotoxicity and genotoxicity (SCEs, micronuclei, mutations) in bone marrow (ATSDR, 2012; U.S. EPA, 2002b). • DEB and EB exposure induced genotoxicity in bone marrow and spleen of mice, hamsters, and rats (U.S. EPA, 2002b). • EBD induced genotoxicity in bone marrow of mice (rats were not tested) (U.S. EPA, 2002b). • Hemoglobin adducts have been observed in mice, rats, and humans (although it is unclear if these are merely markers of exposure) (ATSDR, 2012; U.S. EPA, 2002b). • 1,3-butadiene induces lymphohematopoietic cancers in both mice and humans (ATSDR, 2012; U.S. EPA, 2002b). • An *in vitro* study showed that 1,3-butadiene decreased the ratio of mature to immature bone marrow stem cells (Leiderman et al., 1986).	• Two studies in rats exposed to 1,3-butadiene by inhalation showed no increases in micronuclei or SCEs in bone marrow (ATSDR, 2012; U.S. EPA, 2002b).	*Key findings*: Genotoxicity in bone marrow cells may contribute to 1,3-butadiene-induced hematological effects leading to anemia in mice. Other mechanistic and supporting information suggest that mechanisms underlying development of anemia should be present in humans. *Overall judgement for hematologic effects based on mechanistic evidence*: • Slight	

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Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Lymphohematopoietic cancers				
Evidence for lymphohematopoietic cancers in studies of exposed humans[1]				
• Retrospective cohort studies of styrene-butadiene rubber workers (n>22,000 men and women) (Sathiakumar et al., 2021b; Sathiakumar et al., 2019; Sathiakumar et al., 2015; Sielken and Valdez-Flores, 2011; Graff et al., 2009; Sathiakumar and Delzell, 2009; Cheng et al., 2007; Delzell et al., 2006; Graff et al., 2005; Sathiakumar et al., 2005; Delzell et al., 2001; IISRP, 1999; Delzell et al., 1996; UAB, 1995) OQD=Medium; (Valdez-Flores et al., 2022; Sielken and Valdez-Flores, 2013; Sielken, 2007; Sielken and Valdez-Flores, 2001; IISRP, 1986) OQD=Low. • Retrospective cohort study of butadiene monomer workers (n=2,800 men) (Divine and Hartman, 2001). OQD=Medium. • Case-control study of ALL and AML in children <6 years old, exposure based on ambient air monitoring data at station nearest maternal address during pregnancy (Heck et al., 2014). OQD=Medium. • Case-control study of ALL in children <5 years old, exposure based on modeled air concentration at maternal address at birth (Symanski et al., 2016). OQD=Medium.	• In a large cohort of styrene-butadiene rubber workers, exposure to 1,3-butadiene was associated with increased risk of mortality from leukemia in men and women. The risk increased with magnitude and duration of exposure and remained elevated after control for covariates including styrene exposure, consideration of alternative exposure assessments, and longer follow-up times (Valdez-Flores et al., 2022; Sathiakumar et al., 2021b; Sathiakumar et al., 2019; Sathiakumar et al., 2015; Sielken and Valdez-Flores, 2013, 2011; Graff et al., 2009; Cheng et al., 2007; Graff et al., 2005; Sathiakumar et al., 2005; Delzell et al., 2001; Sielken and Valdez-Flores, 2001; IISRP, 1999; Delzell et al., 1996; UAB, 1995; IISRP, 1986). • The most recent analyses with longest follow-up of this cohort reported an exposure-response trend for lymphoid leukemia but not myeloid leukemia, and trends for B-cell malignancies and NHL in some, but not all, analyses (Sathiakumar et al.,	• In butadiene monomer workers, the increased mortality from lymphohematopoietic cancer was not correlated with employment duration (Divine and Hartman, 2001). • Classification of lymphohematopoietic cancers is complex and has changed over time.	*Key findings:* Extensive analyses of a large cohort of styrene-butadiene rubber workers document a clear association between occupational 1,3-butadiene exposure and exposure-related increases in mortality from leukemia. This finding is supported by studies of smaller cohorts and case-control studies of exposure to ambient air. Subtype analyses suggest the strongest association with lymphoid leukemias. *Overall judgement for lymphohematopoietic system tumors based on human evidence*: • Robust	*Overall judgement for carcinogenicity based on integration of information across evidence streams:* **Based on EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), EPA concludes that 1,3-butadiene is carcinogenic to humans.**

