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Health Assessment of 1,3-Butadiene

National Center for Environmental Assessment–Washington Office
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ABSTRACT

This assessment was conducted to review the new information that has become available since EPA's 1985 health assessment of 1,3-butadiene.

1,3-Butadiene is a gas used commercially in the production of styrene-butadiene rubber, plastics, and thermoplastic resins. The major environmental source of 1,3-butadiene is the incomplete combustion of fuels from mobile sources (e.g., automobile exhaust). Tobacco smoke can be a significant source of 1,3-butadiene in indoor air.

This assessment concludes that 1,3-butadiene is carcinogenic to humans by inhalation, based on the total weight of evidence. The specific mechanisms of 1,3-butadiene-induced carcinogenesis are unknown, however, it is virtually certain that the carcinogenic effects are mediated by genotoxic metabolites of 1,3-butadiene.

Animal data suggest that females may be more sensitive than males for cancer effects; nevertheless, there are insufficient data from which to draw any conclusions on potentially sensitive subpopulations.

The human incremental lifetime unit cancer (incidence) risk estimate is based on extrapolation from leukemias observed in an occupational epidemiologic study. A twofold adjustment to the epidemiologic-based unit cancer risk is then applied to reflect evidence from the rodent bioassays suggesting that the epidemiologic-based estimate may underestimate total cancer risk from 1,3-butadiene exposure in the general population.

1,3-Butadiene also causes a variety of reproductive and developmental effects in mice; no human data on these effects are available. The most sensitive effect was ovarian atrophy observed in a lifetime bioassay of female mice. Based on this critical effect and using the benchmark concentration methodology, an RfC (i.e., a chronic exposure level presumed to be "without appreciable risk" for noncancer effects) was calculated.

In summary, the EPA's conclusions about the health effects of 1,3-butadiene are:

- 1,3-Butadiene is carcinogenic to humans by inhalation.
- The unit cancer risk estimate is 0.08/ppm (based primarily on linear modeling and extrapolation of human data). This incorporates an adjustment factor of 2 to address concerns for sensitive populations. The corresponding estimate of the chronic exposure level of 1,3-butadiene resulting in extra cancer risk of 10^{-6} (i.e., 1 in a million) is 0.01 ppb.
- A chronic RfC (0.9 ppb), an acute reference value (7 ppb), and a subchronic reference value (7 ppb) are presented for noncancer effects.

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PREFACE

This *Health Assessment of 1,3-Butadiene* was prepared to serve as a source document for Agency-wide use. This document was developed primarily for use by the U.S. Environmental Protection Agency's (EPA's) Office of Transportation and Air Quality (OTAQ), previously known as the Office of Mobile Sources (OMS), to support decision making regarding Section 202(l)2 of the Clean Air Act Amendments of 1990. The exposure information included here is an overview of the ambient exposures and exposure to populations adjacent to emission sources, without any actual exposure assessment as such.

In the development of this assessment, relevant scientific literature has been incorporated from the period July 1, 1985, through January 31, 2000. Key studies were evaluated to qualitatively describe and, where possible, quantitatively identify the noncancer and cancer effects of 1,3-butadiene. The assessment also includes a summary, conclusions, and hazard and dose-response characterization. Measures of dose-risk relationships relevant to ambient air exposures are discussed so that the adverse health effects can be placed in perspective with possible exposure levels.

This final document reflects a consideration of all comments received on an External Review Draft dated January 1998 (EPA/600/P-98/001A) provided by the Environmental Health Committee (EHC) of the EPA's Science Advisory Board (at a public meeting held on April 30 and May 1, 1998) and the public.

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1. INTRODUCTION

This chapter presents background information about 1,3-butadiene, including an overview of the scope of this health assessment and a summary of the Environmental Protection Agency's (EPA's) previous health assessment (U.S. EPA, 1985).

1.1. BACKGROUND

1,3-Butadiene (CH_2=CH-CH=CH_2, CAS No. 106-99-0) is a colorless gas produced by three different processes: (1) oxidative dehydrogenation of n-butene (the Oxo-D or O-X-D process), (2) catalytic dehydrogenation of n-butane and n-butene (the Houdry process), and (3) recovery from the C_4 coproduct (by-product) stream from the steam-cracking process used to manufacture ethylene (the ethylene coproduct process). This noncorrosive gas has a boiling point of −4.4°C and a vapor pressure of 1,790 mm Hg at 20°C (Santodonato, 1985). 1,3-Butadiene is highly volatile and has low solubility in water; thus, environmental release results primarily in atmospheric contamination. Atmospheric decomposition of 1,3-butadiene occurs primarily by photoinitiated reactions. A significant amount of decomposition also occurs by the gas-phase reaction with ozone and by reaction with nitrate radicals at nighttime, particularly in urban areas (U.S. DHHS, 1992). The major photooxidation products of 1,3-butadiene are acrolein and formaldehyde (Maldotti et al., 1980).

Approximately 12 billion pounds of 1,3-butadiene are produced annually worldwide, including 3 billion pounds produced in the United States (Morrow, 1990; USITC, 1990). 1,3-butadiene is used as an intermediate in the production of polymers, elastomers, and other chemicals. Its major uses are in the manufacture of styrene-butadiene rubber (SBR) (synthetic rubber) and of thermoplastic resins. Elastomers of butadiene are used in the manufacture of tires, footwear, sponges, hoses and piping, luggage, packaging, and a variety of other molded products. In addition, 1,3-butadiene is used as an intermediate to produce a variety of industrial chemicals, including the fungicides captan and captfol. The primary way the 1,3-butadiene is released into the environment is via emissions from gasoline- and diesel-powered vehicles and equipment. Lesser releases occur from the combustion of other fossil fuels and biomass. Minor releases occur in production processes, tobacco smoke, gasoline vapors, and vapors from the burning of plastics as well as rubber (Miller, 1978).

1.2. OVERVIEW OF THE SCOPE OF THIS HEALTH ASSESSMENT

EPA first published a health assessment of 1,3-butadiene in 1985. The purpose of this current assessment is to review new information on 1,3-butadiene and update the conclusions from the earlier assessment. This reassessment is intended to serve as a source document for risk

assessors inside and outside the Agency, although its development was specifically prompted by a request from EPA's Office Transportation and Air Quality (OTAQ), previously known as Office of Mobile Sources (OMS), to support decision making regarding Section 202(l)2 of the Clean Air Act Amendments of 1990. As requested by OTAQ, the document focuses primarily on the following major health effects: carcinogenicity, mutagenicity, and reproductive/developmental toxicity. A detailed exposure assessment was not requested and was not conducted.

The remainder of Chapter 1 reviews the 1985 health assessment. For background purposes, Chapter 2 provides a brief overview of 1,3-butadiene exposure. Chapter 3 reviews the data on metabolism and pharmacokinetics, while Chapter 4 discusses the evidence on mutagenicity and cytogenetic effects, including dominant lethal effects.

Toxicity of 1,3-butadiene in experimental animals is reviewed in Chapters 5 (reproductive and developmental effects) and 6 (other toxicity, primarily carcinogenicity). From the available data, 1,3-butadiene appears to have low acute toxicity. Most data on acute toxicity precede 1985 and are summarized by Himmelstein et al. (1997); however, they are not reviewed in this document. From available short-term and subchronic studies, the major effects are reproductive (e.g., dominant lethal effects) and developmental (e.g., reduced fetal weight), which are reviewed in Chapter 5, with the exception of dominant lethal effects, which are reviewed in Chapter 4. Some hematological/immunological effects also have been observed in subchronic studies and are discussed briefly in Chapter 6. The major effects observed in chronic experimental studies are carcinogenicity, reviewed in Chapter 6, and ovarian, uterine, and testicular atrophy, reviewed in Chapter 5.

Evidence of carcinogenicity from epidemiologic studies is reviewed in Chapter 7. The epidemiologic studies focus on workers involved in the production of either 1,3-butadiene monomer or styrene-butadiene polymer. Chapter 8 provides a summary and characterization of the hazard attributable to 1,3-butadiene exposure with the scope of the health effects review. Chapter 9 reviews the physiologically based pharmacokinetic (PBPK) models that have been developed for 1,3-butadiene. Chapter 10 presents quantitative risk estimates for 1,3-butadiene effects. Cancer potency estimates are derived from the epidemiologic data, as well as from the cancer bioassays for comparison purposes. Benchmark dose modeling and sample reference concentrations (RfCs) are performed for various reproductive and developmental effects that are candidates for the critical noncancer effect. Ultimately, a chronic RfC is presented based on the critical endpoint of ovarian atrophy. Reference values for acute and subchronic exposures based on the critical endpoint of reduced fetal weight are also presented in Chapter 10. Chapter 11 provides a dose-response characterization based on the information developed in Chapter 10, and the references for the entire document are listed at the end.

1.3. SUMMARY OF EPA'S CARCINOGEN ASSESSMENT (U.S. EPA, 1985)

Pertinent studies reported before 1986 were reviewed in *Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene* (U.S. EPA, 1985). This document was peer-reviewed by experts in the field, as well as in public sessions of the Environmental Health Committee of EPA's Science Advisory Board. The studies presented in the 1985 document are not reviewed in the present document but are briefly summarized below.

EPA reviewed six epidemiologic studies, which included four retrospective cohort mortality studies, one nested case-control study, and an industrial hygiene and hematologic cross-sectional survey. The first cohort study involved 6,678 hourly workers in a rubber tire manufacturing plant in Akron, Ohio (McMichael et al., 1974). The standard mortality ratios (SMRs) were calculated using the 1968 U.S. male population as the reference. Cause-specific mortality was evaluated for 16 different occupational title groups (work areas) within the plant. This study was followed up by a nested case-control study involving 455 of the 1,983 deaths recorded between 1968 and 1973 (McMichael et al., 1976). The second cohort study was conducted in 8,938 male workers in a rubber plant also located in Akron, Ohio (Andjelkovich et al., 1976, 1977). The 1976 study used the U.S. male population as the reference for calculating the SMRs, whereas for the 1977 study, the entire cohort was used to calculate the SMRs for 28 different work areas. The third cohort study included 2,756 workers at two styrene-butadiene rubber facilities in eastern Texas (Meinhardt et al., 1982). The sex, age, race, and calendar-time cause-specific rates of the U.S. population were used to calculate the SMRs. The last and most comprehensive study was conducted in 13,920 workers at one Canadian and seven U.S. styrene-butadiene rubber plants (Matanoski et al., 1982). The SMRs for black and white workers were calculated separately. The cross-sectional survey was conducted on workers in the same styrene-butadiene rubber plant studied by McMichael et al. (Checkoway and Williams, 1982). Blood samples were obtained to evaluate hematology parameters. The survey was not designed to evaluate mortality experience and did not contribute to cancer risk evaluation of 1,3-butadiene.

Of the five epidemiologic studies that evaluated cause-specific mortality, three cohort studies demonstrated statistically significant excess mortality due to cancers of the lymphatic and hematopoietic tissues (Andjelkovich et al., 1976; McMichael et al., 1976; Meinhardt et al., 1982). The fourth cohort study by Matanoski et al. (1982) also showed increased leukemia, but failed to achieve statistical significance. Lastly, the nested case-control study by McMichael et al. (1976) showed statistically significant increased standardized risk ratios for cancers of the lymphatic and hematopoietic tissues among workers with exposures of 5 years or more in one area of the plant (synthetic rubber plant area), compared with either all the other workers or the matched controls. Statistically significant excess cancer mortality was also observed for gastrointestinal tract, respiratory tract, central nervous system, prostate, testicles, and urinary

bladder in one or more studies. However, these excesses were not observed consistently across all the studies.

Although excess mortality due to cancers of the lymphatic and hematopoietic tissues was observed consistently in all the evaluated studies, the methodologic limitations prompted EPA to conclude that the evidence was inadequate for determining a causal association between exposure to 1,3-butadiene and cancer in humans. The methodologic limitations included too few deaths from specific cancers to evaluate the causal association, exclusion of large portions of the population due to lack of records, lack of adjustment for smoking, confounding by other exposures such as benzene or styrene, and excess cancer mortality at other sites.

Two long-term animal studies presented strong evidence for the induction of cancers at multiple anatomical sites in rats (HLE, 1981b) and mice (NTP, 1984). Sprague-Dawley rats were exposed by inhalation to 1,3-butadiene at concentrations of 1,000 or 8,000 ppm 6 hours/day, 5 days/week, for 111 weeks and 105 weeks for males and females, respectively. Statistically significant increased incidences in the following neoplasms were observed at one or both concentrations: mammary gland tumors, thyroid follicular adenomas/carcinomas, and Zymbal gland carcinomas in female rats and Leydig cell adenomas/carcinomas, pancreatic exocrine adenomas, and Zymbal gland tumors in male rats. In addition, gliomas occurred in four high-dose male rats. Non-neoplastic effects due to long-term exposure of rats to 1,3-butadiene included clinical signs of toxicity, increase in liver weight in both sexes, marked to severe nephropathy in 27% of the high-dose male rats compared with 9% or 10% of the controls, and alveolar metaplasia in male rats.

Among B6C3F$_1$ mice exposed by inhalation to 1,3-butadiene at 625 or 1,250 ppm for 6 hours/day, 5 days/week, neoplasms also developed at multiple anatomical sites; this study was terminated at Week 60 to 61 because of high mortality in the treated groups, primarily due to neoplasms. There was an overall increase in the number of animals with primary neoplasms and animals with multiple neoplasms. Neoplasms showing statistically significant increased incidences among both male and female mice were as follows: malignant lymphomas, alveolar/bronchiolar adenomas/carcinomas, hemangiosarcomas of the heart, and forestomach papillomas/carcinomas. In addition, mammary gland acinar cell carcinomas, ovarian granulosa cell carcinomas, and hepatocellular adenomas/carcinomas occurred in female mice. Nonneoplastic effects observed were testicular atrophy, chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium of the nasal cavity in male mice. Ovarian atrophy was observed in female mice. Some discrepancies were noted for this study, but they were not considered to pose a significant impact on the overall interpretation of the study.

EPA also reviewed data from metabolism and mutagenicity studies, concluding that inhaled 1,3-butadiene is metabolized to mutagenic epoxide intermediates.

In addition, EPA reviewed the carcinogenicity of related compounds (4-vinyl-1-cyclohexene, epoxybutene, *dl*-1,2:3,4-diepoxybutane, and *meso*-1,2:3,4-diepoxybutane). 4-Vinyl-1-cyclohexene is carcinogenic in female mice (oral/gavage), based on increased incidences of ovarian and adrenal gland neoplasms. Equivocal evidence was noted for malignant lymphomas and alveolar/bronchiolar adenomas in male mice and clitoral gland neoplasms in female rats (NTP, 1985). Skin painting of mice with *meso*-1,2:3,4-diepoxybutane induced papillomas and squamous cell carcinomas (Van Duuren et al., 1963), and subcutaneous injection with *dl*-1,2:3,4-diepoxybutane caused fibrosarcomas in mice and rats (Van Duuren et al., 1966).

Based on the studies in mice and rats, EPA concluded that there is sufficient evidence for carcinogenicity of 1,3-butadiene in animals. EPA also concluded that evidence from metabolism, mutagenicity, and carcinogenicity studies suggests that 1,3-butadiene presents a genetic risk to humans.

Two developmental toxicity studies were reviewed. One study was conducted using pregnant female Sprague-Dawley rats exposed to 200, 1,000, and 8,000 ppm 6 hours/day on gestation days 6-15 (HLE, 1981a). Developmental effects included slightly decreased fetal weight and mean crown-rump length and increased skeletal variations and malformations. The other study was inadequately reported (Carpenter et al., 1944).

Thus, in 1985, EPA presented the following conclusion regarding the qualitative evaluation of the data for 1,3-butadiene: "On the basis of sufficient evidence from studies in two species of rodents, and inadequate epidemiologic data, 1,3-butadiene can be classified as a probable human carcinogen, Group B2." Using the classification scheme of the International Agency for Research on Cancer (IARC), 1,3-butadiene would also be classified as a "probable" human carcinogen, Group 2B.

The linearized multistage model was used to calculate the maximum likelihood estimate for the incremental risk for 1,3-butadiene based upon the National Toxicology Program (NTP) mouse data (NTP, 1984), the HLE (1981) rat data, and internal dosimetry derived from data on mice and rats exposed to varying concentrations of 1,3-butadiene for 6 hours. The upper-limit unit risk of 6.4×10^{-1} $(\text{ppm})^{-1}$ was a geometric mean of the values calculated for male and female mice separately. This value was used to predict human responses in the epidemiologic studies, which were then compared with the actual responses. According to EPA (U.S. EPA, 1985),

> The comparisons were hampered by a scarcity of information in the epidemiologic data concerning actual exposures, age distribution, and work histories. In

addition, because there was no consistent cancer response across all of the studies, the most predominant response, cancer of the lymphatic and hematopoietic tissues, was chosen as being the target for 1,3-butadiene. Based on the comparisons between the predicted and observed human response, the extrapolated value from the mouse data was consistent with human response, but in view of all the uncertainties and apparent inconsistencies in the epidemiologic data, a fairly wide range of potency estimates and exposure scenarios would also be satisfactory.

2. OVERVIEW OF EXPOSURE TO 1,3-BUTADIENE

The purpose of this chapter is to present an overview of how human exposure to 1,3-butadiene occurs. The chapter summarizes physical/chemical properties, production/use, sources/emissions, and ambient air data. Pathways of exposure are briefly described, but no quantitative estimates of exposure levels and numbers of people exposed are presented. Therefore, this chapter should not be used as a source of data for enforcement purposes.