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Case-control study of leukemia, Hodgkin's disease, and NHL in people <20 years old, exposure based on modeled air concentration at residence at time of diagnosis (Whitworth et al., 2008). OQD=Medium. *Other Human Studies* • Authoritative reviews of older epidemiology data (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002b) have concluded that occupational exposure to 1,3-butadiene was associated with increased mortality from leukemia and NHL. • One semiquantitative study assessed relative levels of male hematopoietic cancer nearby hydrocarbon processing centers in Canada (Simpson et al., 2013).	2021b) • In butadiene monomer workers, exposure to 1,3-butadiene was associated with increased mortality from lymphohematopoietic cancer (Divine and Hartman, 2001). • In case-control studies of non-occupational populations, higher measured or modeled air concentration of 1,3-butadiene was associated with increased odds of leukemia, ALL, and/or AML (Symanski et al., 2016; Heck et al., 2014; Whitworth et al., 2008). • Male hematopoietic cancers were elevated (no statistics provided) near a hydrocarbon processing center with high 1,3-butadiene levels (Simpson et al., 2013)			
Evidence for lymphohematopoietic cancers from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low. Rat studies	• Significant dose-related trends and pairwise comparisons with concurrent controls for histiocytic sarcoma in male and female mice in one study (NTP, 1993). Significant increases remained after adjustment for survival. Significantly increased incidences were seen in male mice in all stop-exposure groups. • Significant dose-related trends and pairwise comparisons with	• No increase in hematopoietic system tumor incidence in rats indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced dose-related increased incidences of hematopoietic system cancers in male and female mice and these cancers were the primary cause of early deaths in exposed mice in both available studies. No increase in hematopoietic system cancer incidence was observed in exposed rats.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	concurrent controls for malignant lymphoma/ lymphocytic lymphoma in male and female mice in both studies and in all groups of the stop-exposure experiment (NTP, 1993, 1984). In the 103-week study, significant increases remained after adjustment for survival. • In both mouse studies, malignant lymphomas occurred as early as week 20-23 and were the primary cause of early death (NTP, 1993, 1984).		*Overall judgement for hematopoietic system tumors based on animal evidence*: • Robust	
Bladder Cancer				
Evidence for bladder cancer in studies of exposed humans[1]				
• Retrospective cohort studies of styrene-butadiene rubber workers (n>22,000 men and women) (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB, 1995). OQD=Medium (IISRP, 1986). OQD=Low. • Retrospective cohort study of butadiene monomer workers (n=2800 men) (Divine and Hartman, 2001). OQD=Medium.	• In the most recent analyses with longest follow-up of styrene-butadiene rubber workers, exposure to 1,3-butadiene was associated with increased risk of mortality from bladder cancer. The increase exhibited an exposure-response trend (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019).	• The association with bladder cancer in styrene-butadiene rubber workers may be confounded by smoking, as data on smoking were not available for the cohort (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019). • No association between 1,3-butadiene exposure and bladder cancer was observed in a smaller cohort of butadiene monomer workers (Divine and Hartman, 2001)	*Key findings:* An association between 1,3-butadiene exposure and exposure-related increase in bladder cancer mortality was observed in styrene-butadiene rubber workers, but lack of data on smoking precluded consideration of this potential confounder. *Overall judgement for bladder tumors based on human evidence*: • Moderate	
Evidence for bladder cancer from *in vivo* mammalian animal studies[2]				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• None	• No increased incidences of tumors originating in bladder tissues were observed in mice or rats (NTP, 1993, 1984; Hazleton Labs, 1981b). Bladder tumors of lymphohematopoietic origin are considered under lymphohematopoietic cancers. • The 103-week study in mice examined bladders only if there were gross abnormalities (NTP, 1993).	*Key findings:* No association between 1,3-butadiene exposure and bladder tumors in high- and medium-quality studies of mice and rats. *Overall judgement for bladder tumors based on animal evidence*: • Indeterminate/no effect	
Central nervous system cancer				
Evidence for central nervous system cancer in studies of exposed humans[1]				
• Retrospective cohort studies of styrene-butadiene rubber workers (n>22,000 men and women) (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005). OQD=Medium (IISRP, 1986). OQD=Low. • Retrospective cohort study of butadiene monomer workers (n=2800 men) (Divine and Hartman, 2001). OQD=Medium. • Ecological study of central nervous system tumors in children and modeled air concentration at residence at time of diagnosis (Danysh et al., 2015). OQD=Medium. *Other human studies*	• Increased incidence rate ratio for astrocytomas other than juvenile pilocytic astrocytoma (JPA) associated with modeled 1,3-butadiene concentrations in quartile 2 (Q2) and Q3, but not Q4 (Danysh et al., 2015). • In another human study, increased odds of primitive neuroectodermal tumors were associated with 1,3-butadiene in ambient air during pregnancy and first year of life (Von Ehrenstein et al., 2016).	• In the study by (Danysh et al., 2015), exposure misclassification is likely given the use of census tract-level estimates to represent individual exposure. In addition, exposure estimates were assigned based on address at time of diagnosis. • In the study by (Danysh et al., 2015), confounding is likely because exposure estimates were higher near major metropolitan areas but, urban/rural status was not evaluated as a potential confounder; and the modeled 1,3-butadiene concentration was highly	*Key findings:* An association between modeled 1,3-butadiene concentration and non-JPA astrocytomas in children was reported in an ecological study but not in the highest quartile of exposure. The study was limited by its design as well as lack of adjustment for important confounders and co-exposures. *Overall judgement for brain tumors based on human evidence*: • Indeterminate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Case-control study of central nervous system tumors in children, exposure based on ambient air monitoring data at station nearest to residence during pregnancy and first year of life (Von Ehrenstein et al., 2016).		correlated with modeled concentrations of other chemicals but confounding by co-exposures was not evaluated (Danysh et al., 2015). • No association was observed between 1,3-butadiene exposure and astrocytomas in the study by (Von Ehrenstein et al., 2016) • No association was observed between 1,3-butadiene exposure and central nervous system cancer and/or central nervous system cancer mortality in occupational populations (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; Divine and Hartman, 2001; IISRP, 1986). • Increased odds of astrocytoma or medullablastoma were not associated with 1,3-butadiene in ambient air during pregnancy and first year of life (Von Ehrenstein et al., 2016).		
Evidence for central nervous system cancer from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High.	• Significant dose-related trend for increased incidence of brain	• No statistically significant pair-wise comparisons with concurrent control group for	*Key findings:* Brain glial cell tumors were observed in exposed male rats	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. <u>Rat studies</u> • ≤8,000 ppm for 105-111 weeks (Hazleton Labs, 1981b). OQD=High.	glial cell tumors in male rats (Hazleton Labs, 1981b). • In the 60-week study, brain gliomas were identified in two male mice at 619 ppm and one male mouse at 1260 ppm, and an ependymoma of the brain was observed in one male mouse at 619 ppm (NTP, 1984). • In the 103-week study, malignant glioma was observed in one male mouse at 199 ppm (NTP, 1993). • In the stop-exposure studies at 619 ppm, malignant gliomas were found in two male mice after 13 weeks exposure and in one male mouse after 26 weeks, and malignant neuroblastomas were identified in two male mice after 13 weeks (NTP, 1993). • Gliomas and neuroblastomas are rare in B6C3F1 mice and were not seen in historical controls according to (NTP, 1993).	male rats. No historical control data were reported (Hazleton Labs, 1981b). • No brain glial cell tumors were observed in female rats (Hazleton Labs, 1981b). No gliomas, ependymomas, or neuroblastomas were observed in female mice (NTP, 1993), indicating a lack of consistency across sexes.	with dose-related trend and low incidences of gliomas, neuroblastomas, and ependymoma in exposed male B6C3F1 mice. These tumors are rare in B6C3F1 mice. *Overall judgement for brain tumors based on animal evidence*: • Slight	
Gastrointestinal tumors				
Evidence for gastrointestinal tumors in studies of exposed humans[1]				
• Retrospective cohort studies of styrene-butadiene rubber workers (n>22,000 men and women) (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB,	• In a retrospective cohort study of a small group of butadiene monomer workers, employment in the rubber reserve unit for at least 2 years was associated with increased mortality from stomach cancer.	• In larger retrospective cohort studies of styrene-butadiene rubber workers (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB, 1995; IISRP,	*Key findings:* The weight of evidence from available studies does not support an association with stomach cancer.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
1995). OQD=Medium. (IISRP, 1986). OQD=Low. • Retrospective cohort study of butadiene monomer workers (n=2,800 men) (Divine and Hartman, 2001). OQD=Medium. • Retrospective cohort study of butadiene monomer workers (n=364 men) (Ward et al., 1996a; Ward et al., 1995).	Exposure levels were not quantified. (Ward et al., 1996a; Ward et al., 1995).	1986) and butadiene monomer workers (Divine and Hartman, 2001), exposure to 1,3-butadiene was not associated with mortality from cancers of the gastrointestinal tract.	*Overall judgement for stomach cancer based on human evidence*: • Indeterminate/no effect	
Evidence for gastrointestinal tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trends and/or pairwise comparisons with concurrent controls for forestomach papilloma or carcinoma incidences in male and female mice in two studies (NTP, 1993, 1984). In the 103-week study, significant increases remained after adjustment for survival. • Significantly increased incidences of forestomach papilloma or carcinoma were also seen in male mice in stop-exposure studies (NTP, 1993).	• No increase in forestomach tumor incidence in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of forestomach papilloma or carcinoma in male and female mice. No increase in forestomach tumor incidence was observed in exposed rats. *Overall judgement for forestomach tumors based on animal evidence*: • Moderate	
Germ cell cancers				
Evidence for germ cell cancers in studies of exposed humans[1]				
• Case-control study of germ cell cancers in children; exposure based on ambient air monitoring data at station nearest to residence during pregnancy (Hall et al., 2019). OQD=Medium.	• Increased odds of all germ cell tumors and yolk sac tumors associated with 1,3-butadiene concentration in ambient air during second trimester (Hall et al., 2019).	• No associations identified for germ cell tumors or yolk sac tumors with 1,3-butadiene concentration in ambient air during first or third trimester (Hall et al., 2019).	*Key findings:* In a single study, an association was observed between 1,3-butadiene concentration in ambient air during pregnancy and all germ cell tumors and yolk sac tumors in children. No	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
		• One known risk factor for germ cell tumors, cryptorchidism, was not accounted for in the study because data were not available for the study population.	other studies of this endpoint were located. *Overall judgement for germ cell tumors based on human evidence*: • Indeterminate	
Evidence for germ cell cancers from *in vivo* mammalian animal studies[2]				
No data. *Overall judgement for germ cell tumors based on animal evidence*: Indeterminate				
Lung cancer				
Evidence for lung cancer in studies of exposed humans[1]				
• Retrospective cohort studies of styrene-butadiene rubber workers (n>22,000 men and women) (Sathiakumar et al., 2019; Sathiakumar et al., 2009; Sathiakumar and Delzell, 2009; Sathiakumar et al., 2005; UAB, 1995). OQD=Medium. (IISRP, 1986). OQD=Low. • Retrospective cohort study of butadiene monomer workers (n=2,800 men) (Divine and Hartman, 2001). OQD=Medium. • Ecological study of lung cancer incidence using TRI data for exposure and SEER data for outcome (Luo et al., 2011). OQD=Low. • Nested case-control study of smokers in cohort of men in Shanghai, exposure based on urinary monohydroxybutyl mercapturic acid (MHBMA) (Yuan et al., 2012). OQD=Medium.	• In a large cohort of styrene-butadiene rubber workers, exposure to 1,3-butadiene was associated with mortality from lung cancer among female workers (Sathiakumar et al., 2019; Sathiakumar et al., 2009; Sathiakumar and Delzell, 2009).	• In female styrene-butadiene rubber workers, there was no exposure-response trend for lung cancer, and the analyses were not adjusted for smoking. The study authors indicated that indirect adjustment for smoking partially explained the increase in mortality among female workers (Sathiakumar et al., 2019). • No association was observed between 1,3-butadiene exposure and lung cancer in male styrene-butadiene rubber workers (Sathiakumar et al., 2019; Sathiakumar et al., 2009; Sathiakumar et al., 2005; Divine and Hartman, 2001; UAB, 1995; IISRP, 1986). • General population studies provided limited information on lung cancer	*Key findings:* An association between 1,3-butadiene exposure and lung cancer mortality was observed in female styrene-butadiene rubber workers, but not male styrene-butadiene rubber workers. The observed association lacked a dose-response relationship and may have been confounded by smoking. *Overall judgement for lung tumors based on human evidence*: • Indeterminate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
		due to ecological study design (Luo et al., 2011) or analysis limited to male smokers (Yuan et al., 2012).		
Evidence for lung cancer from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). • OQD=High.	• Significant dose-related trends and pairwise comparisons with concurrent controls for alveolar/bronchiolar adenoma, adenocarcinoma, and/or carcinoma in male and female mice in two studies (NTP, 1993, 1984). In the 103-week study, incidences exceeded the upper limit for historical control ranges, and significant increases remained after adjustment for survival. Significantly increased incidences were also seen in male mice in all stop-exposure groups (NTP, 1993).	• In the 103-week study, the incidence of alveolar/ bronchiolar adenoma, adenocarcinoma, or carcinoma incidence in concurrent control males exceeded the upper limit for historical controls (NTP, 1993). • No increase in lung tumor incidence in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of lung tumors in male and female mice. No increase in lung tumor incidence was observed in exposed rats. *Overall judgement for lung tumors based on animal evidence*: • Moderate	
Ocular tumors				
Evidence for ocular tumors in studies of exposed humans[1]				
• Retrospective cohort study of male styrene-butadiene rubber workers (IISRP, 1986). OQD=Low. • Case-control study of retinoblastoma in children, exposure based on ambient air monitoring data at station nearest to residence (Heck et al., 2015). OQD=Medium.	• Increased odds of retinoblastoma associated with 1,3-butadiene concentration in ambient air during pregnancy (Heck et al., 2015).	• No association between 1,3-butadiene exposure and mortality from ocular tumors in large cohort of male styrene-butadiene rubber workers (IISRP, 1986).	*Key findings:* In a single study, an association was observed between 1,3-butadiene concentration in ambient air during pregnancy and retinoblastoma in children. No other studies of this endpoint were located. *Overall judgement for ocular tumors based on human evidence*: • Indeterminate	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Evidence for ocular tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• None	• No increased incidences of ocular tumors were observed in mice or rats (NTP, 1993, 1984; Hazleton Labs, 1981b). Harderian gland tumors are considered separately. • Available studies in mice examined the eyes for histopathology only if there were gross abnormalities.	*Key findings:* No association between 1,3-butadiene exposure and ocular tumors in high- and medium-quality studies of mice and rats. *Overall judgement for bladder tumors based on animal evidence*: • Indeterminate/no effect	
Liver tumors				
Evidence for liver tumors in studies of exposed humans[1]				
• Retrospective cohort studies, occupational populations (men and women) (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007; Sathiakumar et al., 2005; UAB, 1995). OQD=Medium. (IISRP, 1986). • OQD=Low.	• None	• No association between 1,3-butadiene exposure and liver cancer in occupational studies of men and women (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007; Sathiakumar et al., 2005; UAB, 1995; IISRP, 1986).	*Key findings:* No association between 1,3-butadiene exposure and liver tumors in several medium-quality and one low-quality studies. *Overall judgement for liver tumors based on human evidence*: • Indeterminate/no effect	
Evidence for liver tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993).	• Significant dose-related trend (NTP, 1984) and pairwise comparisons with concurrent controls for hepatocellular adenoma and/or carcinoma in female mice in two studies (NTP, 1993, 1984).	• No increase in liver tumor incidence in rats indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of hepatocellular adenomas or carcinomas in male and female mice. No increase in liver	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
OQD=Medium. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Survival-adjusted incidences were significantly increased in both male and female mice in the 103-week study (NTP, 1993).		tumor incidence was observed in exposed rats. *Overall judgement for liver tumors based on animal evidence*: • Moderate	
Mammary gland tumors				
Evidence for mammary gland tumors in studies of exposed humans[1]				
• Retrospective cohort follow-up studies, occupational populations (n=4,863 women in each study), quantitative exposure assessment (Sathiakumar and Delzell, 2009; UAB, 2007). OQD=Medium. • Retrospective cohort study, occupational population (n= 17,924 men, 4,861 women), qualitative and quantitative exposure assessment (Sathiakumar et al., 2019). OQD=Medium. • General population, cohort study (n=49,718 women), quantitative exposure assessment (Niehoff et al., 2019). OQD=Medium.	• None	• No association between 1,3-butadiene exposure and breast cancer mortality in occupational studies (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007). • No elevated risk for overall breast cancer or estrogen receptor positive (ER+) invasive breast cancer (Niehoff et al., 2019).	*Key findings:* No association between 1,3-butadiene exposure and breast cancer in several medium-quality studies. *Overall judgement for mammary gland tumors based on human evidence*: • Indeterminate/No effect	
Evidence for mammary gland tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low.	• Significant dose-related trends and/or pairwise comparisons with concurrent control for mammary gland acinar cell carcinoma in female mice (NTP, 1984) and for mammary gland adenoacanthoma, carcinoma, or malignant mixed	• Historical control incidences were not reported for mice or rats.	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of mammary gland tumors in female mice and female rats.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	tumor in female mice (NTP, 1993). In the 103-week study, significant increases in adenoacanthoma or carcinoma incidence remained after adjustment for survival. • Significant dose-related trends and pairwise comparisons with concurrent control for increased incidences of benign and total (benign + malignant) mammary gland tumors in female rats (Hazleton Labs, 1981b).		*Overall judgement for mammary gland tumors based on animal evidence*: • Moderate	
Ovarian tumors				
Evidence for ovarian tumors in studies of exposed humans[1]				
• Retrospective cohort studies, (n = 4,863 women/study), quantitative exposure assessment (Sathiakumar and Delzell, 2009; UAB, 2007). OQD=Medium. • Retrospective cohort study, occupational population (n = 4,861 women), qualitative and quantitative exposure assessment (Sathiakumar et al., 2019). OQD=Medium.	• None	• No association between 1,3-butadiene exposure and ovarian tumors in workers (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007).	*Key findings:* No association between 1,3-butadiene exposure and ovarian tumors in three medium-quality occupational studies. *Overall judgement for ovarian tumors based on human evidence*: • Indeterminate/No effect	
Evidence for ovarian tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low.	• Significant dose-related trends and pairwise comparisons with concurrent control for ovarian granulosa cell tumors in female mice in two studies (NTP, 1993, 1984). In the 103-week study, significant increases remained after adjustment for	• No increase in ovarian tumor incidence in female rats, indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of ovarian granulosa cell tumors in mice. No increase in ovarian tumor incidence was observed in exposed rats.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	survival, and survival-adjusted rates exhibited monotonicity with exposure (NTP, 1993).		*Overall judgement for ovarian tumors based on animal evidence*: • Slight	
Pancreatic tumors				
Evidence for pancreatic tumors in studies of exposed humans[1]				
• Retrospective cohort studies, occupational populations (men and women) (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007; Sathiakumar et al., 2005; Divine and Hartman, 2001; UAB, 1995). OQD=Medium. (IISRP, 1986). OQD=Low.	• None	• No association between 1,3-butadiene exposure and pancreatic cancer in male or female workers (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007; Sathiakumar et al., 2005; Divine and Hartman, 2001; UAB, 1995; IISRP, 1986).	*Key findings:* No association between 1,3-butadiene exposure and pancreatic cancer in several medium- and low-quality studies. *Overall judgement for pancreatic tumors based on human evidence*: • Indeterminate/No effect	
Evidence for pancreatic tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend and pairwise comparison with concurrent control for increased incidence of pancreatic exocrine adenomas in male rats (Hazleton Labs, 1981b).	• No increase in pancreatic tumor incidence in mice (NTP, 1993, 1984), indicating a lack of consistency across species. • No increase in pancreatic tumor incidence in female rats indicating a lack of consistency across sexes of rat (Hazleton Labs, 1981b). • Historical control incidences were not reported (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced increased incidences of pancreatic exocrine adenomas in male rats; no increase in pancreatic tumor incidence was observed in exposed female rats or in exposed male or female mice. *Overall judgement for pancreatic tumors based on animal evidence*: • Slight	
Subcutaneous skin tumors				
Evidence for subcutaneous skin tumors in studies of exposed humans[1]				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Retrospective cohort studies, occupational populations (men) (Divine and Hartman, 2001; UAB, 1995). OQD=Medium. (IISRP, 1986). OQD=Low.	• None	• No association between 1,3-butadiene exposure and skin cancer in male workers (Divine and Hartman, 2001; UAB, 1995; IISRP, 1986).	*Key findings:* No association between 1,3-butadiene exposure and skin tumors in two medium-quality and one low-quality studies. *Overall judgement for skin tumors based on human evidence*: • Indeterminate/no effect	
Evidence for subcutaneous skin tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend and pairwise comparison with concurrent control for increased incidences of subcutaneous skin hemangiosarcoma and neurofibrosarcoma or sarcoma in female mice. Incidences in several groups exceeded the upper limits of the respective historical control ranges (NTP, 1993).	• No increase in subcutaneous skin tumor incidence in male mice in two studies (NTP, 1993, 1984). • No increase in subcutaneous skin tumor incidence in rats indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Increased incidences of subcutaneous skin tumors were observed in female mice exposed to 1,3-butadiene. No increase in subcutaneous skin tumor incidence was observed in exposed male mice or in male or female rats. *Overall judgement for subcutaneous skin tumors based on animal evidence*: • Slight	
Thyroid tumors				
Evidence for thyroid tumors in studies of exposed humans[1]				
• Retrospective cohort study, occupational population (n > 12,000 men), qualitative exposure assessment (IISRP, 1986). OQD=Low.	• None	• No association between 1,3-butadiene exposure and thyroid tumors in male workers (IISRP, 1986).	*Key findings:* None *Overall judgement for thyroid tumors based on human evidence*: • Indeterminate/no effect	
Evidence for thyroid tumors from *in vivo* mammalian animal studies[2]				