2.1. PHYSICAL/CHEMICAL PROPERTIES

1,3-Butadiene (CH_2=CH−CH=CH_2, CAS No. 106-99-0) is a colorless gas with mildly aromatic odor (Sax and Lewis, 1987). It is noncorrosive and has a molecular weight of 54.09. Its boiling point is −4.4°C (Weast, 1989) and its vapor pressure is 1,790 mm Hg (239 kPa) at 20°C (Santodonato, 1985). It is easily liquefied, with a density of 0.6211 g/mL at 20°C/liquefied (Kirshenbaum, 1978; Verschueren, 1983). It is soluble in ethanol, diethyl ether, and organic solvents (Verschueren, 1983; Sax and Lewis, 1987; Budavari, 1989) and very slightly soluble in water, with a solubility of 735 mg/L at 20°C. 1,3-Butadiene has a flash point of −76°C (Sax and Lewis, 1987), is slowly dimerized to 4-vinyl-1-cyclohexene (OSHA, 1990), and may form peroxide upon exposure to air (Kirshenbaum, 1978). Because 1,3-butadiene is a highly volatile gas, it is expected to partition predominantly to the atmosphere and then undergo rapid destruction by photoinitiated reactions. The reaction with photochemically produced hydroxyl radicals has a calculated half-life of approximately 6 hours and is expected to be the dominant pathway for atmospheric removal (U.S. DHHS, 1992). Destruction of atmospheric 1,3-butadiene by the gas-phase reaction with ozone and by the nighttime reaction with nitrate radicals in urban areas is also expected to be significant (U.S. DHHS, 1992). The major photooxidation products of 1,3-butadiene are acrolein and formaldehyde (Maldotti et al., 1980).

Data are limited on the fate of 1,3-butadiene in soil or water. Based on its physical properties, rapid volatilization of 1,3-butadiene from either soil or water to atmosphere is expected to dominate over all other potential environmental processes. Studies performed with pure cultures indicate that 1,3-butadiene may be susceptible to microbial attack. On the basis of estimated values, 1,3-butadiene is not expected to adsorb significantly to soil or sediment, nor is it expected to bioconcentrate in fish or aquatic organisms (U.S. DHHS, 1992).

2.2. PRODUCTION AND USE

1,3-Butadiene was first produced in 1886 by the pyrolysis of petroleum hydrocarbons (Kirshenbaum, 1978). Commercial production of 1,3-butadiene started in the 1930s (Kosaric et al., 1987) and has involved three processes: catalytic dehydrogenation of n-butane and n-butene,

oxidative dehydrogenation of n-butene, and recovery from the C_4 coproduct (by-product) stream from the steam-cracking process used to manufacture ethylene. The ethylene coproduct process accounts for approximately 95% of U.S. and 85% of worldwide production (Morrow, 1990). Approximately 12 billion pounds of this gas are produced annually worldwide and 3 billion pounds in the United States (Morrow, 1990; USITC, 1990).

1,3-Butadiene is used as an intermediate in the production of polymers, elastomers, and other chemicals. The major uses of this chemical are in the manufacture of styrene-butadiene (SB) rubber (synthetic rubber) and of thermoplastic resins. In 1990, 1,3-butadiene was used in the United States for SB rubber (30%), polybutadiene rubber (20%), adiponitrile/ hexamethylenediamine (15%), SB latex (10%), neoprene rubber (5%), acrylonitrile-butadiene-styrene resins (5%), exports (4%), nitrile rubber (3%), and other (including specialty polymers) (8%) (Anonymous, 1991).

2.2.1. SB Latex and Rubber Production

SB latex and rubber production is the major use for butadiene, accounting for 40% of butadiene consumption. SB latex and rubber are used for a variety of products, including automobile tires, textiles, paper, and adhesives.

The 1994 EPA report, *Locating and Estimating Air Emissions From Sources of 1,3-Butadiene*, lists SB latex and rubber production as the major contributor to industrial butadiene emissions (U.S. EPA, 1994a). About 74% of the industrial emissions are from SB latex and rubber production. At least 26 facilities in the United States produce SB latex and rubber (SRI International, 1993).

As stated previously, butadiene has very low water solubility and high vapor pressure; thus, if it were released to an aqueous waste stream, it would immediately evaporate. It is then logical to assume, and the data confirm, that the amount of butadiene found in secondary sources such as wastewater and solid waste is minimal or nonexistent. The majority of the butadiene releases during industrial production occur via process vents, so only emission factors for process vents will be presented. The emission factors, as presented in the 1994 EPA report for process-vent butadiene released during SB latex and rubber production, range from 0.00024 to 94.34 lb butadiene emitted per ton produced (mean of 7.10) measured at 18 facilities (U.S. EPA, 1994a).

2.2.2. Polybutadiene Production

The second largest use for butadiene is in production of polybutadiene, accounting for more than 20% of butadiene consumption. Polybutadiene is used in tire manufacturing and in the high-impact resin industry. Four companies at five locations in the United States currently produce polybutadiene. The estimate for process-vent butadiene emissions from polybutadiene

production, as stated in the 1994 EPA report, ranges from 0.00008 to 36.06 lb butadiene emitted per ton produced (mean of 6.14), measured at six facilities (U.S. EPA, 1994a).

2.2.3. Neoprene Rubber Production

Neoprene, or polychloroprene, rubber production accounts for 5% of butadiene consumption. Neoprene rubber is primarily used in the automotive industry for belts, cables, hoses, and wires. Three facilities currently produce neoprene, although only two use butadiene as a raw material and the other starts with chloroprene. The two facilities identified in the 1994 EPA report that used butadiene as a raw material yield estimated process-vent butadiene emissions from neoprene production ranging from 0.32 to 6.78 lb butadiene emitted per ton produced (mean of 4.04) (U.S. EPA, 1994a).

2.2.4. Acrylonitrile-Butadiene Resin Production

Acrylonitrile-butadiene (ABS) resins are used to make plastic components such as automotive parts, pipes and fittings, appliances, telephones, and business machines, among many other uses. ABS production accounts for 5% of butadiene consumption. Currently, 10 facilities produce ABS resin, only 6 of which use butadiene as a raw material. The estimate for process-vent butadiene emissions from ABS resin production ranges from 0.16 to 10.66 lb butadiene emitted per ton produced (mean of 4.22), measured at three facilities (U.S. EPA, 1994a).

2.2.5. Nitrile Elastomer Production

Nitrile elastomer or nitrile-butyl rubber is a specialty elastomer known for its resistance to oil solvents and chemicals. Some uses include the manufacture of hoses, belts, cables, seals, and gaskets. Nitrile elastomer is produced at nine facilities in the United States and accounts for about 5% of total butadiene consumption. The estimate for process-vent butadiene emissions from nitrile elastomer production ranges from 0.0004 to 17.80 lb butadiene emitted per ton produced, measured at six facilities identified in the 1994 EPA report (U.S. EPA, 1994a).

2.2.6. Adiponitrile Production

Adiponitrile (hexanedinitrile) is primarily an intermediate used in the production of nylon 6,6. Three facilities produce adiponitrile, but only two of these facilities use butadiene in production. This accounts for 12% of butadiene consumption. Despite the large usage of butadiene in adiponitrile production, emissions appear to be fairly small. The estimate for process-vent butadiene emissions from adiponitrile production, based on actual emissions reported at two facilities, is 0.12 lb butadiene emitted per ton produced (U.S. EPA, 1994a).

2.3. SOURCES AND EMISSION

1,3-Butadiene may be released to the environment as an intentional or fugitive emission during its production, use, storage, transport, or disposal. Its sources and emission to the environment can be classified as industrial production and use (1.6%), mobile sources (78.8%), and other miscellaneous combustion sources (19.6%) (U.S. EPA, 1994a).

Industrial butadiene emissions arise from process vents, equipment leaks, and secondary sources such as wastewater treatment. Because butadiene released to aqueous systems or entering treatment plants is likely to evaporate completely, all emissions of butadiene can be considered air emissions. Actual reported emissions of butadiene are available through the Toxic Release Inventory, and the relative contribution of butadiene production to the national butadiene emissions is 0.2% (U.S. EPA, 1994a). The EPA's 1996 Toxic Release Inventory report (U.S. EPA, 2001) summarized the on-site and off-site releases of 1,3-butadiene for all industries for 1988 and 1994–1999. Significant reduction of release has been achieved, as shown in Table 2-1.

2.3.1. Mobile Sources

Butadiene is formed as a product of incomplete combustion of fossil fuels and has been reported in the emissions from gasoline and diesel vehicles as well as aircraft. Emissions of butadiene from combustion sources are commonly represented as a weight percentage of total organic gas emissions. The relative contribution of mobile sources to the national butadiene emissions is 78.8%, which includes both on-road and nonroad engines. Levels of butadiene in gasoline and diesel fuel are expected to be insignificant, because butadiene tends to form a varnish that can be harmful to engines; therefore, refiners try to minimize the butadiene content. Because butadiene is not a component of gasoline, it is not present in mobile-source evaporative or refueling emissions and is found only in exhaust emissions (U.S. EPA, 1992).

It should be noted that a recent reevaluation by Nordlinder et al. (1996) of the Concawe report (1987) found that the concentrations of 1,3-butadiene in gasoline vapors were much lower than had been reported. Two analyses also found negligible amounts of 1,3-butadiene in gasoline vapors (Löfgren et al., 1991; Ramnas et al., 1994). When the investigators compared the concentrations of benzene and butadiene in gasoline, they found concentrations to be 3% to 5% and <0.0005%, respectively. On the basis of these three reports, Nordlinder et al. (1996) concluded that no significant amount of 1,3-butadiene is present in gasoline vapors.

2.3.1.1. *On-Road Mobile Sources*

On-road mobile sources include the following classes of vehicles: light-duty gasoline vehicles (LDGVs), light-duty gasoline trucks, heavy-duty gasoline trucks, light-duty diesel

Table 2-1. 1,3-Butadiene on-site and off-site releases (in pounds).

	On-site releases						Off-site releases	
Year	Fugitive or nonpoint air emissions	Stack or point air emissions	Surface water discharges	Underground injection	On-site land releases	Total on-site releases	Transfers off-site to disposal	Total on- and off-site releases
1988	4,059,260	2,945,362	522,504	1,500	7,817	7,536,443	185,398	7,721,841
1994	1,680,098	1,260,880	7,118	0	396	2,948,492	6,846	2,955,338
1995	1,436,010	1,613,728	5,393	0	277	3,055,408	4,892	3,060,300
1996	1,429,487	1,296,809	11,001	1,000	263	2,300,159	4,790	2,743,350
1998	1,435,139	1,303,975	8,834	732	7998	2,760,284	2,567	2,762,851
1999	1,911,696 for total air emission		1,900	720	127	1,914,443	2,571	1,917,014

vehicles, light-duty diesel trucks, heavy-duty diesel trucks, and motorcycles. On-road mobile sources account for 37.7% of national butadiene emissions.

Although data on the butadiene content of motor vehicle exhaust were lacking until the late 1980s, butadiene emissions from LDGVs are now reasonably well understood. As mentioned previously, butadiene is not a component of gasoline and is not present in evaporative or refueling emissions; thus, only exhaust butadiene emissions are included. Butadiene has been found to be removed effectively from motor vehicle exhaust by catalytic convertors (McCabe et al., 1992). Thus, nearly all on-road motor vehicle butadiene emissions come from older, noncatalyst vehicles; new vehicles with nonfunctional catalysts; cold-start emissions from catalyst vehicles; and diesel vehicles.

The emission factors calculated for all of the vehicles listed above range from 0.01 to 0.09 g/mile (U.S. EPA, 1994b). A composite emission factor of 0.0156 g/mile was calculated for the calendar year 1990 by the EPA Office of Mobile Sources using the MOBILE model. The composite emission factor represents all vehicles classes and is based on the percentage of total vehicle miles traveled (VMT) attributable to each vehicle class (U.S. EPA, 1993a).

2.3.1.2. *Nonroad Mobile Sources*

Nonroad mobile sources include mobile gasoline- and diesel-powered equipment and vehicles and other equipment types. Types of equipment included in this category range from construction, industrial, and agricultural equipment to small engines used in lawnmowers, chain saws, and other gasoline-powered equipment. Nonroad vehicles include motorcycles, snowmobiles, golf carts, all-terrain vehicles (ATVs) used for off-road recreation, and recreational and commercial marine vessels.

Generally, most nonroad engines are in use for many years and are noncatalyst engines. The lack of a catalyst, in conjunction with the engine deterioration associated with increased equipment age, may have profound effects on the amount of butadiene emitted. The EPA 1994 draft denotes that nonroad engines are expected to contribute 41% to the national butadiene emissions (U.S. EPA, 1994a).

2.3.1.3. *Aircraft*

Human exposure to aircraft emissions is considered to be limited to the emissions that occur during aircraft landing and takeoff (LTO). Airborne aircraft are assumed to fly at sufficiently high altitudes that their emissions do not reach the surface. This assumption is likely to be valid for butadiene because of its short atmospheric lifetime.

Butadiene has been reported in aircraft LTO emissions from military, commercial, and general aviation. Based on the EPA SPECIATE database, the butadiene weight percentages for

aircraft LTO hydrocarbon emissions range from 1.57% for general aviation (piston engines) to 1.89% from military aircraft (jet and piston engines). The 1994 EPA report estimates that 0.1% of the national butadiene emissions is attributable to aircraft LTO (U.S. EPA, 1994a).

2.3.2. Miscellaneous Sources

The miscellaneous sources of butadiene emissions are grouped as miscellaneous chemical production, secondary lead smelters, petroleum refining, and combustion sources (especially biomass burning). Emissions from these sources account for 19.6% of the national butadiene emissions.

2.3.2.1. *Miscellaneous Chemical Production*

The 1994 EPA report notes that butadiene is used to produce other elastomers and plastics not mentioned previously, as well as pesticides and fungicides at 19 separate facilities in the United States (U.S. EPA, 1994a). This process accounts for 8% of the butadiene use, but contributes only 0.1% to the national average butadiene emissions. The emission factors for process-vent butadiene released during miscellaneous chemical production range from 0.03 to 440 lb butadiene emitted per ton produced (product varies), measured at only four facilities.

2.3.2.2. *Secondary Lead Smelters*

Secondary lead smelting involves reclamation of scrap automobile batteries to produce elemental and lead alloys. The 23 such facilities in the United States are mostly located near large population centers. The plastic and rubber components of the battery are the source of the butadiene emissions, contributing 0.4% of the national butadiene emissions. The 1994 EPA report lists uncontrolled butadiene emissions measured from a blast furnace yielding an average emission factor of 0.79 lb/ton (U.S. EPA, 1994a).

2.3.2.3. *Petroleum Refining*

The 1992 Toxic Release Inventory (TRI) contains the emission factor 270,389 lb/year for petroleum refining. Using this emission factor would make this source one of the largest emitters of butadiene, contributing 0.2% to the national butadiene emissions. However, a recent TRI report indicates that the release of 1,3-Butadiene from petroleum refining has decreased significantly. The total air emission of this chemical for 1998 and 1999 was 150,705 lbs/yr and 144,222 lbs/yr respectively.

2.3.2.4. *Combustion Sources*

Butadiene is, as mentioned previously, a product of incomplete combustion and has been reported in the emissions from gasoline and diesel vehicles, as well as aircraft. Butadiene is also released during the combustion of tobacco, biomass, and automobile tires, and may be released from home heatings. However, only the combustion of biomass and automobile tires will be discussed in this section because of the scarcity of data.

2.3.2.4.1. ***Tire burning.*** A 1992 report (Lemieux and DeMarini, 1992) showed that approximately 240 million tires are discarded annually, of which only 25% are recycled. The remaining tires are discarded in landfills, stockpiles, or illegal dumps. Tires are combusted through accidental fires at stockpiles, illegal burning, tire-to-energy facilities, cement kilns, tire manufacturing facilities, and as a supplemental fuel in boilers. Butadiene is a major constituent of the tire manufacturing process and is present in emissions from uncontrolled tire burning. Emission factors have been calculated for the open burning of tires (U.S. EPA, 1992; Lemieux and DeMarini, 1992). These emission factors range from 234.28 lb per 1,000 tons of chunk tires to 277.95 lb per 1,000 tons of shredded tires. No emission factor for butadiene from tire incineration has been located. The Rubber Manufacturers Association (RMA) recently indicated that annual scrap tire generation is now in the range of 260 to 270 million units per year (RMA, 1998). RMA claims that approximately 75% of annual scrap tire generation is now directed to markets that make beneficial use of the scrap tire. Of the remaining 25%, most is directed to legal landfilling or monofilling. Only a small number of tires are stockpiled or illegally dumped. RMA also suggests, based on a 1994 EPA study (U.S. EPA, 1994a), that butadiene emissions from the use of tires as a fuel in a well-controlled facility are negligible.

2.3.2.4.2. ***Biomass burning.*** Biomass burning includes residential wood combustion in both fireplaces and wood stoves, open burning such as backyard burning of yard waste, slash burning, land clearing/burning, agricultural burning, forest fires/prescribed burning, structural fires, and other wildfires. Although these fires differ in many important characteristics, the fuels in all cases are composed primarily of wood. The relative contribution of biomass burning to the overall national butadiene emissions was calculated at 18.8% in the 1994 EPA report (U.S. EPA, 1994a).

Emission factor models based on field and laboratory data were developed by the U.S. Forest Service (Peterson and Ward, 1989). These models incorporate variables such as fuel type and combustion type (flaming or smoldering) and correlate butadiene emissions with carbon monoxide emissions to develop emission factors for biomass burning (Campbell and Mangino,

1994). The calculated emission factors range from 0.40 lb/ton of yard waste burned to 0.90 lb/ton for large wood burning in forest fires and prescribed burning.

Butadiene emissions have been reported from the combustion of wood (Sandberg et al., 1975; Ward and Hao, 1992). The data of Ward and Hao (1992), in which both butadiene and benzene were quantified from biomass burning, provide a butadiene:benzene ratio of 0.36 for wood smoke.

2.4. AMBIENT CONCENTRATION OF 1,3-BUTADIENE

2.4.1. Air

The dominant sources for the release of 1,3-butadiene to the atmosphere are fugitive or accidental emissions during its manufacture, use, transport, and storage. Low levels of 1,3-butadiene are constantly emitted to the atmosphere from many sources, including exhaust from motor vehicle engines using petroleum-based fuels (ATSDR, 1991).

2.4.1.1. *Ambient Monitoring Data*

Several EPA databases contain results of various air toxics monitoring programs. These programs have set up monitoring devices that are used to collect air samples all over the United States over a period of months or years. Three of these programs/databases contain data on 1,3-butadiene. This section summarizes the three monitoring programs and presents annual average concentrations of 1,3-butadiene derived from these programs.