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend and pairwise comparison with concurrent control for increased incidence of thyroid follicular cell adenomas in female rats (Hazleton Labs, 1981b).	• No increase in thyroid tumor incidence in male rats (Hazleton Labs, 1981b), indicating a lack of consistency across sexes. • No increase in thyroid tumor incidence in mice (NTP, 1993, 1984), indicating a lack of consistency across species.	*Key findings:* Increased incidences of thyroid tumors were observed in female rats. No increase in thyroid tumor incidence was observed in exposed male rats or mice of either sex. *Overall judgement for thyroid tumors based on animal evidence*: • Slight	
Uterine tumors				
Evidence for uterine tumors in studies of exposed humans[1]				
Retrospective cohort studies, occupational populations (women), qualitative and quantitative exposure assessment (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007). OQD=Medium.	• None	• No association between 1,3-butadiene exposure and uterine tumors in occupational studies in women (Sathiakumar et al., 2019; Sathiakumar and Delzell, 2009; UAB, 2007).	*Key findings:* No association between 1,3-butadiene exposure and uterine tumors in three medium-quality studies. *Overall judgement for uterine tumors based on human evidence*: • Indeterminate/No effect	
Evidence for uterine tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend for increased incidence of uterine sarcomas in female rats (Hazleton Labs, 1981b).	• No significant pairwise comparisons with concurrent control for uterine sarcomas in female rats. No historical control data reported for uterine tumors in female rats (Hazleton Labs, 1981b). • No increase in uterine tumor incidence in female mice (NTP, 1993, 1984),	*Key findings:* A dose-related trend for increased uterine tumors without significant pairwise comparisons was seen in rats. No increase in uterine tumor incidence was observed in exposed mice.	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
		indicating a lack of consistency across species.	*Overall judgement for uterine tumors based on animal evidence*: • Indeterminate	
Heart hemangiosarcomas				
Evidence for heart hemangiosarcomas in studies of exposed humans[1]				
No data. *Overall judgement for heart hemangiosarcomas based on human evidence*: Indeterminate				
Evidence for heart hemangiosarcomas from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks[3] (NTP, 1993). OQD=High. • Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. • ≤10,000 ppm for single 2-hour exposure and followed for 2 years (Bucher et al., 1993). OQD=Low. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trends and pairwise comparisons with concurrent controls for heart hemangiosarcoma in male and female mice in two studies (NTP, 1993, 1984). Significant increases remained after adjustment for survival. Significantly increased incidences were seen in male mice in all stop-exposure groups (NTP, 1993). • Heart hemangiosarcomas are rare in B6C3F1 and were not seen in historical controls according to (NTP, 1993). • In the 103-week study, heart hemangiosarcomas were the second-most common cause of early death (NTP, 1993).	• No increase in heart tumor incidence in rats, indicating a lack of consistency across species (Hazleton Labs, 1981b).	*Key findings:* Exposure to 1,3-butadiene induced dose-related increased incidences of heart hemangiosarcomas in male and female mice and these cancers were the second-most common cause of early deaths in exposed mice in both studies. No increase in heart tumor incidence was observed in exposed rats. *Overall judgement for heart tumors based on animal evidence*: • Robust	
Harderian gland tumors				
Evidence for harderian gland tumors in studies of exposed humans[1]				
Harderian gland tumors are not relevant to humans.				
Evidence for harderian gland tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High.	• Significant dose-related trend and pairwise comparisons with concurrent controls for	• No increase in Harderian gland tumor incidence in rats, indicating a lack of	*Key findings:* Exposure to 1,3-butadiene induced increased incidences	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	Harderian gland adenoma or carcinoma in male mice. Significant increases remained after adjustment for survival. Significantly increased incidences were seen in male mice in all stop-exposure groups (NTP, 1993). • Survival-adjusted incidences of Harderian gland adenoma or carcinoma were significantly increased (pairwise relative to concurrent control) in female mice (NTP, 1993).	consistency across species (Hazleton Labs, 1981b). • The concurrent female mouse control incidence exceeded the upper limit of historical control incidence (NTP, 1993).	of Harderian gland adenoma or carcinoma in male and female mice. No increase in Harderian gland tumor incidence was observed in exposed rats. *Overall judgement for Harderian Gland tumors based on animal evidence*: • Moderate	
Preputial gland tumors				
Evidence for preputial gland tumors in studies of exposed humans[1]				
No data and questionable relevance to humans. *Overall judgement for testicular tumors based on human evidence*: Indeterminate				
Evidence for preputial gland tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. Stop-exposure studies (males only) (NTP, 1993). OQD=Medium.	• Significant pairwise comparisons with concurrent controls for preputial gland adenoma or carcinoma in mice in the stop-exposure experiments with highest cumulative exposures (NTP, 1993). • In the 103-week experiment, the survival-adjusted incidence for preputial gland carcinoma was significantly increased compared to concurrent controls. • Preputial gland carcinomas are rare in B6C3F1 mice and were not observed in historical	• Data on preputial gland tumors are from a single study in one species (NTP, 1993).	*Key findings:* Increased incidences of preputial gland adenomas and/or carcinomas were observed in mice exposed to higher cumulative levels of 1,3-butadiene in a single study. There are no data on this endpoint for rats. *Overall judgement for preputial gland tumors based on animal evidence*: • Slight	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
	controls according to (NTP, 1993).			
Testicular tumors				
Evidence for testicular tumors in studies of exposed humans[1]				
• No data. *Overall judgement for testicular tumors based on human evidence*: Indeterminate				
Evidence for testicular tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend and pairwise comparison with concurrent control for testicular Leydig cell tumors in male rats (Hazleton Labs, 1981b).	• No increase in testicular tumor incidence in male mice (NTP, 1993, 1984), indicating a lack of consistency across species.	*Key findings:* Increased incidences of testicular Leydig cell tumors were observed in rats. No increase in testicular tumor incidence was observed in exposed mice. *Overall judgement for testicular tumors based on animal evidence*: • Slight	
Zymbal gland tumors				
Evidence for zymbal gland tumors in studies of exposed humans[1]				
Zymbal gland tumors are not relevant to humans.				
Evidence for zymbal gland tumors from *in vivo* mammalian animal studies[2]				
Mouse studies • ≤1,250 ppm for 60–61 weeks (NTP, 1984). OQD=High. • ≤619 ppm for 103 weeks (NTP, 1993). OQD=High. Stop-exposure studies (males only) (NTP, 1993). OQD=Medium. Rat studies • ≤8,000 ppm for 105–111 weeks (Hazleton Labs, 1981b). OQD=High.	• Significant dose-related trend for increased incidence of Zymbal gland carcinomas in female rats (Hazleton Labs, 1981b). • Low incidences (1–2 mice) of Zymbal gland adenomas and carcinomas were seen in male and/or female mice in all mouse studies including the stop-exposure studies (NTP, 1993, 1984).	• No significant pairwise comparisons for Zymbal gland carcinomas in female rats. No increase in tumor incidence in male rats. Historical control incidences were not reported (Hazleton Labs, 1981b). • Tumor incidences in mice were not significantly increased over concurrent controls at any exposure	*Key findings:* Zymbal gland tumors were observed in female rats with dose-related trend and at low incidences in male and female B6C3F1 mice. These tumors are rare in B6C3F1 mice. *Overall judgement for Zymbal gland tumors based on animal evidence*: • Slight	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
	• Zymbal gland tumors are rare in B6C3F1 mice and were not seen in historical controls according to (NTP, 1993)	level and there were no significant dose-related trends (NTP, 1993, 1984).		
Evidence in mechanistic studies and supplemental information				
Mechanistic evidence in lymphohematopoietic cells and tissues				
Mutagenic Mode-of-Action (MOA) Key Events (KEs) KE1 Bioactivation to DNA reactive metabolites • Studies of 1,3-butadiene metabolism in multiple species (ATSDR, 2012; Albertini et al., 2010; Kirman et al., 2010a; U.S. EPA, 2002a; Himmelstein et al., 1997). • Genotoxicity studies in lymphohematopoietic tissues using metabolites (ATSDR, 2012; Swenberg et al., 2011; U.S. EPA, 2002a; Cochrane and Skopek, 1994). KE2 Formation of DNA adducts • Studies demonstrating DNA adduct formation *in vitro* and in lymphohematopoietic tissues following *in vivo* exposure (ATSDR, 2012; U.S. EPA, 2002a). • Studies of DNA adducts in lymphohematopoietic cells from occupationally exposed workers (ATSDR, 2012; U.S. EPA, 2002a). KE3 Chromosomal aberrations and/or mutations	• Interspecies variation in cancer susceptibility is consistent with documented differences in 1,3-butadiene metabolism and resulting genotoxicity (Albertini et al., 2010; Kirman et al., 2010a; Himmelstein et al., 1997). • Electrophilic metabolites form DNA adducts, induce DNA strand breaks, stimulate unscheduled DNA synthesis and DNA excision repair, and trigger sister-chromatid exchange (Albertini et al., 2010) • Several studies show a positive correlation between occupational exposure to 1,3-butadiene and levels of DNA adducts in peripheral blood lymphocytes (ATSDR, 2012; U.S. EPA, 2002a). • Mutagenic activity arises from epoxide metabolites (ATSDR, 2012; U.S. EPA, 2002a) and potentially from novel bifunctional metabolites such as chlorinated and ketone/aldehyde derivates (Nakamura et al., 2021; Wu et	• While the weight of evidence sufficiently supports a mutagenic MOA for 1,3-butadiene carcinogenicity, the possibility of alternative or additional MOAs cannot be excluded, although these have not been definitively identified or supported by the existing evidence. • An *in vitro* study showed that 1,3-butadiene decreased the ratio of mature to immature bone marrow stem cells (Leiderman et al., 1986)	*Key findings*: The weight of evidence strongly supports a mutagenic MOA for 1,3-butadiene in the development of lymphohematopoietic malignancies in both rodents and humans. The primary driver of 1,3-butadiene's mutagenic MOA is the formation of electrophilic metabolites which readily react with DNA, causing adduct formation and other types of DNA damage. If not repaired, this persistent damage can lead to mutations, particularly in oncogenes and tumor suppressor genes. The accumulation of mutations in critical genes results in uncontrolled cell proliferation and cancer development. The variability in 1,3-butadiene's mutagenic and carcinogenic potential across species and cancer types is attributed to differences in 1,3-butadiene metabolism, resulting in varying levels and types of	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
• *In vitro* mutation assays conducted on human and rodent lymphohematopoietic cells (ATSDR, 2012; U.S. EPA, 2002a). • Studies of mutagenicity in lymphohematopoietic cells from rodents exposed *in vivo* and in tissues from exposed workers (ATSDR, 2012; Meng et al., 2007; Meng et al., 2004; U.S. EPA, 2002a; Ammenheuser et al., 2001; Ward et al., 2001; Ma et al., 2000; Meng et al., 2000; Meng et al., 1999; Ward et al., 1996b; Cochrane and Skopek, 1994). *Alternative Mode-of-Action Studies* • Study of bone marrow stem cells exposed *in vitro* (Leiderman et al., 1986)	al., 2019; Wang et al., 2018; Elfarra and Zhang, 2012). • 1,3-Butadiene induces specific mutations in key genes involved in cancer development, including oncogenes (*e.g.*, K-ras) and tumor suppressor genes (*e.g.*, Trp53), as well as those in the Wnt signaling pathway (ATSDR, 2012; U.S. EPA, 2002a). • Mice and rats had increased *hprt* locus mutations in splenic T cells (Meng et al., 2007; Meng et al., 2004; Meng et al., 2000; Meng et al., 1999; Cochrane and Skopek, 1994). • In a transgenic mouse, increased *lacI* mutant frequency was observed in both spleen and bone marrow (U.S. EPA, 2002b). • In workers, a potential association was observed between 1,3-butadiene exposure and increased frequencies of *hprt* variants in lymphocytes (Ammenheuser et al., 2001; Ward et al., 2001; Ma et al., 2000; Ward et al., 1996b).		DNA damaging electrophilic metabolites. *Overall judgement for lymphohematopoietic carcinogenicity based on mechanistic evidence*: • Robust	
Mechanistic evidence in other cells and tissues				
• Mutation assays in bacteria and fruit flies (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002a) • Genotoxicity studies in rat skin and	• 1,3-butadiene induced reverse mutations in bacteria in the presence of S9 (ATSDR, 2012; IARC, 2008b; U.S. EPA,	• No increase in *lacZ*$^-$ mutation frequency was observed in the livers of transgenic mice exposed to	*Key findings*: There is generalized evidence of genotoxicity and mutagenicity in bacteria and	