One of these programs is the Aerometric Information Retrieval System (AIRS), which became operational in 1987 and uses a network of monitoring stations called the State and Local Air Monitoring System (SLAMS) (U.S. EPA, 1989a). This network consists of monitoring stations set up by every State in accordance with regulations promulgated in response to requirements of the Clean Air Act. EPA's Office of Air Quality Planning and Standards (OAQPS) administers the AIRS program.

The AIRS program allows State and local agencies to submit local air pollution data and also have access to national air pollution data (U.S. EPA, 1989a). EPA uses data from AIRS to monitor the States' progress in attaining air quality standards for ozone, carbon monoxide, nitrogen oxides, sulfur oxides, and lead through the use of State Implementation Plans (SIPs). In addition to containing information about each monitoring site, including its geographic location and operator, AIRS contains extensive information on ambient levels of many toxic compounds. The AIRS catalogs ambient air pollution data from 18 to 55 monitors in 15 to 23 urban areas, depending on the pollutant. These monitors collect a 24-hour sample every 12 days. However, in some cases not every target compound was detected in every sample. Where this occurred, half the minimum detection limit was used to average the data for this summary. The annual

average ppb for each site was calculated using only those sites that provided four quarters of monitoring data. The cities monitored and the average concentrations determined at each monitoring site are listed in Table 2-2A for 1988 to 1994 and in Table 2-2B for 1992 to 1994.

Another air monitoring program is the Urban Air Toxic Monitoring Program (UATMP), which EPA developed in 1987 to assist State and local agencies in determining the nature and extent of urban air toxic pollution (McAlister et al., 1989, 1990, 1991; Wijnberg and Faoro, 1989). Data from the UATMP also are used in air toxic risk assessment models (U.S. EPA, 1989b,c; 1990a,b). In 1989, the UATMP had 14 monitors in 12 urban areas, and in 1990, the UATMP had 12 monitors in 11 urban areas, of which 9 also participated in the 1989 monitoring program.

In 1989 and 1990, the UATMP network simultaneously monitored 37 nonmethane organic compounds, selected metals, benzo(a)pyrene (1989 only), formaldehyde, acetaldehyde, and acetone for a 24-hour period once every 12 days. The UATMP database lists the data collected from the monitoring network using two methods. In the first method, only the concentrations above the detection limit of the compound are included in the data. In the second method, if the concentration of a compound is below the detection limit, then one-half of the compound's detection limit is incorporated into the data. The second method was used because it seemed more reasonable and allowed a greater number of samples to be averaged. Data collected in 1989 and 1990 were used in this summary. The cities monitored and the average concentrations determined at each monotoring site can be found in Table 2-3.

Monitoring data for the UATMP collected from 1991 to 1994 have not yet been released as separate reports. The data collected in those 4 years were entered into and reported as part of the 1991-1994 AIRS database.

The National Ambient Volatile Organic Compounds (NAVOC) database contains approximately 175,000 records on the concentrations of 320 volatile organic compounds (VOCs) observed in 1-hour air samples taken every 24 hours between 1970 and 1987 (Shah and Heyerdahl, 1988; Hunt et al., 1988). However, only the most current NAVOC data, taken during 1987, are used in this summary. In averaging data for this summary, samples that had nondetects of 1,3-butadiene were included as one-half the detection limit. The NAVOC database includes air samples collected using indoor and outdoor monitoring devices. Personal monitors were also used. The types of locations of outdoor monitoring sites included remote, rural, suburban, and urban areas, as well as near specific point sources of VOCs. Indoor monitoring sites consisted of nonindustrial workplaces and residential environments. Personal monitors also are included in the indoor category. This database was an interim precursor to the air toxics portion of AIRS. For this summary, only the outdoor urban data were used. The cities monitored and the average concentrations determined at each monitoring site can be found in Table 2-4.

Table 2-2A. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1988 to 1991.

Sampling site	Land use of monitor location	Average concentration (ppb)				Number of samples			
		1988	1989	1990	1991	1988	1989	1990	1991
Washington, DC	Commercial/urban & center city		0.13				7		
Ft. Lauderdale, FL	Commercial/urban & center city		0.29				29		
Miami, FL	Commercial/urban & center city		0.14				31		
Miami, FL	Commercial/urban & center city		0.19				6		
Miami, FL	Commercial/urban & center city		0.14				6		
Miami, FL	Commercial/urban & center city		0.13				6		
Chicago, IL	Commercial/urban & center city		0.10				10		
Chicago, IL	Residential/suburban		0.29				25		
Chicago, IL	Mobile/urban & center city		0.73				9		
St. Clair Co., IL	Industrial/suburban		0.22				29		
St. Clair Co., IL	Industrial/Suburban		0.31				4		
St. Clair Co., IL	Industrial/suburban		0.30				4		
St. Clair Co., IL	Industrial/suburban		0.25				4		
Wichita, KS	Residential/suburban		0.13				29		
Wichita, KS	Residential/urban & center city		0.16				8		
Louisville, KY	Commercial/urban & center city	0.44				6			
Baton Rouge, LA	Commercial/urban & center city		0.43				29		
Baton Rouge, LA	Commercial/urban & center city		0.41				4		
Baton Rouge, LA	Commercial/urban & center city		0.53				4		

Table 2-2A. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1988 to 1991 (continued).

Sampling sites	Land use of monitor location	Average concentration (ppb)				Number of samples			
		1988	1989	1990	1991	1988	1989	1990	1991
Baton Rouge, LA	Commercial/urban & center city		0.37				4		
Detroit, MI	Commercial/urban & center city			0.33	0.07			19	28
St. Louis, MO	Commercial/urban & center city		0.12				28		
Camden, NJ	Residential/suburban		0.23				29		
Camden, NJ	Residential/suburban		0.16				4		
Camden, NJ	Residential/suburban		0.18				4		
Camden, NJ	Residential/suburban		0.15				4		
Newark, NJ	Industrial/urban & center city		0.26				9		
Plainfield, NJ	Residential/suburban		0.20				9		
New York, NY	Residential/urban & center city		0.25				9		
New York, NY	Commercial/urban & center city		0.29				9		
Dallas, TX	Commercial/urban & center city		0.11				23		
Houston, TX	Residential/suburban	1.11	0.60	0.72		6	30	4	
Burlington, VT	Commercial/urban & center city	0.47				6			
Arlington Co., VA	Commercial/urban & center city			0.20	0.11			13	18
Henrico Co., VA	Residential/suburban			0.16	0.12			21	12
Hampton, VA	Residential/suburban			0.22	0.11			14	22
Hopewell, VA	Residential/suburban			0.13	0.06			16	15
Roanoke, VA	Residential/suburban			0.24	0.12			14	22
	1,3-Butadiene Average								
		0.67	0.26	0.29	0.10				

Table 2-2B. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1992 to 1994.

Sampling sites	Land use of monitor location	Average concentration (ppb)			Number of samples		
		1992	1993	1994	1992	1993	1994
Jefferson Co., AL	Residential/rural		0.12	0.33		83	79
Jefferson Co., AL	Residential/rural			0.19			9
Tarrant City, AL	Residential/suburban		4.32	0.78		82	81
Tarrant City, AL	Residential/suburban			0.91			10
Shelby Co., AL	Agricultural/rral		0.07	0.15		50	78
Fresno, CA	Residential/suburban		0.30	0.30		30	31
Clovis, CA	Residential/urban & center city			0.54			111
Bakersfield, CA	Residential/urban & center city		0.28	0.27		30	9
Bakersfield, CA	Commercial/urban & center city			0.35			23
Bakersfield, CA	Commercial/urban & center city			0.63			105
Los Angeles, CA	Residential/urban & center city		0.65	0.53		26	30
Roseville, CA	Mobile/suburban		0.16	0.15		23	31
Citris Heights, CA	Residential/suburban		0.41			6	
Sacramento, CA	Residential/suburban			0.53			84
El Cajon, CA	Commercial/suburban		0.34	0.27		28	28
Simi Valley, CA	Residential/suburban		0.18	0.17		28	29
Washington, DC	Commercial/urban & center city		0.36	0.28		10	16
Washington, DC	Commercial/urban & center city		0.32	0.46		13	15
Chicago, IL	Industrial/urban & center city		0.09	0.18		6	14

Table 2-2B. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1992 to 1994 (continued).

		Average concentration (ppb)			Number of samples		
Sampling sites	**Land use of monitor location**	**1992**	**1993**	**1994**	**1992**	**1993**	**1994**
Lemont, IL	Residential/suburban		0.06	0.08		15	13
St. Clair Co., IL	St. Louis metro area		0.10	0.11		10	12
Kansas City, KS	Industrial/urban & center city		0.17	0.26		11	13
Baton Rouge, LA	Commercial/urban & center city		0.42	0.43		14	8
Glen Burnie, MD	Commercial/suburban		0.15	0.22		60	54
Essex, MD	Residential/suburban		0.25	0.26		39	58
Baltimore, MD	Residential/suburban	0.18	0.17		56	59	
Baltimore, MD	Industrial/suburban	0.09			21		
Baltimore, MD	Industrial/urban & center city	0.10	0.12		50	50	
Baltimore, MD	Residential/urban & center city	0.26	0.25	0.28	58	57	48
Baltimore, MD	Industrial/urban & center city	0.09	0.10		39	48	
Alma, MI	Commercial/rural	0.45	0.05		22	14	
Portage, MI	Industrial/suburban	0.06	0.08		25	4	
Midland, MI	Commercial/rural	0.08	0.09	0.07	56	61	60
Midland, MI	Commercial/rural	0.07	0.07	0.07	55	61	22
Midland, MI	Industrial/rural	0.07	0.07	0.07	55	61	61
Midland, MI	Agricultural/rural	0.07	0.07	0.07	55	61	61
Midland, MI	Industrial/rural	0.07	0.08	0.07	55	60	61
Midland, MI	Residential/suburban	0.07	0.07	0.07	28	31	11
Midland, MI	Residential/rural	0.07	0.07	0.07	28	30	11
Detroit, MI	Commercial/urban & center city	0.72	0.08		24	15	

Table 2-2B. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1992 to 1994 (continued).

Sampling sites	Land use of monitor location	Average concentration (ppb)			Number of samples		
		1992	1993	1994	1992	1993	1994
Camden, NJ	Residential/suburban		0.26	0.49		14	13
Newark, NJ	Industrial/urban & center city		0.63	0.73		8	9
Plainfield, NJ	Residential/suburban		0.72	0.79		8	8
Nassau Co., NY	Commercial/suburban			0.70			9
Philadelphia, PA	Residential/suburban		0.19			38	
San Antonio, TX	Residential/suburban			0.08			21
Clute, TX	Residential/suburban		0.24	0.37		29	53
Brownsville, TX	Commercial/urban & center city		0.18	0.21		7	15
Brownsville, TX	Commercial/urban & center city		0.29	0.37		19	59
Dallas, TX	Commercial/urban & center city		0.32			82	
Dallas, TX	Industrial/rural		0.20	0.37		34	58
Odessa, TX	Residential/suburban		0.41	1.68		39	59
Midlothian, TX	Agricultural/rural		0.18	0.37		39	51
El Paso, TX	Commercial/urban & center city		0.45	0.43		14	15
El Paso, TX	Commercial/urban & center city		0.98	1.16		79	79
El Paso, TX	Commercial/urban & center city			1.49			9
El Paso, TX	Commercial/suburban		0.83	1.29		8	4
El Paso, TX	Residential/suburban		0.21	0.38		22	58
Texas City, TX	Residential/urban & center city	0.29	0.22	1.54	5	35	51
Texas City, TX	Residential/suburban		0.35	0.29		12	15
Harris Co., TX	Agricultural/suburban	0.19	0.30	0.56	5	28	59

Table 2-2B. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) for 1992 to 1994 (continued).

		Average concentration (ppb)			Number of samples		
Sampling sites	**Land use of monitor location**	**1992**	**1993**	**1994**	**1992**	**1993**	**1994**
Houston, TX	Industrial/suburban		0.44	0.81		39	54
Houston, TX	Industrial/suburban		0.24	0.64		33	58
Houston, TX	Industrial/suburban	0.05	0.39	0.73	6	37	58
Beaumont, TX	Residential/suburban		0.56			78	
Beaumont, TX	Residential/suburban	0.05	0.25	0.37	7	36	59
Port Arthur, TX	Residential/suburban	0.10	0.23	0.44	6	45	57
Port Neches, TX	Industrial/suburban		0.32	0.43		18	58
Port Neches, TX	Residential/urban & center city			5.95			23
Port Neches, TX	Residential/suburban		5.22	6.11		11	14
Corpus Christi, TX	Commercial/suburban		0.18	0.37		39	52
West Orange, TX	Residential/suburban			0.18			27
Smith Co., TX	Mobile/rural			0.33			32
Fort Worth, TX	Commercial/urban & center city		0.20	0.57		80	77
Fort Worth, TX	Commercial/urban & center city			0.80			8
Fort Worth, TX	Commercial/suburban			0.06			9
Grapevine, TX	Residential/urban & center city			0.05			15
Austin, TX	Commercial/urban & center city			0.13			21
Burlington, VT	Commercial/urban & center city		0.31	0.49		13	15
Rutland, VT	Commercial/urban & center city		0.15	0.44		13	15
Waterbury, VT	Commercial/suburban		0.08	0.09		14	15
		1,3-butadiene average concentrations across sites by year					
		0.16 ppb	0.40 ppb	0.59 ppb			

Table 2-3. Summary of 1,3-butadiene ambient data from the Urban Air Toxics Monitoring Program (UATMP).

Sampling sites	Land use of monitor location	Sampling time/frequency	Average concentration (ppb)[a]		Detected/total	
			1989	1990	1989	1990
Baton Rouge, LA	Urban/center city-commercial	24-hour/every 12 days	0.39	0.36	11/31	8/29
Chicago, IL	Suburban-residential	24-hour/every 12 days	0.24	0.06	10/27	4/29
Camden, NJ	Suburban-residential	24-hour/every 12 days	0.20	0.10	19/32	9/30
Dallas, TX	Urban/center city-commercial	24-hour/every 12 days	0.08		8/25	
Fort Lauderdale,	Urban/center city-commercial	24-hour/every 12 days	0.20		18/31	
Houston, TX	Suburban-residential	24-hour/every 12 days	0.60	0.47	23/34	111/28
Miami, FL	Urban/center city-commercial	24-hour/every 12 days	0.11		7/33	
Pensacola, FL	Suburban-industrial	24-hour/every 12 days		0.06		6/42
St. Louis, MO	Urban/center city-commercial	24-hour/every 12 days	0.09		12/30	
Sauget, IL	Suburban-industrial	24-hour/every 12 days	0.20	0.06	7/31	2/27
Washington, DC-1	Urban/center city-commercial	24-hour/every 12 days	0.11	0.10	9/27	11/30
Washington, DC-2	Urban/center city-commercial	24-hour/every 12 days	0.29	0.15	19/27	12/27
Wichita, KS-1	Urban/Center City-/Residential	24-hour/every 12 days	0.16	0.06	10/31	1/30
Wichita, KS-2	Suburban-residential	24-hour/every 12 days	0.09		7/31	
Port Neches, TX	Suburban-residential	24-hour/every 12 days		11.09		24/28
Orlando, FL	Urban/center city-commercial	24-hour/every 12 days		0.10		8/28
Toledo, OH	Suburban-residential	24-hour/every 12 days		0.06	4/21	
			1,3-butadiene average			
			0.21	1.02		

[a]The arithmetic average concentration of all samples using half minimum detection limit (MDL) value for samples in which compound was not found.
[b]Calculated by averaging all 390 samples taken from 13 sites equally in 1989 and 349 samples from 12 sites in 1990.

Table 2-4. Summary of outdoor urban data from the National Ambient Volatile Organic Compounds (NAVOC) Database.

Sample years	Average concentration used (ppb)[a]	Number of samples	Sampling site[b]	Land use of monitor location
9/22/87 to 10/4/87	0.30	2	Bakersfield, CA	Urban
9/16/87 to 9/28/87	0.35	2	Concord, CA	Urban
10/4/87 to 10/4/87	0.60	1	Fremont, CA	Urban
10/4/87 to 10/4/87	0.40	1	Richmond, CA	Urban
9/9/87 to 10/7/87	0.25	2	San Jose, CA	Urban
9/29/87 to 9/27/87	0.30	1	Stockton, CA	Urban

[a]Overall 1,3-butadiene average concentration: 0.344 ppb. Calculated by averaging all nine samples taken from six cities equally.

[b]Number of samples/sampling time equals 1/24 hours.

Table 2-5 summarizes the average concentrations (in ppb) of 1,3-butadiene found at the monitoring sites of each air monitoring program. The table also shows the total number of observations for each average and the number of sites that monitored the compounds in each program. For AIRS, the average concentrations of 1,3-butadiene are listed separately for 1987 through 1994. Some of the highest averages in the AIRS database were from suburban residential sites in Houston and Port Neches, TX. Both of these cities have high point-source emissions that could be affecting the monitor. The AIRS and UATMP data from Houston and Port Neches were excluded to create alternate annual averages (ppb and $\mu g/m^3$) for the years 1988 through 1994 (where applicable) and are presented in Table 2-5. This alternate annual average may be more representative of areas that are not near strong point sources.

Tables 2-6, 2-7, and 2-8 regroup and summarize Tables 2-2, 2-3, and 2-4 according to the sampling locations, that is, rural, suburban, or urban settings. The data obtained from Port Neches were not included in these averages because of the elevated levels caused by industrial emissions.

It should be noted that methods of averaging the data are not consistent between the air monitoring databases. Also, in the NAVOC monitoring network, samples were taken for 1 hour in a 24-hour period, whereas the other monitoring networks collected a 24-hour air sample every 12 days.

It also should be noted that the ambient levels detected in these three databases are not completely representative of an individual's actual exposure to 1,3-butadiene. Ideally, exposure would be estimated on the concentrations in microenvironments and the times that people spend in these microenvironments.

2.4.1.2. *Ambient Source Apportionment*

Measured concentrations in ambient air include contributions from a variety of source categories. If sufficient information is available, such ambient monitoring data can be adjusted to represent the amount attributed to a particular source using emissions inventory apportionment. Three studies attempt to apportion sources according to their contribution to ambient levels of 1,3-butadiene (U.S. EPA, 1994a; CARB, 1992; Ligocki, 1993). The studies assume that all emissions to the atmosphere contribute proportionally to ambient concentration. These three studies are summarized in Table 2-9. As observed in Table 2-9, the source apportionment conducted by Systems Applications International for the American Automobile Manufacturers Association (Ligocki, 1993) contains the category of biomass burning as a large part of the inventory. The weight percentage of butadiene in total organic gases (TOG) for emissions from residential wood combustion, open burning, forest fires, and other burning that are used in this analysis are derived from a single estimate provided in EPA's SPECIATE

Table 2-5. Summary of air monitoring program results for 1,3-butadiene.

	Annual average ppb (μg/m^3)	Alternate annual average[a] ppb (μg/m^3)
AIRS		
1988 level		
Conc.	0.67 (1.48)	0.46 (1.02)[b]
# Observation	18	12
# Sites	3	2
1989 Level		
Conc.	0.23 (0.57)	0.25 (0.55)[b]
# Observation	399	369
# Sites	30	29
1990 Level		
Conc.	0.29 (0.64)	0.21 (0.46)[b]
# Observation	101	97
# Sites	7	6
1991 Level		
Conc.	0.10 (0.22)	--[c]
# Observation	117	
# Sites	6	
1992 Level		
Conc.	0.16 (0.40)	0.16 (40)[b]
# Observation	656	650
# Sites	20	19
1993 Level		
Conc.	0.40 (0.88)	0.32 (0.71)[d]
# Observation	2069	1,931
# Sites	64	59
1994 Level		
Conc.	0.59 (1.30)	0.42 (0.92)[d]
# Observation	2666	2,401
# Sites	70	64

Table 2-5. Summary of air monitoring program results for 1,3-butadiene (continued).

	Annual average ppb ($\mu g/m^3$)	Alternate annual average[a] ppb ($\mu g/m^3$)
UATMP		
1989 Level		
Conc.	0.21 (0.46)	--[e]
# Observation	390	
# Sites	13	
1990 Level		
Conc.	1.02 (2.25)	0.12 (0.27)[b]
# Observation	349	293
# Sites	12	10
NAVOC		
1987 Level		
Conc.	0.34 (0.75)[f]	no data
# Observation	9	
# Sites	6	

[a]Alternate averages do not include data from Houston and Port Neches, TX, due to impacts from strong point sources.
[b]Average ppb from all 4-quarter data sites, excluding Houston, TX.
[c]Houston, TX, was not monitored during this 4-quarter period.
[d]Average ppb from all sites, excluding Houston and Port Neches, TX.
[e]Port Neches, TX, was not monitored during this 4-quarter period.
[f]All urban California sites.

Table 2-6. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) (Table 2-2A and 2-2B) based on sampling locations.

	Rural area			Suburban area			Urban area		
Year	Range	Average[a]	Total samples/ number of locations	Range	Average	Total samples/ number of locations	Range	Average	Total samples/ number of locations
1988				--	1.11	6/1	0.44-	0.46	12/2
1989				0.13-0.60	0.25	151/12	0.10-	0.27	237/18
1990				0.16-0.72	0.29	95/5	0.20-	0.27	32/2
1991				0.06-0.12	0.10	71/4	0.07-	0.09	46/2
1992	0.07-0.45	0.13	271/6	0.05-0.19	0.10	154/7	0.09-	0.29	176/5
1993	0.05-0.20	0.10	494/10	0.06-4.32	0.41	864/31	0.08-	0.31	580/21
1994	0.07-0.37	0.18	522/11	0.06-1.68	0.45	1135/32	0.05-	0.62	780/24

[a]1,3-Butadiene average concentration in ppb.

Table 2-7. Summary of 1,3-butadiene data from Table 2-3 based on sampling locations.

	Suburban			Urban		
Year	Range	Average [a]	Total samples/ number of locations	Range	Average	Total samples/number of locations
1989	0.09-0.60	0.27	155/5	0.08-0.39	0.18	235/8
1990	0.06-0.47	0.14	177/6	0.06-0.36	0.15	144/5

[a]1,3-Butadiene average concentration in ppb.

Table 2-8. Summary of 1,3-butadiene data from Table 2-4 based on sampling locations.

Urban			
Year	**Range**	**Average [a]**	**Total samples/number of locations**
1987	0.25 - 0.60	0.37	9/6

[a]1,3-Butadiene average concentration in ppb.

Table 2-9. Summary of the relative contributions to ambient 1,3-butadiene emissions given as percentage of total mg/year.

Study	Mobile sources[a]	Stationary point and area sources[b]	Aircraft	Biomass burning[c]
EPA, 1994a	78.7	2.4	0.1	18.8
CARB, 1992	96[d]	4	Included under mobile sources	ND[e]
Ligocki, 1993	57	5	3	35

[a]Mobile sources include on-road and off-road vehicles and generally exclude trains and aircraft.
[b]Area and point sources generally include all manufacturing and industrial process, oil and gas production facilities, commerce, residential fuel combustion, and other stationary fuel combustion.
[c]Biomass burning includes residential wood combustion, incineration, and other biomass burning.
[d]The CARB off-road apportionment of mobile sources includes trains and aircraft.
[e]ND=not determined.

database. An actual measurement of 1,3-butadiene TOG weight percentage for incineration of wood was not found in the literature; therefore, the solid waste incineration TOG weight percentage was used. A great deal of uncertainty is connected with the 1,3-butadiene emission estimates that were developed for the biomass burning as well as for emissions from aircraft. Many of the limitations revolve around the lack of real-world data on actual 1,3-butadiene emissions and exposures for the scenarios mentioned above, as well as the allocation of these scenarios nationwide. The emissions from residential wood combustion and forest fires vary by season and region of the country. The mobile source and stationary source emissions would, for the most part, remain constant throughout much of the year.

2.4.2. Indoor Exposure to 1,3-Butadiene

Information on 1,3-butadiene concentrations in homes or public buildings is limited at this time. Indoor concentrations of 1,3-butadiene depend primarily on the presence of environmental tobacco smoke (ETS) (CARB, 1992). Several studies indicate that on average most individuals spend anywhere from about 60% to 70% of their time each day indoors at their residence (Robinson et al., 1989; U.S. EPA, 1993b). In addition, individuals also spend a lot of time at indoor workplaces. This makes indoor air a major route of exposure to 1,3-butadiene for individuals who are exposed to tobacco smoke. It is also apparent that the potential for indoor exposure can exceed outdoor exposure if ETS is taken into consideration. Löfroth et al. (1989) and Brunnemann et al. (1990) measured 1,3-butadiene emissions in sidestream smoke ranging from 200 to 400 μg/cigarette and 1,3-butadiene levels in smoke-filled bars ranging from 2.7 to

19 μg/m^3. As discussed in Section 2.4.5, heating of cooking oils can release butadiene to the indoor air. Fugitive emissions from wood stoves or fireplaces could also release butadiene to the indoor air. Further research and measurements are needed to quantify typical indoor 1,3-butadiene exposures.

2.4.3. Inside Automobile

The 1993 Motor Vehicle Related Air Toxics Study (U.S. EPA, 1993a) reported that a short-term microenvironment exposures study revealed that the in-vehicle exposure level of 1,3-butadiene was determined to have a mean of 3.0 μg/m^3 and a maximum measured level of 17.2 μg/m^3. Similar levels were found in the air surrounding moving vehicles. This is based on measurements made by a monitor mounted on top of a moving vehicle that showed a mean of 3.0 μg/m^3 and a maximum of 6.9 μg/m^3.

2.4.4. Water

The presence of 1,3-butadiene in drinking water in the United States has been noted in the literature, but no concentrations or frequency of detection are available (U.S. EPA, 1978; Kraybill, 1980).

2.4.5. Food

Certain cooking oils release butadiene to the air on heating. This probably occurs primarily by volatilization, but could also involve formation due to low levels of combustion. For example, 1,3-butadiene emissions are approximately 22-fold higher from unrefined Chinese rapeseed oil than from heated peanut oil. Of three fatty acids tested, heated linolenic acid produced the greatest amount of 1,3-butadiene. Although cooking oils in the United States are refined for purity, U.S. rapeseed oil (canola) also emitted 1,3-butadiene (Shields et al., 1995). Also, levels of <0.2 μg/kg 1,3-butadiene were found in retail soft margarine; the plastic tubs containing the margarine contained from less than 5 μg/kg to less than 310 μg/kg (Startin and Gilbert, 1984).

2.5. MODELED ON-ROAD MOBILE SOURCE BUTADIENE EXPOSURE ESTIMATES

A recently published study by the EPA Office of Mobile Sources discussed the assessment of motor vehicle air toxics emissions and exposure for benzene, acetaldehyde, formaldehyde, 1,3-butadiene, methyl tributyl ether (MTBE), and diesel particulate (U.S. EPA, 1999a). The analysis reflects emissions from only on-road motor vehicles (highway vehicles only) located in nine urban areas: Chicago, Denver, Houston, Minneapolis, New York City, Philadelphia, Phoenix, Spokane, and St. Louis. Modeling was performed for 1990, 1996, 2007,

and 2020, and separate estimates were prepared for winter, spring, summer, and fall. The forecast years include both base and control scenarios.

The results of the exposure analysis for 1,3-butadiene from on-road vehicle emissions are presented in Table 2-10 for all nine urban areas. The fleet-average annual average toxics exposures in the table show significant reductions between 1990 and 2020 with no further vehicle or fuel controls. This is because of fleet turnover resulting in full implementation of the Federal emission control regulations currently in place.

Exposure estimates also were prepared for three different demographic groups: total population, outdoor workers, and children 0-17 years of age. (The estimates given in Table 2-10 are for the total population.) This study found that exposure to air toxics for outdoor workers is generally about 20% higher than for the total population, whereas exposure for children is typically slightly below the total population. This information appears in Table 2-11, which shows the annual average 1,3-butadiene exposure for the three demographic groups analyzed in this study for Chicago under the base control scenario. As seen in the table, 1,3-butadiene exposure is highest for outdoor workers, whereas children and the total population show similar results.

2.6. CONCLUSIONS ABOUT PATHWAYS OF EXPOSURE

Although 1,3-butadiene undergoes rapid destruction in the atmosphere, it is almost always present at very low concentrations (generally in the range of 0.1 to 1.0 ppb) in urban and suburban areas. This is primarily because vehicle exhaust provides a continuous source of 1,3-butadiene release to the atmosphere. Although the data are limited, it appears that butadiene levels in other environmental media and food are very low. On this basis it is generally assumed that inhalation is the dominant route of human exposure.

Butadiene has been measured in cigarette smoke and therefore smokers are likely to have elevated inhalation exposures over nonsmokers. Indoor inhalation exposure may be elevated over outdoor levels because of butadiene emissions from cigarette smoke, wood burning, and cooking operations involving some oils. Elevated inhalation exposures may occur to individuals living near butadiene production facilities or facilities where it is made into polymeric materials. Inhalation exposures are also likely to be higher inside vehicles and near roadways than in other locations. Better data are needed on butadiene levels in these microenvironments (i.e., vehicles and residences) in order to more accurately estimate total exposure for the general population.

Table 2-10. Annual-average exposure results (μg/m^3) for 1,3-butadiene, total population - all on-road vehicles, base control scenario.

Area	Calendar year			
	1990	1996	2007	2020
Chicago	0.100	0.057	0.028	0.025
Denver	0.139	0.104	0.049	0.045
Houston	0.081	0.060	0.031	0.027
Minneapolis	0.190	0.122	0.082	0.089
New York	0.229	0.123	0.063	0.048
Philadelphia	0.130	0.073	0.028	0.023
Phoenix	0.150	0.112	0.045	0.044
Spokane	0.186	0.126	0.055	0.046
St. Louis	0.076	0.071	0.030	0.028

Table 2-11. Annual average exposure results (μg/m^3) for 1,3-butadiene in Chicago by demographic group - all on-road vehicles, base control scenario.

Demographic group	Calendar year			
	1990	1996	2007	2020
Total population	0.100	0.057	0.028	0.025
Outdoor workers	0.120	0.069	0.034	0.030
Children 0-17 years	0.098	0.056	0.028	0.024

Occupational exposure to 1,3-butadiene is expected to occur at facilities that manufacture 1,3-butadiene or convert it into commercial polymers. Toll collectors, bus drivers, or other workers who spend extended time periods near roadways are likely to have higher exposures than the general population.

3. PHARMACOKINETICS

Data from both in vitro and in vivo studies have established that metabolites, not the parent compound, cause the toxic effects of exposure to 1,3-butadiene. Because differences have been noted in the toxic responses to 1,3-butadiene among laboratory species, the pharmacokinetics of 1,3-butadiene and its metabolites are important in assessing the risk of cancer and reproductive and developmental effects associated with exposure to this chemical. The U.S. Environmental Protection Agency (U.S. EPA, 1985) and the International Agency for Research on Cancer (IARC, 1986) previously have reviewed the pharmacokinetics of 1,3-butadiene. This chapter summarizes the recent research that has provided information on the pathways and rates of metabolism of 1,3-butadiene, via both in vitro and in vivo studies, and the in vivo disposition of 1,3-butadiene in several animal species, including molecular dosimetry and urinary metabolites from occupationally exposed humans. This chapter is divided into sections according to the various components of pharmacokinetics: absorption, biotransformation, distribution of 1,3-butadiene and its metabolites, and excretion. In addition, a section is included for the structurally similar compounds isoprene and chloroprene. The research summarized in this chapter supports species differences in pharmacokinetics that may contribute to, but not be solely responsible for, species differences in the toxic responses from exposure to 1,3-butadiene.

The chemical terminology used in the publications reviewed in this chapter has been standardized for consistency. Epoxybutene (EB) is used for 1,3-butadiene monoepoxide, 1,3-butadiene monoxide, 1,2-epoxybutene-3, 1,2-epoxy-3-butene, vinyl oxirane, and 3,4-epoxy-1-butene; diepoxybutane (DEB) is used for 1,2:3,4-diepoxybutane, butadiene diepoxide, and butadiene bisoxide; butenediol (BDdiol) is used for 1,2-dihydroxybut-3-ene and 3-butene-1,2-diol; and epoxybutanediol (EBD) for 3,4-epoxybutanediol, 3,4-epoxybutane-1,2-diol, 3,4-epoxy-1,2-butanediol, and 1,2-dihydroxy-3,4-epoxybutane.

3.1. OVERVIEW OF PHARMACOKINETIC STUDIES

In brief, the primary route of exposure to 1,3-butadiene is via inhalation; therefore, absorption occurs through the respiratory system. 1,3-Butadiene is then widely distributed throughout the body. The first step of metabolism is primarily oxidation of 1,3-butadiene to EB. EB can then be further oxidized to DEB, hydrolyzed to BDdiol, or conjugated with glutathione. DEB can then also be hydrolyzed to EBD or conjugated with glutathione, while BDdiol can be further oxidized to EBD. EBD can be hydrolyzed or conjugated with glutathione. These various metabolites also appear to be well distributed in the body, and some of them have been measured in a number of different tissues. 1,3-Butadiene can be excreted via the respiratory system as the parent compound or as a metabolite. Metabolites of 1,3-butadiene are also excreted in urine and

feces. This qualitative overview appears to apply to the various species that have been studied, including humans; however, the enzymatic rates for specific metabolic routes differ among species (and tissues).

Since the previous reviews by EPA in 1985 and IARC in 1986, considerable data have been generated regarding the pharmacokinetics of 1,3-butadiene in various species. Most of the studies have been of mice and rats; however, there are also some data on monkeys and humans. Recent research has elucidated new metabolic pathways that had not been described previously and has shown differences in tissue concentrations of reactive metabolites of 1,3-butadiene. In vitro studies with liver, lung, and kidney cytosolic and microsomal fractions have elucidated possible metabolic products and yielded estimates of kinetic constants for the various metabolic pathways under controlled conditions. The in vitro studies indicated differences in the rates of metabolism that may result in different concentrations of the reactive metabolites in vivo. In vivo studies, which encompass all processes of pharmacokinetics—absorption, distribution, biotransformation, and excretion—have provided an account of the disposition of 1,3-butadiene during and following inhalation exposures in various animal species. The studies have quantitated kinetic parameters governing disposition in rodents, identified tissue and urinary metabolites, and identified DNA and hemoglobin adducts of reactive metabolites. The in vivo pharmacokinetic studies validated the existence of certain reactive metabolites of 1,3-butadiene and showed differences in tissue concentrations of the metabolites among species. The in vivo studies verified that interspecies differences in rates of metabolism for the various biotransformation pathways result in different tissue doses of reactive metabolites. An extensive summary of the pharmacokinetics of 1,3-butadiene was published recently by Himmelstein et al. (1997), and readers are referred to this reference for a more detailed description of the pharmacokinetic research. This chapter summarizes the relevant research for evaluating the role of 1,3-butadiene pharmacokinetics in the species differences in toxicity following inhalation exposures.

3.2. ABSORPTION

Absorption refers to the rate at which a chemical leaves its site of administration and enters the systemic circulation. The primary route of exposure to 1,3-butadiene is via inhalation; therefore this section focuses on factors that affect absorption of 1,3-butadiene via inhalation.

Absorption via inhalation is dependent on the solubility of the chemical in blood versus air. Several laboratories have reported blood:air partition coefficients, which are ratios of the concentrations of chemical in blood versus air, for 1,3-butadiene and its metabolites in mice and rats. Table 3-1 summarizes the results from various laboratories. 1,3-Butadiene is moderately soluble in blood, with a partition coefficient of approximately 1, which means theoretically in a

Table 3-1. Tissue:blood partition coefficients[a].