Database Summary	Factors that Increase Strength	Factors that Decrease Strength	Summary of Key Findings and Within-Stream Strength of the Evidence Judgement	Inferences across Evidence Streams and Overall Evidence Integration Judgement
embryonic fibroblasts, mouse skin fibroblasts, mouse lung, and rat and mouse germ cells using metabolites (IARC, 2008b; U.S. EPA, 2002a) • Studies of DNA adduct formation, DNA damage, and/or micronuclei in rat and mouse liver and lung following *in vivo* exposure (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002a) • *In vivo* mutation assays in liver and lung of transgenic mice (ATSDR, 2012; U.S. EPA, 2002a).	2002a) • Electrophilic metabolites induced gene mutations, micronuclei, and SCEs in rat and mouse skin fibroblasts, rat embryo fibroblasts, mouse lung, and rat and mouse germ cells (IARC, 2008b; U.S. EPA, 2002a) • *In vivo* studies of rats and mice exposed to 1,3-butadiene demonstrated DNA adduct formation, DNA damage, and/or micronuclei in rat and mouse liver, lung, and germ cells (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002a) • Frequencies of *lacZ*$^-$ and *lacI* mutations were increased in the lungs of transgenic mice exposed to 1,3-butadiene (ATSDR, 2012; IARC, 2008b; U.S. EPA, 2002b).	1,3-butadiene (ATSDR, 2012).	various rodent tissues, however the data for each particular tissue type is limited. *Overall judgement for carcinogenicity in other tissues based on mechanistic evidence*: • Slight	

ALL: acute lymphoblastic/lymphocytic leukemia; AML: acute myeloid leukemia; DNA: deoxyribonucleic acid; NHL: non-Hodgkin's lymphoma; SCE: sister chromatid exchange; SEER: Surveillance, Epidemiology, and End Results database of the National Cancer Institute; TRI: Toxics Release Inventory

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Appendix B BENCHMARK DOSE MODELING RESULTS FOR CRITICAL ENDPOINTS

BMD modeling was conducted with the EPA's BMD software (BMDS 3.3.2). For continuous data, the Exponential, Hill, Linear, Polynomial, and Power Continuous models available within the software were fit employing a BMR of 1 SD for maternal body weight gain and 1 SD, 5 percent RD and 10 percent RD for fetal body weight and mean percent of supernumerary ribs per litter. An adequate fit was judged based on the $\chi 2$ goodness-of-fit p value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (*i.e.*, Test 2; p-value > 0.05 [note: this is a change from previous versions of BMDS, which required variance p-value > 0.10 for adequate fit]), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (p-value < 0.05), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the data (*i.e.*, Test 3; p-value < 0.05), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL has been selected if the BMDLs estimated from different models varied >3-fold; otherwise, the BMDL from the model with the lowest AIC has been selected.

For dichotomous data, the Gamma, Logistic, Log Logistic, Log-Probit, Multistage, Probit, Weibull, and Quantal Linear Dichotomous models available within the software were fit using a benchmark response (BMR) of 5 percent and 10 percent extra risk. Adequacy of model fit has been judged based on the $\chi 2$ goodness-of-fit p-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest BMDL has been selected if the BMDLs estimated from different models varied >3-fold; otherwise, the BMDL from the model with the lowest AIC has been selected.

Dichotomous nested modeling of data was conducted for the number of fetuses with supernumerary ribs and male fetuses with body weight below the 5th or 10th percentiles of control male fetal weight. The Nested Logistic model available within the software was fit using a benchmark response (BMR) of 5 percent and 10 percent extra risk. Litter size was used for the litter-specific covariate (lsc). The models were applied with and without the litter-specific covariate to determine whether or not the litter-specific covariate contributes to a better explanation of the observation. Models are also run with and without the intralitter correlation (ilc) to estimate the degree to which observations within the same litter are correlated. The forms of the models include lsc+ilc+, lsc+ilc-, lsc-ilc+, and lsc-ilc-. The "overall mean" (default) was selected for the litter-specific covariate option. Adequacy of model fit has been judged based on the $\chi 2$ goodness-of-fit p-value ($p > 0.1$). The overall model should be considered questionable if the scaled residuals are greater than 2 or less than −2 for several individual dose and litter-specific covariate combinations, particularly near the control or dose group nearest the BMD. Among the forms of the models providing adequate fit, the model form with the lowest AIC has been selected.

Results for maternal and related developmental toxicity endpoints are presented in Table_Apx B-1. Results for male reproductive system and resulting developmental toxicity endpoints are presented in Table_Apx B-2. Results for hematological endpoints are presented in Table_Apx B-3. Full BMD modeling results including all approaches, endpoints, BMRs, model fits, and statistics are included in *Draft Benchmark Dose Modeling Results for 1,3-Butadiene* (U.S. EPA, 2024a).

Table_Apx B-1. BMD Modeling Results for Maternal and Related Developmental Toxicity Endpoints