	1,3-Butadiene[b]		3,4-Epoxybutene[c]		1,2,3,4-Diepoxybutane[d]	
Tissue	**Rats**	**Mice**	**Rats**	**Mice**	**Rats**	**Mice**
Liver	0.80	1.0	1.4	1.1	NR[e]	1.4
Lung	0.62	1.1	1.1	1.5	NR	NR
Muscle	0.99	3.0	0.39	0.64	NR	1.8
Fat	15	14	2.7	2.5	NR	2.2

[a]Partition coefficients represent the thermodynamic equilibrium of the chemical in the tissue and blood.
[b]Experimentally determined partition coefficients were reported by Medinsky et al. (1994), who measured tissue:air and blood:air by the vial equilibration method of Gargas et al. (1989). The blood:air partition coefficients for BD were 1.34 and 1.49 in mice and rats, respectively, and for EB were 36.6 and 50.4 in mice and rats, respectively.
[c]Experimentally determined partition coefficients were reported by Medinsky et al. (1994), who measured tissue:air and blood:air ratios by the vial equilibration method of Gargas et al. (1989).
[d]Data obtained from Sweeney et al. (1997). Sweeney et al. calculated tissue:blood partition coefficents from the ratio of tissue:hexane partition coefficients versus blood:hexane partition coefficients. This method for measuring partition coefficients has not been previously validated or extensively used to date.
[e]Values for these partition coefficients were not reported.

single breath 50% of the amount of 1,3-butadiene would enter the blood to equilibrate with the 1,3-butadiene present in the air. No significant species differences have been noted among the reported blood:air partition coefficients.

Pharmacokinetic studies have been performed exposing rats, mice, and monkeys to 1,3-[1-^{14}C]butadiene to measure the percentage of the inhaled dose that was retained at the end of exposure. Although the studies indicate qualitatively that 1,3-butadiene is absorbed into the bloodstream from the air, the rate of absorption or amount absorbed cannot be estimated from the collected data. Different protocols were used in the various studies and none completely analyzed the total amount of radioactivity during and after exposures.

Bond et al. (1986) exposed mice and rats by inhalation to 1,3-[1-^{14}C]butadiene for 6 h at exposure concentrations ranging from 0.08 to 1,000 ppm and 0.08 to 7,100 ppm for mice and rats, respectively. Radioactivity measured at the end of exposure in the animals included the amount in exhaled breath, urine, feces, and body. The measured radioactivity excluded the amounts excreted via all routes during exposure. Except for the exposures to 0.8 and 7 ppm 1,3-butadiene, the rats and mice appeared to retain similar percentages of the inhaled 1,3-butadiene, 4–20% and 1.5–17% for mice and rats, respectively. The amount retained at the end of 6 hours was used to estimate an inhaled dose normalized to body weight for each species of animal. The mice received approximately 2.5–11 times the inhaled dose of 1,3-butadiene per kg body weight as rats in the experiments. The measured minute ventilation of mice normalized to the body weight in this study was approximately threefold higher than rats, 96 versus 34 L/h/kg, respectively.

Dahl et al. (1991) exposed monkeys for 2 h by inhalation to 1,3-[1-^{14}C]butadiene at concentrations of 10, 300, or 8,000 ppm and quantitated ^{14}C in exhaled breath, urine, and feces during and up to approximately 96 h postexposure. The amount of radioactivity recovered was 1.5–2.9% of the inhaled amount of radiolabeled 1,3-butadiene; however, the data did not include residual ^{14}C still present in the monkeys at the end of 96 h or the amount of ^{14}C exhaled as 1,3-butadiene either during or following exposures.

The higher dose in mice would be due partly to higher ventilation per kg of body weight of mice versus rats or monkeys, which would allow greater absorption of the chemical into the blood. In addition, more extensive metabolism of 1,3-butadiene by the mice would enhance the amount of 1,3-butadiene absorbed in the blood.

3.3. BIOTRANSFORMATION

This section highlights the major findings from several in vitro and in vivo studies that have elucidated the pathways of 1,3-butadiene biotransformation, identified responsible enzymes, and quantitated kinetic parameters that govern the rates of metabolism. The pathways of 1,3-

butadiene biotransformation are illustrated in Figure 3-1 and identified in Table 3-2. Several of the metabolites, which are indicated by brackets, are electrophilic and capable of reacting with DNA and protein.

Table 3-3 summarizes the intermediate metabolites that have been identified either in vitro or in vivo from the proposed biotransformation pathways and the corresponding animal species in which the metabolite has been identified. The detection of the metabolite in the urine only is indicated as such in the table. Some compounds have been detected in vivo only as adducts of DNA or hemoglobin adduct, which is noted in the table.

3.3.1. Pathway Elucidation

The initial and predominate biotransformation step of 1,3-butadiene is oxidation to form EB. EB has been detected in vitro during incubations of 1,3-butadiene with hepatic postmitochondrial fractions from mouse, rat, monkey, or human (Schmidt and Loeser, 1985); with hepatic microsomes from rats, mice, or humans (Cheng and Ruth, 1993; Csanády et al., 1992; Duescher and Elfarra, 1994; Elfarra et al., 1991; Malvoisin et al., 1979a; Nieusma et al., 1997); with lung postmitochondrial fractions from mouse and rat (Schmidt and Loeser, 1985); with lung microsomes from mice, rats, and humans (Csanády et al., 1992); with lung tissue from airways of mice and rats (Seaton et al., 1996); with kidney microsomes from mice (Sharer et al., 1992); with bone marrow from humans and mice (Maniglier-Poulet et al., 1995); with chloroperoxidase from *Caldoriomyces fumago* (Elfarra et al., 1991); with human myeloperoxidase (Duescher and Elfarra, 1992; Maniglier-Poulet et al., 1995); and with cDNA-expressed human cytochrome P450s (Duescher and Elfarra, 1994). EB was identified in vivo in blood and tissues of mice, rats, and monkeys that were exposed by inhalation to 1,3-butadiene (Bechtold et al., 1995; Bond et al., 1986; Himmelstein et al.,1994; Himmelstein et al., 1995; Leavens et al., 1996; Leavens et al., 1997; Thornton-Manning et al., 1995a, 1997, 1998); and exhaled EB has been measured in the atmosphere of closed chambers containing rats and mice exposed by inhalation to 1,3-butadiene (Bolt et al., 1983; Filser and Bolt, 1984; Kreiling et al., 1987).

EB can be oxidized further to form another reactive metabolite, DEB. DEB has been detected in vitro during incubations of 1,3-butadiene with rat liver microsomes (Malvoisin and Roberfroid, 1982; Cheng and Ruth, 1993) and during incubations of EB with mouse, rat, and human liver microsomes (Krause and Elfarra, 1997; Malvoisin et al., 1979b; Nieusma et al., 1997; Seaton et al., 1995). DEB has been detected in vivo in blood and tissues of mice and rats exposed by inhalation to 1,3-butadiene (Bechtold et al., 1995; Bond et. al., 1986 ; Himmelstein et al., 1994; Himmelstein et al., 1995; Thornton-Manning et al., 1995a,b; 1997, 1998).

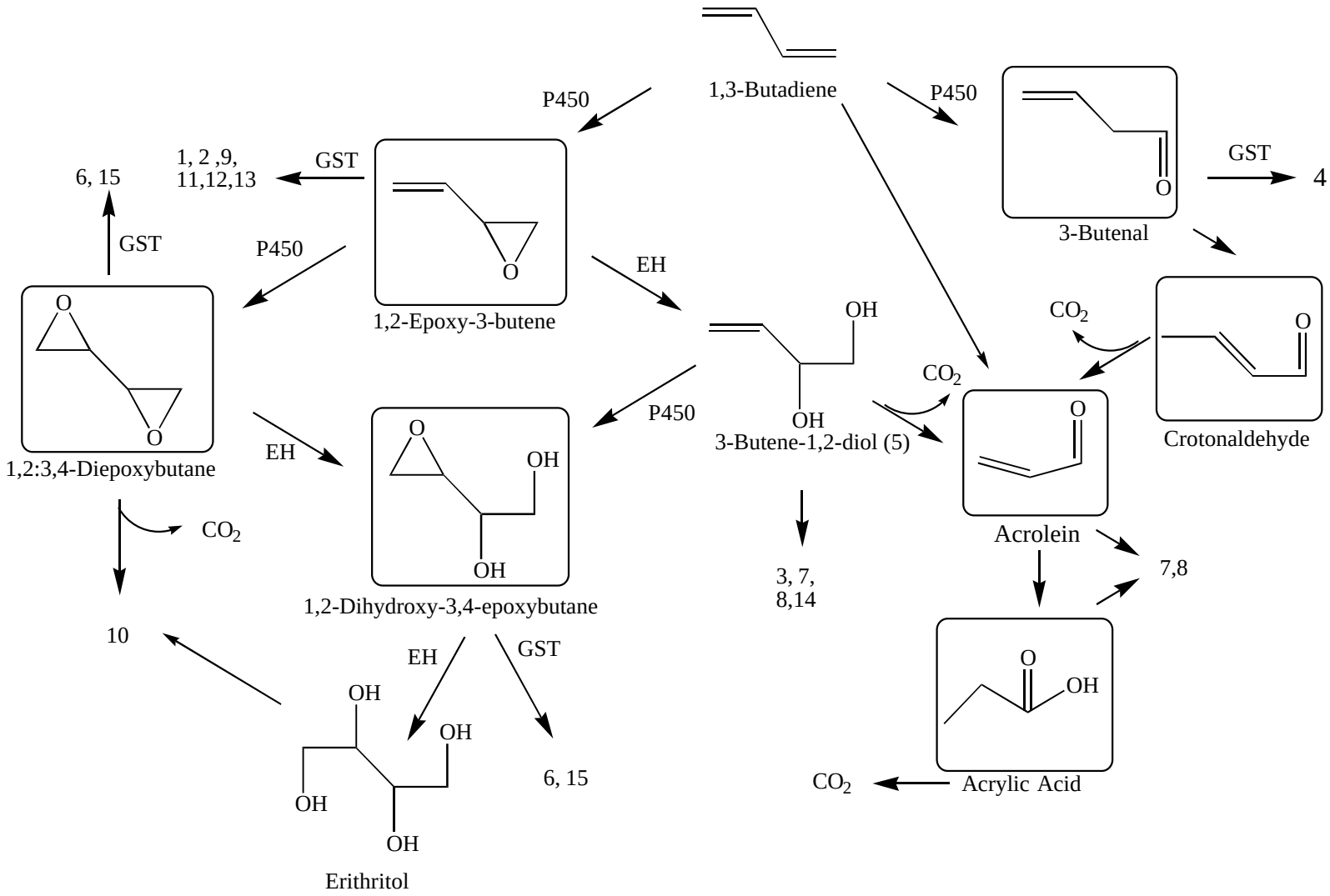

Figure 3-1. Schematic of 1,3-butadiene metabolism[a].

[a]P450 stands for cytochrome P450, EH stands for epoxide hydrolase, GST stands for glutathione transferase, and GSH stands for glutathione. The reactive metabolites are shown inside boxes. The urinary metabolites are numbered and listed in Table 3-1.

Table 3-2. Identification of urinary metabolites in Figure 3-1.

Urinary metabolite number	Urinary metabolite name
1	*N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine, *S*-(2-hydroxy-3-buten-1-yl)-*N*-acetyl-L-cysteine, or 1-(*N*-Acetyl-L-cystein-*S*-yl)-2-hydroxybut-3-ene
2	*N*-acetyl-*S*-(1-(hydroxymethyl)-2-propenyl)-L-cysteine, or *S*-(1-hydroxy-3-buten-2-yl)-*N*-acetyl-L-cysteine, or 2-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-3-ene
3	1,2-dihydroxy-4-(*N*-acetyl-L-cysteinyl)butane, 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*)-butane, or 4-(*N*-acetyl-L-cystein-*S*-yl)-1,2-dihydroxybutane
4	*N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine
5	3-Butene-1,2-diol
6	*N*-Acetyl-*S*-(1-(hydroxymethyl)-2,3-dihydroxypropyl)-L-cysteine or 3-(*N*-acetyl-L-cystein-*S*-yl)-1,2,4-trihydroxybutane
7	3-(*N*-acetyl-L-cystein-*S*-yl)propan-1-ol
8	3-(*N*-acetyl-L-cystein-*S*-yl)propanoic acid
9	*S*-(1-(Hydroxymethyl)-2-propenyl)-L-cysteine
10	1,3-Dihydroxypropanone or dihydryoxyacetone
11	4-(*N*-acetyl-L-cystein-*S*-yl)-1-hydroxybut-2-ene
12	*S*-(1-hydroxy-3-buten-2-yl)mercaptoacetic acid
13	*S*-(2-hydroxy-3-buten-1-yl)mercaptoacetic acid
14	4-(*N*-acetyl-L-cystein-*S*-yl)-2-hydroxybutanoic acid
15	4-(*N*-acetyl-L-cystein-*S*-yl)-1,2,3-trihydroxybutane

Table 3-3. Identified 1,3-butadiene metabolites[a].

Species	Butadiene metabolite					
	3-Butenal	Crotonaldehye	EB	DEB	BDdiol	EBD
			In vitro			
Mice	−	+	+	+	+	ND
Rats	−	−	+	+	+	+
Monkeys	ND	ND	+	ND	ND	ND
Humans	−	+[b]	+	+	+	ND
			In vivo			
Mice	ND	ND	+	+	+[c]	+[d]
Rats	ND	ND	+	+	+[c]	+[d]
Monkeys	ND	ND	+	+	ND	ND
Humans	ND	ND	+[e]	+[f]	ND	+[f]

[a]This table includes the intermediate metabolites that are shown in Figure 3-1 except for acrolein and acrylic acid, which have not been studied specifically in any tissue in vitro or in vivo but have been suggested as intermediates for 3-carbon urinary metabolites identified by Nauhaus et al. (1996). In the table + indicates the metabolite has been detected, − indicates the metabolite has not been detected, and ND indicates that the presence of the metabolite has not been examined.
[b]Detected in incubations with human myeloperoxidase but not human tissues.
[c]Detected as a urinary metabolite in animals following exposure to 1,3-butadiene and in blood or tissues of animals administered EB by intraperitoneal injection.
[d]Hemoglobin and DNA adducts that may have been formed from EBD detected following exposure to 1,3-butadiene.
[e]Hemoglobin and DNA adducts detected in occupationally exposed humans.
[f]Hemoglobin and DNA adducts that may have been formed from either DEB or EBD detected in occupationally exposed humans.

In addition, EB can be hydrolyzed chemically or by epoxide hydrolase and conjugated with glutathione chemically or by glutathione transferases. EB is hydrolyzed to BDdiol, which has been detected in vitro with incubations of 1,3-butadiene with rat liver microsomes (Cheng and Ruth, 1993); with incubations of EB in the absence of NADPH with hepatic microsomes from rats, mice, and humans (Krause et al., 1997; Malvoisin and Roberfroid, 1982); with incubations of EB in the absence of NADPH with liver cytosols from mice and humans (Krause et al., 1997); and with cDNA-expressed human microsomal epoxide hydrolase (Krause et al., 1997). BDdiol has been detected in vivo in urine from mice and rats exposed by inhalation to 1,3-butadiene (Nauhaus et al., 1996) and from rats and mice administered EB by intraperitoneal injection (Krause et al., 1997).Glutathione conjugates of EB have been detected in the bile from rats administered EB intraperitoneally (Sharer and Elfarra, 1992). Mercapturic acids from glutathione conjugation with EB have been identified in urine of mice and rats exposed by inhalation to 1,3-butadiene (Bechtold et al., 1994; Nauhaus et al., 1996; Osterman-Golkar et al., 1991; Sabourin et al., 1992); in urine of mice and rats administered EB intraperitoneally (Elfarra et al., 1995; Richardson et al., 1998); and in workers exposed occupationally (Bechtold et al., 1994; Ward et al., 1994).

Because of its electrophilic nature, EB also can form DNA and hemoglobin adducts in both rats and mice. DNA adducts of EB have been detected in blood and tissues of mice and rats exposed by inhalation to 1,3-butadiene (Jelitto et al., 1989; Koc et al., 1999; Koivisto et al., 1997; Koivisto et al., 1998; Tretyakova et al., 1998). Hemoglobin adducts derived from EB have been detected in blood of mice and rats exposed by inhalation to 1,3-butadiene (Albrecht et al., 1993; Osterman-Golkar et al., 1991, 1998; Sun et al., 1989a; Swenberg et al., 1999; Tretyakova et al., 1996) and in workers occupationally exposed to 1,3-butadiene (Osterman-Golkar et al., 1996).

BDdiol, the hydrolysis product of EB, can be oxidized or conjugated with glutathione. The oxidation of BDdiol has been studied in vitro in hepatic cytosols from rats, mice, and humans (Kemper and Elfarra, 1996); in microsomes from mice, rats, and humans (Krause et al., 1997); and in vivo in mice (Kemper et al., 1998). A mercapturic acid derived from glutathione conjugation of BDdiol was detected in the urine of mice and rats exposed by inhalation to 1,3-butadiene (Bechtold et al., 1994; Nauhaus et al., 1996; Sabourin et al., 1992).

DEB, the oxidation product of EB, can be hydrolyzed chemically or by epoxide hydrolase and conjugated with glutathione chemically or by glutathione transferases. EBD, which can be formed by the hydrolysis of DEB or the oxidation of BDdiol, has been identified from in vitro incubations of 1,3-butadiene with rat liver microsomes (Cheng and Ruth, 1993; Malvoisin and Roberfroid, 1982). Formation of EBD from 1,3-butadiene has not been studied in microsomal fractions from other species. Other metabolites of hydrolysis that have been measured in vitro

include erythritol in mouse, rat, and human microsomes incubated with DEB; and anhydroerythritol in human microsomes incubated with DEB, although these products represented only a small percentage of the DEB that was hydrolyzed (Boogaard and Bond, 1996). Mercapturic acids that could have been derived from glutathione conjugation of DEB were detected in the urine of mice and rats exposed by inhalation to 1,3-butadiene (Nauhaus et al., 1996). Boogaard et al. (1996) identified two glutathione metabolites and the corresponding trihydroxy metabolites that were formed from glutathione conjugation of DEB in liver cytosols of mice and rats. Dihydropropanone, which could be formed through the pentose phosphate pathway from DEB, was detected in the urine of rats exposed by inhalation to 1,3-butadiene (Nauhaus et al., 1996).