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Mouse data (Battelle PNL, 1987b)												
Maternal absolute body weight gain (GD 11–16) (g)	Exponential 5 (constant variance)	58.2	10.4	NA	NA	NA		NA				The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, only the Exponential 5 model provided an adequate fit to the means (test 4 p-value > 0.1); therefore, this model was selected.
Maternal extragestational weight gain (g) (gravid uterus adjusted) GD 0–18	Exponential 3 (constant variance)	337	193	NA	NA	NA		NA				The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, only the Exponential 3 model provided adequate fit to the means (test 4 p-value > 0.1); therefore, this model was selected.
Mean fetal body weight in male fetuses/litter (g)	ND	ND	ND	ND	ND	ND	ND	NA		NA		Both the constant and nonconstant variance models provide adequate fit to the variance data; however with either variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Mean fetal body weight in male fetuses/litter (g) – **highest concentration dropped**	Exponential 3 (constant variance)	19.6	15.3	13.1	10.7	26.8	22.1	NA		NA		The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, the Exponential 3, Polynomial 2-degree, Power, and Linear models provided adequate fit to the means (test 4 p-value > 0.1). The goodness of fit test for the means (test 4) could not be calculated for the Exponential 5 and Hill models because the models were saturated (degree of freedom = 0). BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Mean fetal body weight - male fetuses (g)	ND	ND	ND	ND	ND	ND	ND	NA		NA		Neither the constant nor nonconstant variance provided an adequate fit to the variance data. This data set is not suitable for BMD modeling; no model selected.
Mean fetal body weight - male fetuses (g) - **highest concentration dropped**	ND	ND	ND	ND	ND	ND	ND	NA		NA		Neither the constant nor nonconstant variance provided an adequate fit to the variance data. This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Male fetuses with body weight below 5th percentile of control male fetal weight - **Nested model**	Nested Logistic (lsc-ilc+); overall mean	NA		NA		NA		5.49	2.52	10.4	5.32	The model forms applying the intralitter correlation (ilc +) provided adequate fit to the data (chi-square p-value > 0.1) both with and without the litter-specific covariate (lsc) applied. Model forms without the intralitter correlation (ilc-) did not provide adequate fits. Between the Nested Logistic (lsc+ilc+) and Nested Logistic (lsc-ilc+), the Nested Logistic (lsc-ilc+) had the lower AIC; therefore this model form is selected.
Male fetuses with body weight below 10th percentile of control male fetal weight – **Nested model**	Nested Logistic (lsc-ilc+); overall mean	NA		NA		NA		3.41	1.20	6.09	2.53	The model forms applying the intralitter correlation (ilc +) provided adequate fit to the data (chi-square p-value > 0.1) both with and without the litter-specific covariate (lsc) applied. Model forms without the intralitter correlation (ilc-) did not provide adequate fits. Between the Nested Logistic (lsc+ilc+) and Nested Logistic (lsc-ilc+), the Nested Logistic (lsc-ilc+) had the lower AIC; therefore this model form is selected.
Mean fetal body weight – males and females combined (g)	ND	ND	ND	ND	ND	ND	ND	NA		NA		Neither the constant nor nonconstant variance provided an adequate fit to the variance data. This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Mean fetal body weight - males and females combined (g) - **highest concentration dropped**	ND	ND	ND	ND	ND	ND	ND	NA		NA		Neither the constant nor nonconstant variance provided an adequate fit to the variance data. This data set is not suitable for BMD modeling; no model selected.
Number of litters with supernumerary ribs	Multistage 3-degree	NA		NA		NA		14.2	1.38	18.0	2.84	All models provided adequate fit to the data (chi-square p-value > 0.1). The Weibull model was considered unusable because the BMDL computation failed. BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Number of fetuses with supernumerary ribs	ND	NA		NA		NA		ND	ND	ND	ND	None of the models provided an adequate fit to the data (chi-square p-value > 0.1). This data set is not suitable for BMD modeling; no model selected.
Number of fetuses with supernumerary ribs – **highest concentration dropped**	Gamma	NA		NA		NA		34.7	10.7	38.2	16.7	The Gamma and Multistage 2-degree models provided adequate fit to the data (chi-square p-value > 0.1). The goodness of fit test (x^2 p-value) could not be calculated for the Dichotomous Hill, Log-logistic, Weibull, and Log-probit models because the models were saturated (degree of freedom = 0). BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Number of fetuses with supernumerary ribs – **nested model**	Nested Logistic (lsc-ilc+); overall mean	NA		NA		NA		6.31	2.9	11.9	6.13	The model forms applying the intralitter correlation (ilc +) provided adequate fit to the data (chi-square p-value > 0.1) both with and without the litter-specific covariate (lsc) applied. Model forms without the intralitter correlation (ilc-) did not provide adequate fits. Between the Nested Logistic (lsc+ilc+) and Nested Logistic (lsc-ilc+), the Nested Logistic (lsc-ilc+) had the lower AIC; therefore this model form is selected.
Mean % of supernumerary ribs per litter	ND	ND	ND	ND	ND	ND	ND	NA		NA		Both the constant and nonconstant variance models provide adequate fit to the variance data; however with either variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Mean % of supernumerary ribs per litter – **highest concentration dropped**	Polynomial 2-degree (nonconstant variance)	34.5	22.2	ND	ND	9.43	1.37	NA		NA		The constant variance model did not provide adequate fit to the variance data, but the nonconstant variance model did. With the nonconstant variance model applied, only the Polynomial 2-degree model provided adequate fit to the means (test 4 p-value > 0.1). The goodness of fit test for the means (test 4) could not be calculated for the Exponential 3 and 5, Hill, and Power models because the models were saturated (degree of freedom = 0). The polynomial 2-degree model was selected for BMRs of 1 SD and 10% RD. When applying a BMR of 5% RD, the Polynomial 2-degree model was considered questionable because the BMDL value was 10 times lower than the lowest non-zero dose; no model was selected for this BMR.
Rat data (Hazleton Labs, 1981a)												
Absolute body weight gain in maternal SD rats for GD 6–15	Hill (constant variance)	101.3	48.9	NA		NA		NA		NA		The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, only the Exponential 5 and Hill models provided adequate fit to the means (test 4 p-value > 0.1). The BMDLs for the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Mean uterine-adjusted body weight in maternal SD rats for GD 20	Exponential 3 (constant variance)	2321	1528	NA		4701	1995	NA		NA		The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, the Exponential 3, Polynomial 3-degree, Power, and Linear models provided adequate fit to the means (test 4 p-value > 0.1); the BMD computation failed for the Exponential 5 and Hill models. BMDLs for the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Uterine-adjusted body weight gain in maternal SD rats for GD 0–20	ND	ND	ND	NA		NA		NA		NA		Both the constant and nonconstant variance models provide adequate fit to the variance data; however with either variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). The goodness of fit test for the means (Test 4) could not be calculated for the Exponential 5 and Hill models because the models were saturated (degree of freedom = 0). This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		5% RD		10% RD		5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Uterine-adjusted body weight gain in maternal SD rats for GD 0–20 – **Highest concentration dropped**	Linear (constant variance)	295	193	NA		NA		NA		NA		The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, only the Linear model provided adequate fit to the means (test 4 p-value > 0.1); therefore this model was selected. The goodness of fit test for the means (test 4) could not be calculated for all other models because the models were saturated (degree of freedom = 0).

[a] Modeled concentrations were duration adjusted for 6 hours/day
ND = not determined, no model selected; NA = BMR not applied

Table_Apx B-2. BMD Modeling Results for Male Reproductive System and Resulting Developmental Toxicity Endpoints

Endpoint (Studies)	Recommended Model	5% ER		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Incidence of all deaths – (Anderson et al., 1996)	Log-Logistic	54.2	41.9	114	88.5	All models provided adequate fit to the data (chi-square p-value > 0.1) except for the Dichotomous Hill and Log-Probit models; these models were saturated (degree of freedom = 0) and considered questionable. BMDLs of the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected.
Incidence of all deaths – combined (Brinkworth et al., 1998; Anderson et al., 1996)	Log-Probit	13	4.83	55.0	30.1	Only the Log-Probit and Dichotomous Hill models provided adequate fit to the data (chi-square p-value > 0.1). Between these two models, the BMDLs were sufficiently close (differed by <3-fold); therefore, the model with the lowest AIC was selected.

[a] Modeled concentrations were duration adjusted for 6 hours/day, 5 days/week

Table_Apx B-3. BMD Modeling Results for Hematological Endpoints

Endpoint	Recommended Model	1 SD		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Erythrocyte counts in male mice (10^6/µL)	ND	ND	ND	ND	ND	The constant variance model did not provide adequate fit to the variance data, but the nonconstant variance model did. With the nonconstant variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.
Erythrocyte counts in male mice (10^6/µL) – **highest concentration dropped**	BMR: 1 SD: Exponential 5 (constant variance); BMR: 10% RD: Exponential 3 (constant variance)	10.7	8.07	46.7	35.7	The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models provided adequate fit to the means (test 4 p-value > 0.1) when using the BMR of 1SD. The BMDLs for the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected (Exponential 5). When applying a BMR of 10% RD, the BMD computation failed for the Exponential 5 and Hill models, and they were unusable. Among the remaining models, the BMDLs were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected (Exponential 3); using a BMR of 10% RD resulted in BMD and BMDL values being higher than the maximum modeled concentration.
Hemoglobin concentration in male mice (g/dL)	ND	ND	ND	ND	ND	The constant variance model did not provide adequate fit to the variance data, but the nonconstant variance model did. With the nonconstant variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.
Hemoglobin concentration in male mice (g/dL) – **highest concentration dropped**	ND	ND	ND	ND	ND	The constant variance model did not provide adequate fit to the variance data, but the nonconstant variance model did. With the nonconstant variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.
Hemoglobin concentration in male mice (g/dL) – **two highest concentrations dropped**	Power (constant variance)	10.9	7.95	11.6	11.3	The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models, except for the Exponential 5 and Hill models, provided adequate fit to the means (test 4 p-value > 0.1); the goodness of fit test for the means (test 4) could not be calculated for the Exponential 5 and Hill models because the models were saturated (degree of freedom = 0). The BMDLs for the fit models were sufficiently close (differed by < 3-fold); therefore, the model with the lowest AIC was selected. Using a BMR of 10% RD resulted in BMD and BMDL values being (slightly) higher than the maximum modeled concentration.
Packed red cell volume in male mice (mL/dL)	ND	ND	ND	ND	ND	The constant variance model did not provide adequate fit to the variance data, but the nonconstant variance model did. With the nonconstant variance model applied, none of the models provided adequate fit to the means (test 4 p-value < 0.1). This data set is not suitable for BMD modeling; no model selected.

Endpoint	Recommended Model	1 SD		10% ER		Notes[a]
		BMD (ppm)	BMDL (ppm)	BMD (ppm)	BMDL (ppm)	
Packed red cell volume in male mice (mL/dL) – **highest concentration dropped**	BMR: 1 SD: Hill (constant variance); BMR 10% RD: Exponential 3 (constant variance)	10.8	3.91	62.5	41.9	The constant variance model provided an adequate fit to the variance data. With the constant variance model applied, all models provided adequate fit to the means (test 4 p-value > 0.1) when using the BMR of 1SD. The BMDLs for the fit models were not sufficiently close (differed by >3-fold); therefore, the model with the lowest BMDL was selected (Hill, which also had the lowest AIC). When applying a BMR of 10% RD, the BMD computation failed for the Hill model, and it was unusable. Among the remaining models, the BMDLs were sufficiently close (differed by <3-fold); therefore, the model with the lowest AIC was selected (Exponential 3); using a BMR of 10% RD resulted in BMD and BMDL values being higher than the maximum modeled concentration.

[a] Modeled concentrations were duration adjusted for 6 hours/day, 5 days/week
ND = not determined; no model selected

Appendix C BLADDER CANCER INHALATION UNIT RISK DERIVATION

Seventy-three epidemiologic publications were identified in the TSCA Systematic Review process (U.S. EPA, 2024h). Of those 73 publications, 35 publications that included exposure-response analyses and cumulative exposure were prioritized. Of the 35 publications, 7 publications investigated bladder cancer. All seven publications used data from the original U.S.-Canadian SBR worker cohort study (Delzell et al., 1996). Details of this SRB cohort study in terms of study population, exposure assessment and concentrations, and statistical analysis are described in the above paragraphs in Section 5.4.1.

Table_Apx C-1 summarizes the study populations, exposure levels, and results of seven bladder cancer epidemiological publications. All study participants were adults and all dose-response associations were inconsistent. Three (Valdez-Flores et al., 2022; Sathiakumar et al., 2021a; Sathiakumar et al., 2019) publications found statistically significant relationships. In contrast, three publications (UAB, 2007; Sathiakumar et al., 2005; Delzell et al., 1996) showed negative associations and one reported both significant SMRs for workers in residual operation and non-significant SMRs for SBR workers. Overall, male-only populations did not show a statistically significant association between 1,3-butadiene exposure and bladder cancer, but combined male and female populations had a statistically significant association.

The inconsistent association for bladder cancer could be relevant to three reasons. First, styrene exposure in the SBR cohort. The independent effects of butadiene and styrene could not be determined because exposures to the two monomers were highly correlated. However, 1,3-butadiene and styrene exposures were not consistently associated with the other outcomes analyzed (Sathiakumar et al., 2021a). Second, since the association between 1,3-butadiene and bladder cancer is not adjusted for smoking, potential residual confounding by smoking is an uncertainty. And lastly, the number of female bladder cancer cases (10) in the Valdez-Flores study (2022) causes low statistical power in the analysis. Sathiakumar et al. (2019) indicated that the interpretation of results for bladder cancer is limited because of the inability to control smoking directly and inadequate or inconsistent support from other studies for an association between butadiene and bladder cancer. Due to the inconsistent study results, uncertainty about deriving bladder cancer is moderate.

Table_Apx C-1. Summary of Bladder Cancer Epidemiological Studies that Provided at Least Two Exposure Levels (+ a Reference Level) or Continuous Exposure Levels

Data Source	Reference	Study Period or Follow-up	Exposure Range in the Dose-Response Model	Health Outcomes	Statistically Significant Result?	Systematic Review Score
SBR Cohort	Delzell et al. (1996)	1943–1992	0 to 200+ ppm-years	Bladder cancer mortality	No significant associations.	Medium
SBR Cohort	Sathiakumar et al. (2005)	1944–1998	No quantitative data reported	Bladder cancer mortality	No significant associations.	Medium
SBR Cohort	UAB (2007)	1943–2003	0 to >56.3 ppm-years	Bladder cancer mortality	No significant associations.	Medium
SBR Cohort	Sathiakumar and Delzell (2009)	1943–2003	No quantitative data reported	Bladder cancer mortality	Significant SMR for residual operation and no significant SMR for SBR workers	Medium

Data Source	Reference	Study Period or Follow-up	Exposure Range in the Dose-Response Model	Health Outcomes	Statistically Significant Result?	Systematic Review Score
SBR Cohort	Sathiakumar et al. (2019)	1943–2009	No quantitative data reported	Bladder cancer mortality	Significant SMR	Medium
SBR Cohort	Sathiakumar et al. (2021a)	1943–2009	0 to >328.79 ppm-years	Bladder cancer mortality	Significant positive associations	Medium
SBR Cohort	Valdez-Flores et al. (2022)	1943–2009	0 to 7,900 ppm-years	Bladder cancer mortality	Significant positive associations	Low

C.1 Study Selection

To select the appropriate study to derive bladder cancer IUR, EPA considered three factors to evaluate the seven publications: (1) publications after 2009 because the study's follow-up period ended in 2009; (2) inclusion of all male and female study participants; and (3) rating in the SR as high or medium. After considering all three factors, only two publications (Sathiakumar et al., 2021a; Sathiakumar et al., 2019) were selected for further consideration. Sathiakumar et al. (2021a) provided the regression coefficient, which is required for lifetable analysis, but Sathiakumar et al. (2019) only included bladder cancer standardized mortality ratio (SMR) data. As a result, Sathiakumar et al. publication (2021a) is selected for bladder cancer IUR derivation.