Because of their electrophilic nature, both DEB and EBD form trihydroxybutyl DNA adducts and hemoglobin adducts. Trihydroxybutyl adducts of DNA have been identified in vivo in blood and tissues of mice and rats exposed to 1,3-butadiene by inhalation (Jelitto et al., 1989; Koc et al., 1999; Tretyakova et al., 1998). Trihydroxybutyl hemoglobin adducts have been identified in blood from rodents administered EB, EBD, or DEB intravenously (Pérez et al., 1997; Swenberg et al., 1999) and from humans exposed occupationally to 1,3-butadiene (Pérez et al., 1997; Swenberg et al., 1999).

An alternative but minor biotransformation pathway, which has only been demonstrated definitively in vitro, is oxidation of 1,3-butadiene to 3-butenal. 3-Butenal has been detected in incubations with chloroperoxidase from *Caldariomyces fumago* at a pH of 6.0 (Duescher and Elfarra, 1993). At the physiological pH of 7.4, 3-butenal is thought to be tautomerized rapidly to crotonaldehyde, which has been detected in vitro in hepatic microsomal incubations from mice only (Elfarra et al., 1991; Sharer et al., 1992); in lung and kidney microsomal incubations from mice (Sharer et al., 1992); in chloroperoxidase incubations (Elfarra et al., 1991); and in incubations with human myeloperoxidase (Duescher and Elfarra, 1992). In the mouse microsomes, crotonaldehyde was formed at higher protein concentrations than those used in the incubations in which only EB was detected, 1.7–2.0 mg protein/mL versus 0.3–0.6 mg protein/mL, respectively. In addition, crotonaldehyde concentrations were approximately 30% of EB concentrations in microsomal and chloroperoxidase incubations and approximately 6% of EB concentrations in human myeloperoxidase incubations. Neither 3-butenal nor its product crotonaldehyde were biotransformed from EB, as incubations with EB failed to produce either compound. Although myeloperoxidase was shown to oxidize 1,3-butadiene to crotonaldehyde, incubations with either human or mouse bone marrow failed to produce detectable quantities of crotonaldehyde (Maniglier-Poulet et al., 1995). Experiments have not been conducted to specifically identify 3-butenal or crotonaldehyde in blood or tissues in vivo. However, urinary metabolites, which may result from 3-butenal or further biotransformation of crotonaldehyde to

acrolein, have been detected in the urine of mice but not rats exposed by inhalation to 1,3-butadiene, and represented 13.9% of total urinary metabolites that were quantitated (Nauhaus et al., 1996). But, the metabolites attributed to acrolein biotransformation pathways (10.2% of urinary metabolites) could also be attributed to biotransformation of EB.

Another potential metabolic pathway that was identified in vitro is biotransformation of 1,3-butadiene to 1-chloro-2-hydroxy-3-butene (chlorhydrin). Duescher and Elfarra (1992) detected chlorhydrin by gas chromatography mass spectrometry (GC-MS) in incubations of 1,3-butadiene with physiological concentrations of KCl and human myeloperoxidase purified from human leukocytes. Chlorhydrin concentrations exceeded EB concentrations at KCl concentrations greater than 50 mM. Chlorhydrin was not produced in the absence of KCl or by liver microsomes, chloroperoxidase, or horseradish peroxidase and was not a product of EB or crotonaldeyde because incubation with these two chemicals did not produce chlorhydrin. This particular product has not been specifically investigated in vivo, but as with 3-butenal, it may be relevant to potential toxic effects in the lymphohematopoietic system.

Another chlorine metabolite that was identified in phosphate buffered saline in which EB was incubated in the absence of tissues is 1,2-dichloro-3,4-epoxybutane (Cheng et al., 1994). Cheng and co-workers noted the disappearance of EB from the headspace of solutions in phosphate-buffered saline and identified the product by comparing the fragmentation pattern obtained from electron ionization GC-MS of the solvolysis product with synthetic standard. Although the production of 1,2-dichloro-3,4-epoxybutane was formed nonenzymatically at physiological Cl concentrations, the relevancy of this pathway in vivo is not known.

3.3.2. Enzyme Identification

The major group of enzymes involved in the oxidation of 1,3-butadiene, EB, and BDdiol are cytochromes P450. The generation of EB from 1,3-butadiene in a NADPH-generating system was first demonstrated by Malvoisin et al. (1979a). The role of cytochromes P450 in the biotransformation of 1,3-butadiene was demonstrated in vitro by induction of EB formation in liver microsomes from rats pretreated with phenobarbital (Bolt et al., 1983; Malvoisin et al., 1979a) and 20-methylcholanthrene (Bolt et al., 1983) and by inhibition of the reaction following the addition of SKF525-A (Bolt et al., 1983; Malvoisin et al., 1979a) and dithiocarbamate to the incubation with rat liver microsomes (Bolt et al., 1983). The role of cytochromes P450 in the biotransformation of 1,3-butadiene was demonstrated in vivo by induction of the rate of metabolism in rats pretreated with Arochlor (Bolt et al., 1984) and by reduced uptake of 1,3-butadiene from the atmosphere in closed inhalation chambers by rats and mice pretreated with pyrazole (Medinsky et al., 1994). Elfarra et al. (1991) suggested the role of cytochromes P450 in the oxidation of 1,3-butadiene to both EB and crotonaldehyde in mouse liver microsomes by the

dependence of the reactions on the addition of NADPH to the incubation and by inhibition of the reactions with the addition of 1-benzylimidazole, a selective cytochrome P450 inhibitor, to the incubations. The role of cytochrome P450 in the oxidation of BDdiol was suggested in vivo, since pretreatment of male $B6C3F_1$ mice with benzylimidazole, a cytochrome P450 inhibitor, decreased the clearance of BDdiol administered intraperitoneally (25 mg/kg BW) by 44%.

Several isoforms of cytochrome P450 have been shown to be involved in the biotransformation of 1,3-butadiene. The specific P450 isoforms that have been identified thus far as important in the biotransformation of 1,3-butadiene to EB include P450 2E1 and P450 2A6. Csanády et al. (1992) suggested a significant role of cytochrome P4502E1 in the oxidation of 1,3-butadiene through the use of correlation analysis with chlorzoxazone. Duescher and Elfarra (1994) used microsomes from β-lymphoblastoid cells expressing human cDNA for specific P450 isozymes to demonstrate that both P450 2E1 and P450 2A6 oxidize 1,3-butadiene. Other isoforms oxidized 1,3-butadiene but were not major contributors to the overall activity when normalized for the amount of enzyme present in the incubation. Cytochrome P450 2E1 appeared to be a high-affinity, low-capacity pathway, while cytochrome P450 2A6 was lower affinity and higher capacity.

The major isoform of cytochrome P450 that oxidizes EB to DEB is cytochrome P450 2E1; however, 2A6, 2C9, and 3A4 have been shown to oxidize EB to DEB to some extent (Krause and Elfarra, 1997; Seaton et al., 1995). Seaton et al. (1995) demonstrated from human cDNA-expressed cytochromes P450 in human b-lymphoblastoid cells that P450 2E1 oxidized EB to DEB in incubations with 80 μM EB and that P450 2E1 and 3A4 were the most active in incubations with 5 μM EB. Small amounts of DEB were formed in incubations with 5 μM EB and cytochromes P450 1A1, 1A2, 2A6, 2D6, and 2F1; however, the rates were similar to the rates of oxidation with control incubations of microsomes prepared from the parent cell line used to express the cytochromes P450. Krause and Elfarra (1997) demonstrated, from human cDNA-expressed cytochromes P450 in human β-lymphoblastoid cells incubated with 5 μM EB, that the predominate cytochrome that oxidized EB to DEB was P450 2E1. Although not as active as cytochrome P450 2E1, cytochromes P450 2A6 and 2C9 also catalyzed the oxidation of EB to DEB. Krause and Elfarra did not detect DEB in incubations with cytochrome P450 3A4.

Lewis et al. (1997) attempted to provide explanations for species differences in 1,3-butadiene metabolism among mice, rats, and humans through molecular modeling of the putative active site region on P450 2E1. The molecular stucture for cytochrome P450 2E1 was constructed on the basis of the CYP102 crystal structure. Differences were noted among species for some of the amino acids in the binding pocket, which for mice and rats would facilitate hydrogen bonding and increase the probability of a second epoxidation. The changes in residues were thought to bring about a change in the size of the binding pocket. The mouse also has an

additional methylene group at the active site to allow it to bind EB more strongly and increase the probability of a second epoxidation.

Another enzyme capable of oxidizing BDdiol to EBD is alcohol dehydrogenase. Kemper and Elfarra (1996) demonstrated that purified horse liver alcohol dehydrogenase oxidized BDdiol in vitro. The involvement of alcohol dehydrogenase was indicated by inhibition of BDdiol oxidation by methylpyrazole, as measured by NADH generation, in incubations of mouse, rat, and human liver cytosols with BDdiol (Kemper and Elfarra, 1996). The role of alcohol dehydrogenase in the oxidation of BDdiol was indicated in vivo, since pretreatment with 4-methylpyrazole, a nonselective inhibitor of alcohol dehydrogenase, decreased clearance of BDdiol administered to mice intraperitoneally by 82%.

The major detoxication enzymes for EB and DEB appear to be epoxide hydrolase and glutathione transferase. The role of epoxide hydrolase was indicated by inhibition of EB metabolism in rat liver microsomes incubated with styrene oxide and vinylcyclohexene oxide, substrates for epoxide hydrolase (Malvoisin and Roberfroid, 1982), and by increased EB formation in NADPH-fortified rat liver microsomes incubated with 1,1,1-trichloropropeneoxide, another substrate for epoxide hydrolase (Bolt et al., 1983). Urinary metabolites that were formed from 1,3-butadiene intermediates are predominately products of hydrolyis or glutathione conjugation (Bechtold et al., 1994; Elfarra et al., 1995; Nauhaus et al., 1996; Osterman-Golkar et al., 1991; Richardson et al., 1998; Sabourin et al. 1992). The involvement of glutathione transferase in vivo in the biotransformation of 1,3-butadiene is evident by the depletion of glutathione in the tissues of animals exposed by inhalation to 1,3-butadiene (Deutschmann and Laib, 1989; Himmelstein et al., 1995; Kreiling et al., 1987; Laib et al., 1990).

As noted in Section 3.3.1 on metabolic pathways, other enzymes such as myeloperoxidase and chloroperoxidase have been shown to be capable of oxidizing 1,3-butadiene. The myeloperoxidase-mediated pathway is consistent with the ability of bone marrow to oxidize 1,3-butadiene (Maniglier-Poulet et al., 1995), especially since Genter and Recio (1994) did not detect cytochrome P450 2E1 in mouse bone marrow. Oxidation of 1,3-butadiene by myeloperoxidase has not been studied extensively, particularly in vivo, to evaluate its relevancy for determining a local tissue dose of reactive metabolites that could result in a toxic effect on the lymphohematopoietic system.

3.3.3. Rates of Metabolism

Qualitatively the metabolic pathways are similar among species; however, quantitatively the metabolism of 1,3-butadiene varies among species. Several investigators have noted differences among species in enzymatic activities for several of the pathways of 1,3-butadiene biotransformation shown in Figure 3-1. For example, differences among species exist in the

kinetic constants for oxidation of 1,3-butadiene to EB, oxidation of EB to DEB, and detoxication of EB and DEB by epoxide hydrolase and glutathione transferase. The kinetic parameters for the various biotransformation pathways have been estimated from several experiments, including in vitro incubations with 1,3-butadiene, EB, DEB, and BDdiol in microsomal and cytosolic fractions from tissues of animals including humans; in vivo inhalation exposures of rodents to 1,3-butadiene and the reactive metabolite EB; and in vivo exposures of rats administered EB and DEB intravenously. Based on estimates of intrinsic clearance rates of activation versus detoxication in the liver that were calculated from in vitro experiments and on kinetic parameters that were estimated from in vivo experiments, the tissue and blood concentrations of the reactive 1,3-butadiene metabolites such as EB, EBD, and DEB would be expected to be greater in mice than in either rats or humans exposed by inhalation to 1,3-butadiene.

3.3.3.1. *In Vitro*

Kinetic differences in the various pathways in the metabolism of 1,3-butadiene among species have been demonstrated in vitro with tissue fractions from several organs. This section summarizes the various studies that quantitated enzymatic activities as well as estimated kinetic parameters (Tables 3-4 to 3-9) that governed the rates of reaction for the biotransformation pathways shown in Figure 3-1. The kinetic parameters from the liver were used to estimate intrinsic clearance rates (Table 3-10) to quantitatively compare the metabolism of 1,3-butadiene among species. The clearance rates were compared because the metabolism of 1,3-butadiene and its metabolites would be expected to be first order at the low 1,3-butadiene concentrations likely to be encountered environmentally (see Section 3.3.3.3 on in vivo metabolism). In this section the studies that measured enzymatic activities are discussed first, followed by a description of the experiments that estimated in vitro kinetic constants for the various biotransformation pathways. The enzymatic activity studies are divided by species, gender, and tissue differences; chronic effects; and stereochemistry.

3.3.3.2. *Enzymatic Activities*

3.3.3.2.1. *Species, gender, and tissue differences.* The mouse consistently had the highest enzyme activities compared with rat, human, or monkey. The tissue activities varied, but no single tissue consistently had the greatest activity among species or genders.

One of the first in vitro studies to address species differences in the kinetics of 1,3-butadiene metabolism was that of Schmidt and Loeser (1985). Enzyme activities calculated from the rate of accumulation of EB, which in this study was a function of both formation from 1,3-butadiene and removal of EB, were measured in liver and lung postmitochondrial fractions from male and female mice (NMRI and B6C3F$_1$), male and female rats (Wistar and Sprague-Dawley),

Table 3-4. In vitro Michaelis-Menton kinetic parameters for mouse liver.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	2.6	2
	Duescher and Elfarra (1994)	9.2	160
EB to DEB	Csanády et al. (1992)	0.2	15.6
	Seaton et al. (1995)	1.4	141
	Krause and Elfarra (1997)	4.3	5,600
EB GSH conjugation	Sharer et al. (1992)	107	4,700
	Csanády et al. (1992)	500	35,300
EB hydrolysis	Kreuzer et al. (1991)	19	1,500
	Csanády et al. (1992)	5.79	1,590
	Krause et al. (1997)	4	500
BDdiol oxidation	Kemper and Elfarra (1996)	10.4	20,000
DEB GSH conjugation	Boogaard et al. (1996)	162	6,400
DEB hydrolysis	Boogaard and Bond (1996)	32	8,100

Table 3-5. In vitro Michaelis-Menton kinetic parameters for rat liver.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	0.59	3.74
	Duescher and Elfarra (1994)	2	120
EB to DEB	Seaton et al. (1995)	0.41	145
	Krause and Elfarra (1997)	3.5	16,300
EB GSH conjugation	Sharer et al. (1992)	71	3,100
	Csanády et al. (1992)	241	13,800
EB hydrolysis	Kreuzer et al. (1991)	17	700
	Csanády et al. (1992)	2.48	260
	Krause et al. (1997)	24.4	700
BDdiol oxidation	Kemper and Elfarra (1996)	21.4	32,700
DEB GSH conjugation	Boogaard et al. (1996)	186	24,000
DEB hydrolysis	Boogaard and Bond (1996)	52.9	2,760

Table 3-6. In vitro Michaelis-Menton kinetic parameters for human liver.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	1.18	5.14
	Duescher and Elfarra (1994)	10.4	200
	Duescher and Elfarra (1994)	22.8	390
EB to DEB	Seaton et al. (1995)	1.2	320
	Seaton et al. (1995)	0.38	304
	Seaton et al. (1995)	1.2	880
	Krause and Elfarra (1997)	6.4	32,900
	Krause and Elfarra (1997)	0.8	6,600
EB GSH conjugation	Csanády et al. (1992)	45.1	10,400
EB hydrolysis	Kreuzer et al. (1991)	14	500
	Csanády et al. (1992)	58.1	1,650
	Csanády et al. (1992)	18.5	580
	Csanády et al. (1992)	9.2	240
	Krause et al. (1997)	55.8	2,400
	Krause et al. (1997)	26.7	300
BDdiol oxidation	Kemper and Elfarra (1996)	11.6	18,300
	Kemper and Elfarra (1996)	10.6	19,600
	Kemper and Elfarra (1996)	9.1	22,500
DEB GSH conjugation	Boogaard et al. (1996)	3.7	1,040
	Boogaard et al. (1996)	5.5	1,270
	Boogaard et al. (1996)	7.8	3,600
	Boogaard et al. (1996)	4.7	3,180
	Boogaard et al. (1996)	23.6	9,400
	Boogaard et al. (1996)	19	4,500
DEB hydrolysis	Boogaard and Bond (1996)	229	6,860
	Boogaard and Bond (1996)	133	3,640
	Boogaard and Bond (1996)	74	3,740
	Boogaard and Bond (1996)	270	5,470
	Boogaard and Bond (1996)	119	3,590
	Boogaard and Bond (1996)	120	6,670

Table 3-7. In vitro Michaelis-Menton kinetic parameters for mouse lung.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	2.31	5,001
EB GSH conjugation	Sharer et al. (1992)	12	4,400
	Csanády et al. (1992)	273	36,500
DEB GSH conjugation	Boogaard et al. (1996)	38.5	1,700
DEB hydrolysis	Boogaard and Bond (1996)	49.8	7,500

Table 3-8. In vitro Michaelis-Menton kinetic parameters for rat lung.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	0.16	7,750
EB GSH conjugation	Sharer et al. (1992)	3	4,700
	Csanády et al. (1992)	44.2	17,400
DEB GSH conjugation	Boogaard et al. (1996)	17.1	4,200
DEB hydrolysis	Boogaard and Bond (1996)	19.3	7,100

Table 3-9. In vitro Michaelis-Menton kinetic parameters for human lung.

Reaction	Source	Vmax (nmol/mg prot/min)	Km (μM)
1,3-Butadiene to EB	Csanády et al. (1992)	0.15	2,000
DEB hydrolysis	Boogaard and Bond (1996)	29.5	2,830
	Boogaard and Bond (1996)	29.3	1,800
	Boogaard and Bond (1996)	23	5,000
	Boogaard and Bond (1996)	17.2	2,440
	Boogaard and Bond (1996)	18.1	1,910
	Boogaard and Bond (1996)	22.5	3,120

Table 3-10. Estimated intrinsic clearance for liver (Vmax/Km)[a].