C.2 Selection for Statistical Model and Data

Sathiakumar et al. (2021a) showed that their analyses of exposure-response relations in the SBR cohort by UAB researchers improved and extended their previous analyses, including those that informed the IRIS assessment. The cohort was expanded and updated to include women and 18 additional years of follow-up, which added 418,546 person-years of observation and 5,000 deaths (Sathiakumar et al., 2021a) and included the revised exposure estimates (Macaluso et al., 2004). The analytical framework in Sathiakumar et al. (2021a) was also updated by replacing classical grouped Poisson regression models with proportional hazards models (Cox regression model), which can allow analysts to avoid bias from grouping and assigning exposure values.

Table_Apx C-2 shows that Sathiakumar et al. (2021a) used various models to estimate the association between butadiene exposure and bladder cancer. Since the purpose of IUR derivation is for 1,3-butadiene exposure and bladder cancer, the first three models that include the unexposed population are not under consideration. Between models 4 and 5, model 4 was statistically significant. Therefore, the results from model 4 were ultimately selected for lifetable analysis and IUR derivation.

Table_Apx C-2. Summary of Crucial Cox Regression Models to Analyze Exposure-Response Relations in Sathiakumar et al. (2021a)

Statistical Model	Lag Time (years)	β (Beta-Coefficient)	Upper 95% Confidence Bound on β	Trend P-Value
1. All person-time (untrimmed, including unexposed)	0	3.84E−04	6.12E−04	0.001
2. All person-time (untrimmed, including unexposed)	10	3.87E−04	6.21E−04	0.001
3. All person-time (untrimmed, including unexposed)	20	4.22E−04	6.80E−04	0.001
4. Exposed person-time (exclude unexposed)	0	3.50E−04	5.95E−04	0.005
5. Exposure person time ≤95th percentile: Restricted cubic spline (RCS) Cox regression model (trim to restrict data)	0	4.72E−04	13.79E−04	0.308

C.3 Lifetable Analysis

The lifetable analysis for bladder cancer uses the same method as that for leukemia that is described in Section 5.4.3.2. This method was used by EPA IRIS to derive 1,3-butadiene IUR for leukemia (U.S. EPA, 2002a). The data inputs and outputs are described below.

A. Data Input

Three kinds of inputs are essential to be used in the lifetable analysis:

1. Population statistics include U.S. age-specific all-cause mortality and cause-specific incidence/mortality: U.S. age-specific all-cause mortality rates for deaths in 2019 among all race and gender groups combined are retrieved from CDC's Multiple Cause of Death (final) Database of the Wide-ranging ONline Data for Epidemiologic Research (WONDER) database (CDC, 2024). For 1,3-butadiene lifetable analysis, the bladder cancer-specific incidence was obtained from the Surveillance, Epidemiology, and End Results (SEER) 22 from the National Cancer Institute (NCI), National Institutes of Health (NIH, 2024). Both the U.S. all-cause mortality and bladder cancer incidence are age-specific, but rates above the age of 85 years are not included because bladder cancer-specific incidence did not list for those ages. Therefore, EPA assumed 84.99 years of exposure for the lifetable analysis.

2. Epidemiological studies with cumulative exposure model from the linear or log-linear model for exposure: In epidemiological studies that provide exposure-response analyses, β and upper 95 percent confidence bound (CB) on β can best characterize the hazard and be incorporated into the lifetable analysis. The beta is the estimate of the increase in bladder cancer that results from an increase of one unit of exposure to 1,3-butadiene. If the dose-response had an exposure lag (years, *e.g.*, 0, 10 years), that must be included in the lifetable analysis. The latency period of bladder cancer could range widely, from 14 to over 30 years (Böthig et al., 2021; Saginala et al., 2020; Clin et al., 2014; Yamaguchi et al., 1982; Mazeman, 1972). Since the latency appears rarely to fall under 20 years, 20 years is considered the minimum latency period after the start of exposure. (Clin et al., 2014; Mazeman, 1972) Thus, 20 years is incorporated as the lagged exposure in the lifetable analysis.

3. Selection of Benchmark Response (BMR): BMR is usually set as 1 percent for cancer data, except for rare cancer (U.S. EPA, 2012b). Since the selected health outcome is bladder cancer, 1 percent is used for BMR.

B. Data Output

The lifetable analysis aims to find the 95 percent lower confidence limit of the exposure concentration (LEC_{BMR}) that results in bladder cancer's extra risk (ER) after exposure to 1,3 butadiene. ER is a calculation of the risk of adverse effects, which adjusts for background incidence rates of the same effect by estimating risk at dose only among the fraction of the population not expected to respond to the background causes (U.S. EPA, 2024i). The target extra risk in this lifetable analysis is set as 0.01 since 1 percent is usually used for cancer BMR (U.S. EPA, 2012b). LEC_{BMR} is the cumulative lifetime exposure levels that yield extra risk as 0.01 by interpolating the exposure level corresponding to the 95 percent upper bound on β. Through an iterative process that evaluates the risk levels resulting from selected exposure levels, the exposure expected to result in a 1 percent excess risk can be determined.

C.4 IUR and UR Calculation

Epidemiologic data are used in the 1,3-butadiene IUR derivation because of the rich and good-quality data collected from a 66-year SBR worker cohort study. The method to calculate IUR for bladder cancer is the same as that for leukemia, which is described in Section 5.4.3.3. The equation for IUR calculation is:

UR at 95% upper bound = BMR_{01}/LEC_{01} = 0.01 / $LEC_{01.}$

Where BMR is set as 1 percent for bladder cancer, which means the change in response rate is 1 percent over the background of the BMR, and LEC_{01} is the 95 percent lower confidence limit of the exposure concentration associated with a 1 percent increased risk.

EPA has determined that 1,3-butadiene is "Carcinogenic to Humans" and exhibits a mutagenic mode of action in Section 5.3. In accordance with the Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens, the following ADAFs were applied to the adult unit risk: 10 for children ages less than 2 years; 3 for children ages 2 to 15; and 1 for persons aged 16 to 78. (Barton et al., 2005). The weighted sum of these three partial unit risks is the ADAF-adjusted lifetime IUR (Barton et al., 2005).

C.5 IUR and UR Results

Using the above equation for computation, the LEC_{01} calculated by lifetable analysis is 5.0 ppm, and the UR at 95% upper bound based on ages from less than 1 to 84.9 years old is 0.002 per ppm (0.90×10^{-6} per µg/m^3) (Table_Apx C-3). Due to the carcinogenic mode of action of 1,3-butadiene, the age-dependent adjustment factor (ADAF) is applied to UR at 95 percent upper bound to yield IUR. After applying the ADAF to the UR at 95 percent upper bound, the IUR is computed to be 0.0032 per ppm (1.4×10^{-6} per µg/m^3) (Table_Apx C-4) The interpretation of IUR (1.40×10^{-6} per µg/m^3) is that 1.4 excess bladder cancer cases (as the upper bound estimate) are expected to develop per 1,000,000 people if exposed daily for a lifetime to 1 µg of 1,3-butadiene per m^3 of air.

Table_Apx C-3. Calculation of Bladder Cancer Unit Risk Estimate

Model of the Beta-Coefficient (β), Reference	β		Exposure Concentration Associated with BMR (1% Extra Risk) Starting Exposure at Age <1 years (µg/m^3)		Unit Risk	
	MLE[a]	95% UB[b]	EC_{01} MLE	LEC_{01} 5% LB[c]	MLE	95% UB[b]
Cox regression model (Sathiakumar et al., 2021a)	3.50E−04	5.56E−04	7.90 ppm	5.0 ppm	0.0013 per ppm	0.002 per ppm

[a] MLE means Maximum Likelihood Estimate, a statistical method for estimating a population parameter most likely to have produced the sample observations.
[b] UB means the upper bound estimate.
[c] LB means the lower bound estimate

Table_Apx C-4. Incorporation of Age-Dependent Adjustment Factors for General Population Risk Estimation

Age	ADAF Adjustment[a]	Adjusted Partial Life and General Population IUR
0 to <2	10×	0.002 × 10 × (2/78) = 0.00051
2 to <16	3×	0.002 × 3 × (14/78) = 0.0011
≥16[b]	1×	0.002 × 1 × (62/78) = 0.0016
0 to 78		**0.0032 per ppm (1.4E−6 per µg/m^3)**

[a] ADAFs are applied based on the determination of a mutagenic MOA (Section 5.3) and in accordance with (U.S. EPA, 2005b).
[b] Adjusted IUR value is based on an assumption of 78 years lifetime (U.S. EPA, 2011).
[c] The unit risk was corrected as described in *1,3-Butadiene: Corrected lifetable analyses for leukemia and bladder cancer* (U. S. EPA, 2024a).

Appendix D OTHER HAZARD OUTCOMES

This appendix discusses organ systems that have weaker evidence integration conclusions and insufficient information to develop a detailed evidence integration table. None of these outcomes were considered for dose-response. See full data extraction for all relevant studies in *Data Extraction Information for Human Health Hazard Animal Toxicology and Epidemiology* (U.S. EPA, 2024b) and *Further Filtering Results for Human Health Hazard Animal Toxicology and Epidemiology* (U.S. EPA, 2024c)).

D.1 Neurotoxicity, Sensory Effects, and Non-cancer Mortality

Human Evidence

1,3-butadiene has demonstrated only very mild acute toxicity in human subjects. Eye irritation and impaired visual focus were observed in two single subjects following 7 hours of exposure to 2,000 or 4,000 ppm 1,3-butadiene, without any more severe symptoms observed at 8,000 ppm. No effects on psychomotor responses were observed (Carpenter et al., 1944). In a cross-sectional study of adults, increased urinary 1,3-butadiene metabolite levels was statistically significantly associated with hearing loss, based on data collected during the 2011 to 2012 cycle of the U.S. National Health and Nutritional Examination survey (NHANES) (Pudrith and Dudley, 2019). As previously described in Section 4.1.2.1.1, environmental *in utero* exposure to 1,3-butadiene has also been associated with increased risk for autism (von Ehrenstein et al., 2014).

Laboratory Animal Evidence

A majority of rabbits died following only 23 minutes of exposure and demonstrated central nervous system (CNS) depression following less than 2 minutes of exposure to the very high dose of 250,000 ppm 1,3-butadiene (Carpenter et al., 1944), while the lethal concentration resulting in death to 50 percent of test subject (LC50) values are 122,000 ppm for mice exposed for 2 hours and 129,000 for rats exposed for 4 hours. According to the interim Acute Exposure Guideline Levels (AEGL) document for 1,3-butadiene (NAC/AEGL, 2009), guinea pigs demonstrated 100 percent mortality following 10 hours of exposure to 89,000 ppm 1,3-butadiene but no mortality following 30 min exposure to 200,000 ppm. Rabbits also survived a 25-minute exposure to 200,000 ppm while two of five rats died following a 30-minute exposure to the same concentration.

As summarized by ATSDR (2012), no mortality was observed in rats following 13 weeks of 8,000 ppm or 8 months of 6.700 ppm exposure. Guinea pigs, rabbits, and dogs also did not die following 8 months of 6.700 ppm exposure. The lowest-observed-adverse-effect concentration (LOAEC) for increased mortality in mice was 5,000 ppm (no-observed-adverse-effect concentration [NOAEC] = 2,500 ppm) exposure for 6 hours/day, 5 days/week for 14 weeks (NTP, 1984). Chronic exposure resulted in increased mortality to both mice and rats; however, this is associated with cancer and is discussed in Section 5.1.

No effects on neuromuscular function or observed histopathology were observed in rats exposed for 13 weeks to as high as 8,000 ppm (Crouch et al., 1979). Reduced balance/locomotor function and decreased brain weight in females were observed following 1,000 or 8,000 ppm exposure for 2 years (Hazleton Labs, 1981b); however, tumors were also present at these doses and may have impeded mobility. No functional, measured, or histopathological effects were observed for any other parameter following 2 years of exposure to 619 ppm (NTP, 1993).

Mechanistic and Supporting Evidence

EPA did not identify any reasonably available information to provide any mechanistic support for potential neurotoxicity.

Evidence Integration Summary and Conclusions

Evidence *suggests but is not sufficient to conclude* that 1,3-butadiene causes neurotoxicity and/or sensory effects in humans under relevant exposure circumstances. This conclusion is based on slight evidence of functional and developmental neurotoxicity outcomes in limited human studies, slight evidence in animals based on inconsistent effects observed greater or equal to 1,000 ppm and indeterminate mechanistic data.

For acute/intermediate non-cancer mortality, *strong evidence supports no effect* in humans under relevant exposure circumstances. Only mild irritation effects were observed in humans at exposures several orders of magnitude higher than any realistic occupational or general population exposure. In animals, rodent acute LC50s (the amount of a substance that is lethal to half of a test population) were similarly above 100,000 ppm and intermediate exposures resulted in death only in mice but not other model species at or above 5,000 ppm.

Based on the evidence integration conclusions and absence of strong dose-response data for these endpoints, dose-response analysis is not considered for neurotoxicity or non-cancer mortality.

D.2 Respiratory Toxicity

Human Evidence

Individual case reports of qualitative occupational exposure to 1,3-butadiene mention irritation of respiratory tissues including nose, throat, and lungs (as well as eyes). Some workers also experienced coughing, fatigue, and drowsiness (ATSDR, 2012). Details on exposure levels were not provided.

Laboratory Animal Evidence

As summarized by ATSDR (2012), chronic exposure to 1,250 ppm 1,3-butadiene in mice caused inflammation of the nasal cavity along with fibrosis, metaplasia, and atrophy of olfactory epithelium and hyperplasia of the respiratory epithelium (NTP, 1993, 1984). In contrast, respiratory effects were not observed in rats, guinea pigs, rabbits, or dogs exposed to as much as 6.700 ppm 1,3-butadiene for 8 months (Carpenter et al., 1944) or rats exposed to 8,000 ppm for approximately 3 months (Crouch et al., 1979). However, 2 years of 8,000 ppm exposure did cause lung metaplasia in rats (Hazleton Labs, 1981b). Hyperplasia of the epithelium was increased following as little as 20 ppm exposure for 15 months to 2 years of exposure in females (at higher doses in males); however, the outcome was not dose- or duration-responsive (NTP, 1993).

Mechanistic and Supporting Evidence

EPA did not identify any reasonably available information to provide any mechanistic support for potential respiratory toxicity. However, indications of human respiratory irritation suggest that either parent 1,3-butadiene or metabolites may be cytotoxic to respiratory tissue—leading to subsequent proliferation, repair, and other responses as observed in animals.

Evidence Integration Summary and Conclusions

Evidence *suggests but is not sufficient to conclude* that 1,3-butadiene causes respiratory toxicity in humans under relevant exposure circumstances. This conclusion is based on slight qualitative evidence of irritation in human case studies, moderate evidence in animals due to multiple adverse effects

observed in mice and metaplasia observed at a high dose following 2-years exposure in rats, and indeterminate mechanistic data.

Based on the evidence integration conclusions and absence of strong dose-response data for these endpoints, dose-response analysis is not considered for respiratory toxicity.

D.3 Liver Toxicity

Human Evidence

In 1 study on 82 male elastomer/polymer workers with mean duration of employment over 21 years, 39 percent of acrylonitrile, 1,3-butadiene, and styrene-exposed workers had elevated serum cytokeratin 18 levels indicative of liver disease, but cumulative 1,3-butadiene exposure was higher for healthy workers (Cave et al., 2011).

Laboratory Animal Evidence

Effects on liver were not observed after 3 months of exposure to 1,3-butadiene in rats or mice (NTP, 1984; Crouch et al., 1979). The only observed liver effects in rats was increased liver weight following 2 years of exposure to at least 1,000 ppm (Hazleton Labs, 1981b) but without any indication of adversity from histopathology.

1,3-Butadiene did cause increased liver necrosis in mice following 14 to15 months of exposure to at least 625 ppm (NTP, 1993, 1984) and following 2 years of exposure to 20 to 62.5 ppm (statistical significance unclear) (NTP, 1993). Absolute weights were correspondingly increased only in females dosed to at least 62.5 ppm (NTP, 1993) and neither sex showed consistent other evidence of histopathology.

Mechanistic and Supporting Evidence

EPA did not identify any reasonably available information to provide any mechanistic support for potential liver toxicity.

Evidence Integration Summary and Conclusions

Evidence *suggests but is not sufficient to conclude* whether 1,3-butadiene exposure may cause liver toxicity in humans under relevant exposure circumstances. This conclusion is based on indeterminate evidence in humans, slight evidence in animals (with supporting evidence primarily from mice), and indeterminate mechanistic evidence.

Based on the evidence integration conclusions and absence of strong dose-response data for these endpoints, dose-response analysis is not considered for liver toxicity.

D.4 Kidney Toxicity

Human Evidence

EPA did not identify any reasonably available information assessing effects of 1,3-butadiene exposure on kidney in humans.

Laboratory Animal Evidence

Blood chemistry assessment and urinalysis of rats, guinea pigs, rabbits, and dogs did not demonstrate any atypical measurements for nitrogen, bilirubin, glucose, albumin, sugar, or casts following 8 months of exposure to as high as 6,700 ppm 1,3-butadiene in a poorly described study (Carpenter et al., 1944). Both rats and mice did not demonstrate any signs of kidney toxicity after approximately 3 months of

exposure to as high as 8,000 ppm (NTP, 1984; Crouch et al., 1979). In chronic studies, nephrosis and increased kidney weight was seen in male rats after 2 years of exposure to the highest dose of 8,000 ppm (Hazleton Labs, 1981b) and increased absolute kidney weight was observed in females after 2 years of exposure to 625 ppm in mice (NTP, 1993). The adversity of the increased kidney weight in mice is uncertain because histopathology was not observed at any dose after either 14 weeks or 2 years of exposure (NTP, 1993, 1984).

Mechanistic and Supporting Evidence

EPA did not identify any reasonably available information to provide any mechanistic support for potential kidney toxicity. The observations in male rats was not associated with an increase in alpha 2 globulin (measured at 3, 6, and 12 months)—although globulins were not measured at the 2-year termination of the experiment when the kidney effects were observed (Hazleton Labs, 1981b). Therefore, the significance of the male kidney effects has some uncertainty because α2u-globulin-mediated kidney toxicity in male rats is not relevant to humans.

Evidence Integration Summary and Conclusions

Evidence is inadequate to assess whether 1,3-butadiene exposure may cause kidney toxicity in humans under relevant exposure circumstances. This conclusion is based on indeterminate human, animal, and mechanistic evidence. The only indication of adverse effects on kidney is from male rats, for which the relevance to humans cannot be confirmed due to the absence of measurements at relevant timepoints for α2u-globulin.

Based on the evidence integration conclusions and absence of strong dose-response data for these endpoints, dose-response analysis is not considered for kidney toxicity.

Appendix E HUMAN HEALTH HAZARD CONFIDENCE SUMMARY AND ALTERNATIVE ANALYSES

E.1 Human Health Hazard Confidence Summary

Table_Apx E-1 summarizes the confidence ratings for each factor for the critical human health hazard endpoints and associated hazard values considered for acute, intermediate, and chronic non-cancer scenarios, and cancer lifetime exposure scenarios. The bolded rows in the table are the most robust and sensitive health effect for each exposure scenario and will be used to calculate risks for 1,3-butadiene.

Table_Apx E-1. Confidence Summary for Human Health Hazard Assessment

Hazard Domain	Evidence Integration Conclusion	Selection of Most Critical Endpoint and Study	Relevance to Exposure Scenarios	Dose-Response Considerations	PESS Sensitivity	Overall Hazard Confidence
Acute non-cancer						
None	Not applicable					**Indeterminate to Slight**
Intermediate/chronic non-cancer						
Maternal/ Developmental	+ +	+ + +	+ +	+ + +	+ +	**Robust**
Male Reproductive/ Developmental	+ +	+ + +	+ +	+ +	+ +	Moderate
Hematological	+ +	+ + +	+ +	+ +	+	Moderate
Lifetime cancer						
Leukemia	+++	+++	+++	+++	++	**Robust**

+ + + Robust confidence suggests thorough understanding of the scientific evidence and uncertainties. The supporting WOSE outweighs the uncertainties to the point where it is unlikely that the uncertainties could have a significant effect on the hazard estimate.
+ + Moderate confidence suggests some understanding of the scientific evidence and uncertainties. The supporting scientific evidence weighed against the uncertainties is reasonably adequate to characterize hazard estimates.
+ Slight confidence is assigned when the weight of scientific evidence may not be adequate to characterize the scenario, and when the assessor is making the best scientific assessment possible in the absence of complete information. There are additional uncertainties that may need to be considered.

EPA performed supplemental dose-response analysis on bladder cancer for comparison purposes (Appendix C). Therefore, only an overall confidence score is provided. There is only moderate human evidence of an association with 1,3-butadiene exposure without corresponding evidence from animal toxicity studies. Additionally, the publication utilized for dose-response analysis (Sathiakumar et al., 2021a) did not sufficiently account for confounders such as smoking and there is insufficient mechanistic information to support any particular mode of action. Therefore, there is only moderate overall confidence in the hazard assessment for bladder cancer.

E.2 Sensitivity Analysis for Potential Acute PODs

As discussed in Section 4.2.1.1, EPA has determined that there is not an acute POD with sufficient confidence appropriate for risk estimation. Nonetheless, there are some options for acute hazard values

based on endpoints that could plausibly result from a single exposure. These are presented below in Table_Apx E-2.

Table_Apx E-2. Potential Acute PODs Compared to the Intermediate POD

Endpoint	Study POD (ppm)	HEC (ppm) [UF]	POD/UF	Source
Potential acute PODs				
Difficulty to focus	LOAEC = 2,000	670 [UF=3]	223 ppm	(NAC/AEGL, 2009)
Abnormal sternebrae	NOAEC = 200	50 [UF=30]	1.7 ppm	(Battelle PNL, 1987b)
Dominant lethality	NOAEC = 130	32.5 [UF=30]	1.1 ppm	(Adler et al., 1998)
Intermediate/chronic POD used for risk estimation				
Reduced fetal body weight	LOAEC = 40 ppm	2.5 ppm [UF=30]	0.08 ppm	(Battelle PNL, 1987b)

E.3 Other Uncertainty Factors Not Applied in this Assessment

LOAEL-to-NOAEL Uncertainty Factor (UF_L)

A UF_L is applied when adverse effects are identified at the lowest dose/concentration tested and the POD cannot be refined through BMD modeling. A value of 3 or 10 can be applied based on the magnitude of the observed effect and the dose-response curve. The POD chosen to calculate intermediate, and chronic risks is a BMDL and therefore, EPA did not apply this UF.