	Metabolic reaction						
Species	**1,3-Butadiene to BMO**	**BMO to BDE**	**BMO hydrolysis**	**BMO GSH conjugation**	**BDE hydrolysis**	**BDE GSH conjugation**	**BDdiol oxidation**
Mouse	2.5–56	0.43–0.55	0.16–0.55	4.4–7.0	0.17	7.8	0.16
Rat	0.84–8.0	0.011–0.14	0.05–1.22	5.7–7.4	0.97	2.5	0.21
Human	1.4–6.2	0.034–0.037	0.76–1.0	0.36	0.48–1.34	0.13–0.36	0.033–0.053

[a]Ratio was determined from Michaelis-Menton parameters determined from in vitro incubations with liver microsomal and cytosolic fractions (see Tables 3-2 to 3-4). The values cytosolic and microsomal protein concentrations and liver fractions as a percentage of body weight were taken from Boogaard and Bond (1996). The cytosolic protein concentrations were 83, 108, and 45 mg protein/g liver for mouse, rat, and human, respectively. The microsomal protein concentrations were 11.6, 16.8, and 14.5 mg protein/g liver for mouse, rat, and human, respectively. The liver was assumed to be 6.2%, 5%, and 3.1% of the body weight in mice, rats, and humans, respectively. Units for clearance are L/h/kg body weight.

male and female rhesus monkeys, and one human (gender not specified). EB was detected in incubations with liver postmitochondrial fractions from all species, whereas only mouse and rat lungs formed measurable amounts of EB. The sequence of liver activities (nmol/g tissue/45 min) quantitated from headspace amounts of EB that accumulated during the incubation with 30,000 ppm 1,3-butadiene were mouse>rat≅human>monkey. The mouse had approximately sixfold higher activity in the lung compared with the rat. In addition, female mice had higher liver activities and lower lung activities than the male mice, whereas male rats had higher activities in both the liver and lung. The activity was greater in the lungs compared with the liver of male mice, whereas they were approximately the same in female mice. In rats the lung activities of both males and females were less than liver activities. Sharer et al. (1992) measured the formation of EB by microsomes from liver, lungs, and kidneys of male $B6C3F_1$ mice and Sprague-Dawley rats. The microsomes were incubated in vials with headspace pressures of 1,3-butadiene ranging from 48 to 52 mm Hg. The authors did not report the total pressure in the headspace or to what molar concentration of 1,3-butadiene in the headspace or media that this pressure was equivalent. Assuming that air was present in the headspace for the reaction and that the headspace was at atmospheric pressure, the concentration of 1,3-butadiene in the headspace was approximately 63,158 ppm. In all three tissues the concentration of EB (nmol/mg protein) versus time was greater in mice than in rats. In mice the sequence of activities for the tissues was kidney>liver≅lung, 23.8 ± 2.9, 6.4 ± 1.2, and 6.1 ± 0.4 nmol EB/mg protein/min (mean ± sd), respectively. In rats the sequence of activities for the tissues was liver>lung>kidney, 3 ± 0.6, 1.5 ± 0.2, and 0.5 ± 0.02 nmol EB/mg protein/min (mean ± sd), respectively. Again, in these studies the concentration of EB in the incubation would be a function of formation and removal; however, this study compensated for removal of EB from the system by calculating percentage of recovery.

Because bone marrow is a target tissue for 1,3-butadiene toxicity, Maniglier-Poulet et al. (1995) quantitated the formation of EB from 1,3-butadiene by hydrogen-peroxide-fortified bone marrow cells. EB was not detected in bone marrow cell lysates incubated with NADPH. Mouse bone marrow cell lysates were incubated with 50,000 ppm 1,3-butadiene in the headspace and 1 mM hydrogen peroxide at 37°C and pH 7.4, and hepatic microsomes from male $B6C3F_1$ mice were incubated in the presence of 1 mM NADPH and 50,000 ppm 1,3-butadiene in the headspace for up to 20 min. EB was quantitated in the headspace of each vial. The formation of EB by mouse liver microsomes was 281 times greater than formation from the bone marrow cell lysates. The relevancy of metabolism of 1,3-butadiene by bone marrow lysates to in vivo exposures cannot be accessed since the concentration of hydrogen peroxide in bone marrow cells is not known. Granulocytic respiratory burst is known to generate superoxide and hydrogen peroxide.

Even though bone marrow oxidation of 1,3-butadiene to EB may not add significantly to systemic concentrations of reactive metabolites, it may affect local tissue concentrations.

Seaton et al. (1996) measured the concentrations of EB in media with isolated airway sections from male B6C3F_1 mouse and Sprague-Dawley rat lungs incubated with 10,000 ppm 1,3-butadiene in the headspace of vials. The authors did not find dramatic differences in the concentrations measured at 60 min of incubation between the mice and rats.

The role of epoxide hydrolase in the detoxication of EB and DEB also differs among species. Nieusma et al. (1997) measured greater differences in the concentrations of DEB in hepatic microsomes from rats pretreated with trichloropropenic oxide compared with microsomes without trichloropropylene oxide. Nieusma and co-workers did not note the nearly twofold increase in DEB concentrations with microsomes from B6C3F_1 mice.

3.3.3.2.2. ***Chronic effects***. The effect of repeated exposures on the metabolism of 1,3-butadiene was quantitated in a study that measured enzyme activities in vitro in tissues from animals exposed to 1, 3-butadine in vivo (Bond et al., 1988). Bond and co-workers exposed male Sprague-Dawley rats and B6C3F_1 mice to either 7,600 ppm (252 μmol/L) or 760 ppm (252 μmol/L) 1,3-butadiene, respectively, for 6 h/d for 5 days. Microsomes were prepared from the lungs and livers of both species and from nasal tissue from rats at 18 h postexposure. The assay measured the disappearance of 1,3-butadiene in the headspace of the reaction vial. In the lungs of both species the rate of metabolism of 1,3-butadiene was reduced 50% in animals repeatedly exposed by inhalation to 1,3-butadiene compared with animals exposed to air only. The liver was unaffected in these experiments. The reported activities were not consistent with previous work because the activities in the liver were less than the lung in both species, and the activities in the lung and liver of the rat were greater than previously reported activities in the mouse. However, no units were given for the activities, so it is unclear whether they were normalized, and if they were normalized, whether it was per g of protein or g of tissue weight.

3.3.3.2.3. ***Stereochemistry*****.** A few investigators have examined the role of stereochemistry in species differences in metabolism and toxicity of 1,3-butadiene. There are stereochemical differences in vitro among species, but the significance of the differences in vivo is not known.

Nieusma et al. (1997) incubated hepatic microsomes from male B6C3F_1 mice and Sprague-Dawley rats with headspace concentrations of 25,000 ppm 1,3-butadiene, (R)-EB, or (S)-EB. Total EB from the 1,3-butadiene incubations were quantitated from the headspace by gas chromatography-flame ionization detector (GC-FID), and after extraction from the media the specific enantiomers were quantitated by GC-FID. Concentrations of the racemic and meso forms of DEB were measured in the incubation media by GC-MS. Consistent with previous

studies, microsomes from mice had higher enzyme activities, with approximately twice the concentration of EB at 30 min of incubation. Mice formed approximately the same proportion of (S)-EB to (R)-EB during the incubation, whereas rats initially formed three times more (S)-EB than (R)-EB, but by the end of incubation the ratio was less than one. In hepatic microsomes incubated with (S)-EB or (R)-EB, mice had higher DEB formation than rats. In addition, more DEB was formed from (S)-EB than from (R)-EB in microsomes from mice. At 5 min of incubation, the rat liver microsomes had significantly greater amounts of DEB formed from (R)-EB than (S)-EB. The trend towards more DEB from (R)-EB was present at other timepoints, but the variability in the data made the differences insignificant. There were also regiochemical differences in hydrolysis, with microsomes from mice having epoxide ring opening occurring at the C2 of (S)-EB but not (R)-EB, whereas microsomes from rats had no ring opening at C2 of either enantiomer of EB.

Nieusma et al. (1998a) quantitated the formation of (R)-EB and (S)-EB from purified reconstituted cytochrome P450 2E1 from rabbits, rats, and humans. Solutions containing the enzymes were incubated with headspace concentrations of 250,000 ppm 1,3-butadiene, and EB was measured in the headspace while the specific enantiomers of EB were measured in extracts of the enzyme solutions. The oxidation rate of 1,3-butadiene to EB was greatest by P450 2E1 from rabbits, followed by humans, then rats: 7.79, 5.0, and 4.21 nmol EB/pmol P450 2E1/min, respectively. The ratio of (S)-EB to (R)-EB was not significantly different among the species. The authors did not account for further metabolism of EB to DEB during the incubations.

Nieusma et al. (1998b) investigated the stereochemical aspects of conjugation of EB, DEB, and EBD with glutathione in rat liver cytosols and freshly isolated rat hepatocytes. They quantitated the percentage of glutathione depletion during incubations with 5 mM of the two enantiomers of EB, the three stereoisomers of DEB, or the four stereoisomers of EBD. Based on the amount of glutathione that was depleted, (S)-EB was more reactive than (R)-EB with glutathione transferase, and meso-DEB was the least reactive of the three DEB stereoisomers. Only (SR)-EBD and the (SS)-EBD appeared to be enzymatically conjugated with glutathione in both cytosolic fractions and hepatocytes, since the percentage of glutathione depletion with the other two stereoisomers was the same in incubations with or without hepatic cytosol and was not significant in hepatocytes. (SS)-EBD appeared more reactive than (SR)-EBD in both cytosolic-mediated reactions and in freshly isolated hepatocytes.

3.3.3.2.4. ***Kinetic constants.*** Several investigators have shown species differences in the kinetic constants estimated for in vitro metabolism of 1,3-butadiene and its metabolites. There is variability in the results from the studies, potentially because of differences in experimental methods. Some studies quantitated only the racemic form of DEB while others quantitated the

racemic and meso forms of DEB. Some studies did not control or account for loss of substrate or product through volatilization and further metabolism. In addition, the analysis of the data varied among studies. In general the results from all the studies indicated that mice have greater rates of oxidation of 1,3-butadiene to EB and EB to DEB than do humans or rats. There was some variability among the studies as to whether the rates were greater in humans or rats. The in vitro studies of detoxication of the reactive 1,3-butadiene metabolites EB and DEB via glutathione conjugation or hydrolysis have shown that the predominate pathway of detoxication depends on the species. For mice and rats, the rate of metabolism of EB and DEB via glutathione conjugation in the liver is greater than hydrolysis. In humans, hydrolysis dominated removal of both EB and DEB. A comparison of the metabolism of 1,3-butadiene is illustrated in Table 3-10 with estimates of the ranges of intrinsic clearance for the various biotransformation pathways in the liver for mice, rats, and humans. Overall, mice appear to have greater formation of the reactive metabolites EB and DEB versus detoxication via epoxide hydrolase and glutathione transferase compared with rats and humans.

Csanády et al. (1992) quantitated kinetic constants in liver and lung cytosolic and microsomal fractions from mice, rats, and humans for the oxidation of 1,3-butadiene to EB, EB to DEB, EB hydrolysis, and EB glutathione conjugation. The demographics were not given for the human subjects (n=12 for liver; n=5 for lung) from which tissue samples were obtained. Csanády measured loss of either 1,3-butadiene or EB from the headspace or formation of glutathione conjugates in the media of vials containing the tissue fractions, and estimated the parameters for each pathway with a two-compartment kinetic model that described distribution of the compound between the vial headspace and media and elimination via metabolism. The concentrations of the substrates used in the experiments were 600–25,000 ppm 1,3-butadiene and 20–200 ppm EB. For human liver, all 12 samples were used to estimate the kinetic constants for the oxidation of butadiene, and for human lung, three of the five samples were pooled and used to estimate kinetic constants. For both hydrolysis and glutathione conjugation, the human liver samples were ranked according to enzymatic activity. Kinetic constants were determined for two samples with highest and lowest activity for glutathione conjugation and for three samples with high, median, and low activity for hydrolysis. The metabolic pathways in all species were described by Michaelis-Menton kinetics except for hydrolysis of EB in lung microsomes from all three species and glutathione conjugation of EB in human lung. V_{max} and K_m for the various pathways are summarized in Tables 3-3 to 3-8. The first order rate constants for hydrolysis of EB in the lung microsomal fractions were 0.0013 and 0.00186 min^{-1}· mg $protein^{-1}$ for rats and mice, respectively. For the three human lung samples that were used, the first-order rate constants for hydrolysis of EB were 0.004, 0.0032 and 0.0076 min^{-1}· mg $protein^{-1}$. For the human lung the second-order rate constant for glutathione conjugation of EB in the cytosol was 0.00026

$mmol^{-1}$· min^{-1}·mg $protein^{-1}$. The values for glutathione conjugation in the microsomal fractions of the liver and lung are not listed here because they were approximately equal to spontaneous glutathione conjugation. Csanády and co-workers detected DEB in incubations of EB with microsomal fractions from livers of mice but not from livers of rats or humans or lungs from all three species. The strengths of this study are lower concentration ranges used for the reactions, estimates of the kinetic constants that are not subject to error from loss of substrate during exposure, and estimates that are not linear approximations. However, the estimates of the Michaelis-Menton parameters according to the method used in the paper are interrelated. The standard deviations reported in the paper are not presented here because they represent deviations in estimates of the parameters but not intraspecies variability for a given reaction. The authors used the data to estimate in vivo hepatic clearances for oxidation of 1,3-butadiene to EB and for the clearance of EB via glutathione conjugation and hydrolysis. From these values (corrected in erratum, Csanády et al., 1993) the activation-to-deactivation ratios for each species are 12, 4.5, and 1.3 for mouse, human, and rat, respectively.

Duescher and Elfarra (1994) also estimated Michaelis-Menton kinetic constants for oxidation of 1,3-butadiene to EB by liver microsomes from male Sprague-Dawley rats, male $B6C3F_1$ mice, and two male and four female humans. Duescher and Elfarra measured the generation of EB in microsomes incubated with 1,3-butadiene at pressures up to 52 cm Hg, which the authors estimated would correspond to concentrations ranging from approximately 167 to 4,400 μM 1,3-butadiene in the incubation media. In these studies the accuracy of the measurement of 1,3-butadiene concentrations both in the headspace and media is uncertain. To account for loss of EB from the media via volatilization or further metabolism, the authors corrected for recovery of EB, which was 40% to 60% for human samples and approximately 60% for mice and rat samples. Double reciprocal plots of enzymatic activity versus 1,3-butadiene concentration in the media were used to estimate apparent kinetic constants. These values are listed in Tables 3-4 to 3-9. As seen in the tables, the estimated V_{max} and K_m for each species are greater than the values estimated by Csanády et al. (1992). In this study, oxidation of 1,3-butadiene to EB was similar in mice and humans and greater in mice or humans than in rats. The discrepancies in data between the two studies may be due to different methods for calculating the apparent kinetic constants, different incubation concentrations of 1,3-butadiene, and different human samples. Because Duescher and Elfarra also found higher chlorxazone oxidation by the human samples in their study compared with Csanády et al. (1992), they hypothesized that some of the discrepancy between the two studies, at least in human tissues, was due to variability in expression of P450 enzymes. However, this does not address the differences noted with the rodent samples, which may be due to differences in experimental design between the two studies. Unlike Csanády et al. (1992), this study did not estimate kinetic parameters for glutathione

conjugation or hydrolysis in the same tissues, so activation-to-deactivation ratios cannot be estimated from this study.

Glutathione conjugation of EB occurs primarily in the cytosolic fraction in vitro. Sharer et al. (1992) measured activity of EB conjugation in rat liver and found that the activity measured from cytosolic fraction was approximately 96% of the measured activity from the whole liver homogenate (from reported data of one rat). In addition, Sharer et al. determined V_{max} and K_m for glutathione conjugation of EB in liver, lung, kidney, and testes from both male $B6C3F_1$ mice and Sprague-Dawley rats. The authors did not account for loss of EB into the headspace during the incubations, which could cause an error when calculating the Michaelis-Menton kinetic constants from double reciprocal plots. Data for the liver and lung in both species is contained in Tables 3-4 to 3-8. In addition the V_{max} for kidney was 16 ± 2 and 7 ± 3 nmol conjugates/mg protein/min (mean ± sd) in mice and rats, respectively, and for testes 30 ± 9 and 51 ± 14 nmol conjugates/mg protein/min (mean ± sd) in mice and rats, respectively. The K_m for kidney was approximately 6,600 and 5,800 μM in mice and rats, respectively, and for testes 5,500 and 10,500 μM in mice and rats, respectively. Therefore estimated clearance (V_{max}/K_m) of EB via glutathione conjugation would be approximately twofold higher in kidneys of mice compared with rats, and there would be no difference in the testes between the species.

Kreuzer et al. (1991) studied the kinetics of EB in liver microsomal and cytosolic fractions from male NMRI mice, Sprague-Dawley rats, and one male human. Kreuzer and co-workers found no NADPH-dependent metabolism in the incubations of microsomes with EB. First-order metabolism of EB was detected in the cytosol due to conjugation of EB with glutathione. Epoxide hydrolase activity was found in the microsomes only and Michaelis-Menton parameters (Tables 3-4 to 3-6) were estimated from a two compartment model that described the distribution and metabolism of EB during the incubations.

Seaton et al. (1995) estimated Michaelis-Menton kinetic parameters for the oxidation of EB to DEB in microsomal fractions from livers of male $B6C3F_1$ mice, Sprague-Dawley rats, male and female adult humans, and a male child (Tables 3-4 to 3-6). The microsomal fractions were incubated with media concentrations of 5–1,000 μM EB. DEB concentrations were quantitated in the media by GC-MS and nonlinear regression was used to obtain values for V_{max} and K_m. In addition Seaton et al. measured the enzymatic activity of conversion of EB to DEB in liver microsomes from 10 human donors. The microsomes were incubated with 80 μM EB in the media. The activity varied by 60-fold, spanning the range from rats to mice. Some limitations of the studies include failure to control for volatilization of EB and further metabolism of EB and DEB by hydrolysis or glutathione conjugation. In addition, only the racemic form of DEB was quantitated, not the meso form.