Subchronic-to-Chronic Uncertainty Factor (UF_S)

A UF_S may be justified when a POD from a shorter study is used to characterize a longer duration. For 1,3-butadiene, the intermediate PODs were all based on intermediate developmental and/or pre-mating exposures and therefore no extrapolation across durations was required. For chronic exposures, these intermediate PODs are directly applicable because additional exposure is not expected to be relevant outside the developmental/pre-mating windows. The hematological effects are from a chronic study and are also directly applicable to chronic durations.

Database Uncertainty Factor (UF_D)

EPA may consider application of a UF_D on a case-by-case basis when the available quantitative data may insufficiently account for expected adverse effects from chemical exposure. For 1,3-butadiene EPA is utilizing the most sensitive and well-supported POD from the more sensitive species for risk estimates. There is insufficient evidence of neurological effects in animals (Appendix D.1) to indicate that a neurodevelopmental study would result in a lower POD and a there was no increased sensitivity or severity of outcomes in an OECD 421 reproductive study (WIL Research, 2003) in rats compared to a 10-day developmental toxicity study (Hazleton Labs, 1981a). Therefore, a UF_D is not applied for this assessment.

Appendix F Corrected Unit Risks

F.1 Modified Lifetable Analysis and Unit Risks for Leukemia

The modifications for lifetable analysis and IUR calculation are because of the addition of two new assumptions that were not included in the previous draft: (1) Assume no child exposure from the occupational setting, and (2) Assume the initiation of occupational exposure is at age 16 years old for people working in the occupational setting. Based on these two new assumptions, two changes were made in the lifetable below:

(i) Assumption of no child exposure from the occupational setting: Set zero for exposure duration for ages 0 to 15 years in column I in the lifetable;
(ii) Assumption of initiating occupational exposure at 16 years old: For exposure duration for ages 16 to 85 years in column I in the lifetable, the exposure duration starts at age 16, and the exposure duration for age 16 is set to be 0.495 years. Accordingly, the exposure duration for age 17 is 1.495 years, and for age 18, it is 2.495 years, etc. Due to the assumption of no child exposure before age 16, two unit risks, 'adult-exposure-only' unit risk and 'adult-based' unit risk, need to be derived. The detailed process to derive the updated unit risks and IUR is as follows.

The above two changes were included in the lifetable to modify the lifetable analysis, and the updated EC_{01} and LEC_{01} were derived in the same way using the lifetable procedure. EC_{01} and LEC_{01} were then divided into the benchmark response of 1 percent to calculate the 'adult-exposure-only' unit risk estimates. Afterward, the 'adult-exposure-only' unit risk estimates were multiplied by 78 ÷ 62 to rescale the 62-year adult period to 78 years and to yield the 'adult-based' lifetime unit risk. The last step is to apply the ADAF to the 'adult-based' unit risk at 95 percent UB to obtain the lifetime IUR. The last step is shown in Table_Apx F-3, modified from Table 8-3. These modified unit risks and IUR are shown in Table_Apx F-1, Table_Apx F-2, and Table_Apx F-3 below, which are modified from Table 5-8, Table 8-2, and Table 8-3, respectively. Even though the lifetable analysis and unit risk calculation were modified, the IUR is still the same at 0.00098 per ppm, so Table_Apx F-3 stays the same as the original Table 8-3. However, the chronic occupational unit risk is modified to 0.0049 per ppm, and Table_Apx F-2 presented below, modified from Table 8-2, reflects the modified chronic occupational unit risk.

Table_Apx F-1. Modified Table 5-8 Calculation of Cancer Unit Risk Estimate for Leukemia

Model of the Beta-Coefficient (β), Reference	β		Exposure Concentration Associated with BMR (1% Extra Risk) Starting Exposure at Age 16 Year		Adult-exposure-only Unit Risk (62 year)[d]		Adult-based Unit Risk (78 year)[e]	
	MLE[a]	95% UB[b]	EC_{01} (16+) MLE	LEC_{01} (16+) 5% LB[c]	MLE	95% UB[b]	MLE	95% UB[b]
Cox regression model Sathiakumar et al. (2021b)	0.00094	0.0018	3.69 ppm	2.046 ppm	0.0027 per ppm	0.0049 per ppm	0.0034 per ppm	0.0062 per ppm

[a] MLE means Maximum Likelihood Estimate, a statistical method for estimating a population parameter most likely to have produced the sample observations. This will be used for potential benefits analysis.
[b] UB means the upper bound estimate. This is the IUR to be used for risk estimation.
[c] LB means the lower bound estimate.
[d] Adult-exposure-only Unit Risk (62 year) means the unit risks for the 62-year period between age 16 years and age 85 years (OPPT assumption of a lifetime).
[e] Adult-based Unit Risk (78 year) means to rescale the 'adult-exposure-only' unit risk from 62-year adult period to 78-years by multiplying 78÷62.

Table_Apx F-2. Modified Table 8-2 Cancer Hazard Values for Occupational Cancer Risk Estimation for Leukemia

Chronic Occupational Unit Risk	Reference	Overall Quality Determination
0.0049 per ppm (2.2E−03 per mg/m^3) (2.2E−06 per µg/m^3)	(Sathiakumar et al., 2021b)	Medium

[a] EPA considers a range of extra cancer risk from 1E−04 to 1E−06 to be relevant benchmarks for risk assessment (U.S. EPA, 2017); however, these are not considered bright lines for unreasonable risk determination.

Table_Apx F-3. Modified Table 8-3 Incorporation of Age-Dependent Adjustment Factors for General Population Risk Estimation

Age	ADAF Adjustment[a]	Adjusted Partial Life and General Population IUR
0 to <2	10×	0.0062 × 10 × (2/78) = 0.0016
2 to <16	3×	0.0062 × 3 × (14/78) = 0.0033
≥16[b]	1×	0.0062 × 1 × (62/78) = 0.0049
0 to 78	**1.59**	**0.0098 per ppm (4.4E−06 per µg/m^3)**

[a] ADAFs are applied based on the determination of a mutagenic MOA (Section 5.3) and in accordance with (U.S. EPA, 2005b).
[b] Adjusted IUR value is based on an assumption of 78 years lifetime (U.S. EPA, 2011).

The above-described assumptions and unit risk calculation are consistent with IRIS methodology to conduct lifetable analysis and derive unit risks (U. S. EPA, 2024b). The changes in the lifetable analysis

are shown in *Modified Lifetable Analysis of Leukemia and Bladder Cancer for 1,3-Butadiene* (U. S. EPA, 2024c).

F.2 Modified Lifetable Analysis and Unit Risks for Bladder Cancer

The modifications for lifetable analysis and IUR calculation are mainly because of the addition of two new assumptions which were not included in the previous draft: (1) Assume no child exposure from the occupational setting, and (2) Assume the initiation of occupational exposure is at age 16 years old for people working in the occupational setting. Based on these two new assumptions, two changes were made in the lifetable below:

(i) Assumption of no child exposure from the occupational setting: Set zero for exposure duration for ages 0 to 15 years in column I in the lifetable;
(ii) Assumption of initiating occupational exposure at age 16 years old: For exposure duration for ages 16 to 85 years in column I in the lifetable, the exposure duration starts at age 16, and the exposure duration for age 16 is set to be 0.495 years. Accordingly, the exposure duration for age 17 is 1.495 years, and for age 18, it is 2.495 years, etc. Due to the assumption of no child exposure before age 16, two unit risks, 'adult-exposure-only' unit risk and 'adult-based' unit risk, need to be derived.

In addition to the above two assumptions, another modification for the IUR for bladder cancer is to change the bladder cancer latency from 20 to 0 years in the lifetable analysis. In the modified lifetable analysis (see *Modified Lifetable Analysis of Leukemia and Bladder Cancer for 1,3-Butadiene* (U. S. EPA, 2024c)), the lag of 0 years is used because of two reasons: (1) the model (Sathiakumar et al., 2021a) that EPA chose to adopt the beta coefficient for lifetable analysis used the lag of 0 years; (2) the modeling of different lags time in exposure showed little effect on beta coefficients, and it could be because the SBR cohort study had been following up for many years after study participants stopped being exposed to 1,3-butadiene. (*e.g.*, when the exposure ceased to be monitored, the follow-up continues until much later).

The detailed process to derive the updated unit risks and IUR is as follows. The above two changes were included in the lifetable to modify the lifetable analysis, and the updated EC_{01} and LEC_{01} were derived in the same way using the lifetable procedure. EC_{01} and LEC_{01} were then divided into the benchmark response of 1 percent to calculate the 'adult-exposure-only' unit risk estimates. Afterward, the 'adult-exposure-only' unit risk estimates were multiplied by 78 ÷ 62 to rescale the 62-year adult period to 78 years and to yield the 'adult-based' lifetime unit risk. The modified unit risks are shown in Table_Apx F-4, modified from Table_Apx C-3. The last step is to apply the ADAF to the 'adult-based' unit risk at 95 percent UB to obtain the lifetime IUR. After applying ADAF to the adult-based unit risk, the IUR for bladder cancer is 0.0045 per ppm (2.03×10^{-6} per $\mu g/m^3$) (Table_Apx F-5 modified from Table_Apx C-4).

Table_Apx F-4. Modified Table_Apx C-3 Calculation of Bladder Cancer Unit Risk Estimate

Model of the Beta-Coefficient (β), Reference	β		Exposure Concentration Associated with BMR (1% Extra Risk) Starting Exposure at Age 16 Year		Adult-exposure-only Unit Risk (62 year)[d]		Adult-based Unit Risk (78 year)[e]	
	MLE[a]	95% UB[b]	EC_{01} (16+) MLE	LEC_{01} (16+) 5% LB[c]	MLE	95% UB[b]	MLE	95% UB[b]
Cox regression model (Sathiakumar et al., 2021a)	0.00035	0.000556	7.09 ppm	4.46 ppm	0.0014 per ppm	0.0022 per ppm	0.0018 per ppm	0.0028 per ppm

[a] MLE means Maximum Likelihood Estimate, a statistical method for estimating a population parameter most likely to have produced the sample observations. This will be used for potential benefits analysis.
[b] UB means the upper bound estimate. This is the IUR to be used for risk estimation.
[c] LB means the lower bound estimate.
[d] Adult-exposure-only Unit Risk (62 year) means the unit risks for the 62-year period between age 16 years and age 85 years (OPPT assumption of a lifetime).
[e] Adult-based Unit Risk (78 year) means to rescale the 'adult-exposure-only' unit risk from 62-year adult period to 78-years by multiplying 78÷62.

Table_Apx F-5. Modified Table_Apx C-4 Incorporation of Age-Dependent Adjustment Factors for General Population Risk Estimation for Bladder Cancer

Age	ADAF Adjustment[a]	Adjusted Partial Life and General Population IUR
0 to <2	10×	0.0028 × 10 × (2/78) = 0.00072
2 to <16	3×	0.0028 × 3 × (14/78) = 0.0015
≥16[b]	1×	0.0028× 1 × (62/78) = 0.0022
0 to 78	**1.59**	**0.0045 per ppm (2.03E−06 per µg/m^3)**

[a] ADAFs are applied based on the determination of a mutagenic MOA (Section 5.3) and in accordance with (U.S. EPA, 2005b).
[b] Adjusted IUR value is based on an assumption of 78 years lifetime (U.S. EPA, 2011).

The above-described assumptions and unit risk calculation are consistent with IRIS methodology to conduct lifetable analysis and derive unit risks. (U. S. EPA, 2024b). The changes in the lifetable analysis are shown in the supplemental file (U. S. EPA, 2024c).