Krause and Elfarra (1997) also estimated Michaelis-Menton parameters for oxidation of EB to DEB (Tables 3-4 to 3-6). This study quantitated formation of both racemic and meso-DEB in microsomes from male B6C3F_1 mice and Sprague-Dawley rats and a male and female human. DEB was quantitated by GC-FID in incubations of the microsomes with 0.25–40 μM EB. This study also did not control for volatilization of EB or loss of EB or DEB by hydrolysis. The authors compensated for the loss by calculating percent recoveries of DEB, which were as low as 16% in some samples. The data were analyzed both by linear transformation and by nonlinear curve fitting.

The values obtained in the two studies are variable, probably because of differences in experimental methods and data analysis. Both studies show mice to have greater oxidation of EB to DEB, but Seaton et al. (1995) found greater rates of oxidation in humans compared with rats, while Krause and Elfarra (1997) found greater oxidation in rats compared with humans. The differences in human samples may be due to differences in cytochrome P450 content.

Boogaard and Bond (1996) studied hydrolysis of DEB in liver and lung fractions from mice, rats, and humans. In this study only racemic DEB was studied, not the *meso* form of DEB. Microsomal and cytosolic fractions from livers and lungs of male B6C3F_1 mice and Sprague-Dawley rats and male and female humans were incubated with 0.18 to 15 mM (±)DEB. The human liver samples were obtained from trauma victims, and lung samples were obtained from cancer patients. Depletion of DEB from the incubation media was used to quantitate enzymatic hydrolysis of DEB. The authors reported greater hydrolysis in microsomes compared with cytosols, 51% versus 7.7% depletion of DEB in rats, and 29% versus 6.2% depletion in mice (not determined in humans). The depletion of DEB in liver microsomes from all three species was well described by Michaelis-Menten kinetics (Tables 3-4 to 3-6). The kinetics in the lung was less well described by Michaelis-Menton equations, but the fits obtained were highly significant (Tables 3-7 to 3-9). As with the data of Csanády, V_{max} and K_m estimated in this study are interrelated from the parameter estimation. Standard deviations are not given in the tables because the deviations listed by Bogaards and Bond supposedly were deviations in the parameter estimates, not interanimal variability (although the authors stated for the liver that multiple samples were done for each DEB concentration). Species differences were noted for hydrolysis of DEB. Using the ratio of V_{max}/K_m for comparison, hydrolysis in the liver was greatest in the human followed by rats then mice, whereas hydrolysis in the lung was greatest in humans followed by mice then rats. Only the mouse had higher hydrolysis in the lung compared with the liver. The variability of V_{max}/K_m in human liver microsomes was fourfold; however, the sample size was not large enough to address either gender or age-related diffferences. To assess nonenzymatic hydrolysis of DEB, depletion of DEB was measured in buffer, in heat-inactivated liver microsomes from rats, and in liver microsomes and cytosols from mice and rats in the

presence of 1,2-epoxycyclohexane, an inhibitor of microsomal epoxide hydrolase. The estimated half-life for nonenzymatic hydrolysis of DEB in 0.1 M potassium phosphate buffer was 21.8 ± 0.4 h (n = 4). No depletion of DEB was detected at 60 min of incubation either with heat-inactivated liver microsomes from rats or with liver microsomes from mice in the presence of 1,2-epoxycyclohexane. Depletion of DEB was 9.3% of the initial concentrations in incubations with liver microsomes from rats in the presence of 1,2-epoxycyclohexane. Although the authors reported that depletion of DEB in the cytosols of both species was not affected by 1,2-epoxycyclohexane, the data given in the article do not support these conclusions. DEB was not depleted during incubations with cytosols from mice and rats in the presence of 1,2-epoxycyclohexane, versus 6.2% and 7.7% depletion with cytosols from mice and rats, respectively, in the absence of 1,2-epoxycyclohexane. Some potential limitations of this study include problems with the lung data, in that limited concentration ranges were used for the experiments because of limited amounts of available samples and lower hydrolytic activity in the lung. In addition, the authors only quantitated erythritol and anhydroerythritol, which is a rearrangement product of EBD, but not EBD. The amount of erythritol detected in incubations from all three species and anhydroerythritol detected from humans was not great enough to balance the amount of DEB lost during the incubations; the authors did not give the percentage of disappearance of DEB that was accounted for by the generation of erythritol and anhydroerythritol.

Boogaard et al. (1996) studied glutathione conjugation of DEB in fractions from mice, rats, and humans. Again, in this study only racemic DEB was studied, not the meso form of DEB. Liver and lung cytosols from male B6C3F_1 mice and Sprague-Dawley rats and liver cytosols from male and female humans were incubated with 0.1 to 100 mM (±)DEB and 10 mM glycine-[2-^{3}H]glutathione. ^{13}C nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy were used to identify two isomeric conjugates, S-(1-(hydroxy-methyl)-2,3-epoxypropyl)glutathione and S-(2-hydroxy-3,4-epoxy-butyl)glutathione, which rapidly hydrolyzed to trihydroxy conjugates. The total conjugates were quantitated by high-pressure liquid chromatography (HPLC). The glutathione conjugation of DEB displayed Michaelis-Menton kinetics and the constants are given in Tables 3-4 to 3-8. The same limitations for data analysis from Boogaard and Bond (1996) are also applicable to this study. Conjugation of DEB with glutathione is more efficient in mice than in either rats or humans. DEB was shown to chemically react with glutathione in buffer at rates that averaged from approximately 1.3% to 50% of the enzymatically measured rates in tissues, depending on the species and tissue.

A recent study has estimated V_{max} and K_m for the kinetics of BDdiol oxidation in vitro in liver cytosols from male B6C3F_1 mice and Sprague-Dawley rats and male and female humans (Kemper and Elfarra, 1996) and found no significant species differences. The values of the

Michelis-Menten parameters are given in Tables 3-4 to 3-6. This oxidation represents only metabolism of BDdiol by alcohol dehydrogenase, although as shown in other studies, multiple pathways are responsible for biotransformation of this intermediate.

3.3.3.3. *In Vivo*

This section describes kinetic parameters estimated from in vivo exposures of mice and rats to 1,3-butadiene and its metabolites EB and DEB. Some of the parameters that will be discussed also describe rates of uptake and excretion, but are included in this section for clarity. The rates of metabolism that are discussed are for overall metabolism of 1,3-butadiene, EB, DEB, or BDdiol. In vivo kinetic parameters for specific biotransformation pathways have not been quantitated. This section excludes discussion of physiologically based pharmacokinetic models and the parameter values used in the models, because both are discussed in detail in Chapter 9. Consistent with the in vitro studies, mice have greater rates of metabolism of 1,3-butadiene and EB in vivo, which are due to differences in physiological parameters as well as biochemical parameters.

3.3.3.3.1. ***1,3-Butadiene.*** Bond et al. (1986) exposed mice and rats by inhalation to 1,3-[1-^{14}C]butadiene for 6 h at exposure concentrations ranging from 0.08 to 1,000 ppm and 0.08 to 7,100 ppm for mice and rats, respectively. Total radioactivity that was retained by the body was measured at the end of exposure in the animals. The measured radioactivity excluded the amounts excreted via all routes during exposure. The amount of radioactivity recovered from the animals at the end of the 6-h exposure was 4% to 20% and 1.5% to 17% of the inhaled amount of radioactivity for mice and rats, respectively. The authors defined the amount inhaled as a product of measured alveolar ventilation and air concentration of 1,3-butadiene. The percentage decreased with increasing exposure concentration, indicating saturation of metabolism.

Bolt et al. (1984) measured the concentrations of 1,3-butadiene in a closed inhalation chamber containing male Sprague-Dawley rats exposed by inhalation to initial concentrations of 1,3-butadiene ranging from 1,000 to 4,000 ppm or by intraperitoneal injections of approximately 1,000 mL gas/g body weight (approximately 41 nmol/g body weight). The data were used to estimate kinetic constants that described the disappearance of 1,3-butadiene from the chamber by uptake and metabolism in the rat according to a classical two compartment model (Filser and Bolt, 1979; Filser and Bolt, 1981; Bolt et al., 1981; Filser and Bolt, 1983). Bolt et al. showed that metabolism of 1,3-butadiene by the rat appears saturated above approximately 1,000 ppm. The inhalation uptake ($k_{12}V_1$) of 1,3-butadiene is similar to the metabolic clearance (Cl_{tot}) by the rats, 5,750 mL/h versus 4,490 mL/h, respectively, indicating that metabolism in the rat is

practically limited by the uptake of 1,3-butadiene. The maximum metabolic elimination rate (V_{max}) was 220 μmol/h/kg; K_m was not estimated from the experiments.

Kreiling et al. (1986b) measured the concentrations of 1,3-butadiene in a closed system that contained mice exposed by inhalation to initial concentrations of 1,3-butadiene ranging from 10 to 5,000 ppm, and analyzed the data as described by Bolt et al. (1984). In mice the pharmacokinetics were also first order below approximately 1,000 ppm 1,3-butadiene. As with rats, Cl_{tot} was similar in mice to $k_{12}V_1$, 7,300 versus 10,280 mL/h, respectively. The rates in mice were approximately twice the rates in rats. In addition, V_{max} was 400 μmol/h/kg, approximately twice the rate of rats. The coefficient of dynamic distribution (K_{st}) was also twice as high in mice compared with rats, 1.0 versus 0.5, respectively, which would mean that under exposure conditions where metabolism is first order the steady-state concentrations of 1,3-butadiene in the body of mice would be approximately twofold higher compared with rats. Overall, the results from these studies suggest that under low exposure concentrations, higher metabolic clearance of 1,3-butadiene to other metabolites will be expected in mice compared with rats because of higher respiration frequency and higher lung and liver perfusion.

Species differences have been noted in the extent of glutathione depletion in the tissues of animals exposed to 1,3-butadiene in vivo. Kreiling et al. (1987) exposed male $B6C3F_1$ mice and Sprague-Dawley rats in a closed inhalation system to saturable concentrations of 1,3-butadiene (2,000–4,000 ppm) for 15 h. The hepatic nonprotein sulfhydryl content in BD-exposed animals versus air-exposed control animals was 4% and 72% of unexposed controls, respectively, for mice and 76% and 88% of unexposed controls, respectively, for rats. Kreiling et al. (1988) and Laib et al. (1990) reported depletion of nonprotein sulfhydryl content in male $B6C3F_1$ mice, Sprague-Dawley rats, and Wistar rats exposed to saturable concentrations of 1,3-butadiene (>2,000 ppm) for 7 or 15 h in a closed inhalation system. The content in exposed animals was 78%, 66%, and 24% of unexposed controls at 7 h and 88%, 60%, and 4% of unexposed controls at 15 h, for Sprague-Dawley rats, Wistar rats, and $B6C3F_1$ mice, respectively. Deutschmann and Laib (1989) measured the effects of 1,3-butadiene exposure concentration on nonprotein sulfhydryl content in liver, lung, and heart tissue of male $B6C3F_1$ mice and Sprague-Dawley rats. The animals were exposed by inhalation for 7 h to 10, 50, 100, 250, 500, 1,000, or 2,000 ppm 1,3-butadiene, and nonprotein sulfhydryl content was determined in the tissues immediately following exposure. Mice showed dose-dependent depletion in all tissues, with the lung showing the greatest loss. In rats significant depletion was noted only in liver at the highest exposure concentration. Himmelstein et al. (1995) measured glutathione depletion in the liver and lungs of mice exposed to 62.5, 625, or 1,250 ppm 1,3-butadiene or in rats exposed to 62.5, 625, 1,250, or 8,000 ppm 1,3-butadiene. There was not a significant difference between rats and mice in depletion of glutathione in the liver; glutathione concentrations in the liver of exposed animals

were approximately 60% of controls in both species. In the lung there was a significant species difference in the depletion of glutathione. The depletion was reduced significantly at all exposure concentrations in mice, with the maximal depletion occuring at the 6 h timepoint in mice exposed to 1,250 ppm 1,3-butadiene. The maximum depletion resulted in glutathione concentrations that were approximately 26% of controls. In rats significant depletion of glutatione in the lung occurred during exposures to 1,250 and 8,000 ppm 1,3-butadiene, with maximal depletion occuring at the 6 h timepoint of both exposures. The maximum depletion resulted in glutathione concentrations that were 74% of controls.

3.3.3.3.2. ***EB.*** Filser and Bolt (1984) measured EB in the atmosphere of a closed-chamber inhalation system containing male Sprague-Dawley rats that were exposed either by inhalation chamber to initial concentrations of EB ranging from approximately 500 to 1,500 ppm or by intraperitoneal injection of 5.7 nmol EB/g body weight. In addition, the generation of EB was measured in the atmosphere of a closed inhalation system while 1,3-butadiene was maintained at a constant concentration of 1,500 ppm. A saturable level of metabolism of EB was not attained in the exposed rats. As with 1,3-butadiene, the uptake of EB was not limited by metabolism since $k_{12}V_1$ was similar to Cl_{tot}, 13,800 versus 13,400 mL/h, respectively. When the kinetic parameters from uptake studies with 1,3-butadiene were used with kinetic parameters for EB to predict concentrations of EB in the chamber atmosphere during exposures to 1,3-butadiene, the concentrations of EB were overpredicted by approximately fivefold. Filser and Bolt hypothesized that this may be due to an "intrahepatic first pass effect," where the EB formed from 1,3-butadiene is preferentially oxidized, hydrolyzed, or conjugated with glutathione and is not available systemically.

Kreiling et al. (1987) estimated in vivo pharmacokinetic parameters for EB in male $B6C3F_1$ mice that were exposed in closed chambers by inhalation to initial concentrations of 100–2,000 ppm EB or constant concentrations of 2,000–4,000 ppm 1,3-butadiene. The kinetic parameters for the previously described two-compartment system, $k_{12}V_1$ and Cl_{tot} , were 33,500 and 24,900 mL/h, respectively, indicating that unlike in rats, metabolism of EB, not uptake, limits clearance in mice. In addition, unlike in rats, metabolism of EB in mice appeared to be saturable above atmospheric concentrations of 500 ppm EB. As with the kinetics of 1,3-butadiene, mice had a higher K_{st} than rats, approximately 10-fold higher, because of higher inhalation uptake and lower elimination than rats. Therefore mice would be expected to have a higher body burden of EB than rats.

Valentine et al. (1997) quantitated classical pharmacokinetic parameters in male Sprague-Dawley rats that were administered 71, 143, and 286 μmol EB/kg body weight by intravenous injection. The generation of DEB in the blood was below the limit of detection for the method of

analysis. The AUC_0^∞ was increased significantly at the highest EB concentration indicating saturation of metabolism; however, neither the systemic clearance (Cl_s) nor the volume of distribution at steady state (V_{ss}) were decreased significantly. Since the V_{ss} and the volume of central compartment (V_c) were similar, EB was not distributed significantly from the central compartment. In addition Cl_s was equal to or greater than estimated liver perfusion in the rat at all doses indicating that extrahepatic clearance such as exhalation contributed to elimination of EB from the rat. The systemic clearance of EB in rats was less than the metabolic clearance reported by Kreiling et al. (1987), 67–114 mL/min/kg versus 223 mL/min/kg, respectively. The estimated terminal half life of EB ranged from 5.7 to 8.5 min. Species comparisons cannot be done with the work of Valentine et al. (1997) because only rats were studied.

3.3.3.3.3. ***DEB.*** Only one study to date has evaluated the in vivo pharmacokinetics of DEB (Valentine et al., 1997). Valentine and co-workers administered 523 μmol/kg DEB to male Sprague-Dawley rats intravenously and quantitated DEB in venous blood versus time. The data were used to estimate classical two-compartment pharmacokinetic parameters. The kinetic parameters for DEB were similar to EB in rats. DEB was not distributed significantly from the central compartment because the V_{ss} was similar to V_c. In addition Cl_s was approximately the estimated liver perfusion in the rat, indicating that clearance of DEB in rat was not limited by metabolism. The estimated terminal half-life of DEB was 13.8 min. Unfortunately, species comparisons cannot be done with the work of Valentine et al. because only rats were studied.

3.3.3.3.4. ***BDdiol.*** One study to date has quantitated the kinetics of BDdiol in vivo. Kemper et al. (1998) administered 10, 25, 50, 100, and 250 mg/kg body weight BDdiol to male $B6C3F_1$ mice intraperitoneally. Blood from the animals was collected at time points up to 4–8 h following dosing and was analyzed for BDdiol; urine was collected for 24 h postexposure and analyzed for BDdiol and metabolites. In addition mice were pretreated with either 4-methylpyrazole, an alcohol dehydrogenase inhibitor and P450 inhibitor, or benzylimidazole, a cytochrome P450 inhibitor, and administered 25 mg/kg BDdiol to study the effects of inhibition on BDdiol clearance. Although results were difficult to interpret from intraperitoneal data, Kemper et al. reported that plasma clearance of BDdiol appeared saturated at the higher administered doses. Hepatic and renal reduced nonprotein thiol was significantly depleted in mice exposed to 250 mg/kg BDdiol. Pretreatment with methylpyrazole provided some protection from thiol depletion.

3.4. DISTRIBUTION

To determine if differences in tissue concentrations of reactive metabolites correlate with differences in responses among species and tissues, several studies have measured the blood and tissue concentrations of 1,3-butadiene and its reactive metabolites EB and DEB during and following inhalation exposures to 1,3-butadiene (Bechtold et al., 1995; Bond et al., 1987; Dahl et al., 1991; Himmelstein et al., 1994, 1995; Thornton-Manning et al., 1995a,b; 1997, 1998). The results from the studies indicate that higher concentrations of reactive metabolites in target tissues will be achieved in mice versus rats because of differences in the rates of metabolism of 1,3-butadiene and its metabolites, which have been noted from in vitro and in vivo metabolism studies. In addition, investigators also have quantitated hemoglobin and DNA adducts of reactive metabolites in mice, rats, and humans exposed to 1,3-butadiene and found differences among the species.

The following section summarizes the research that illustrates species differences in the distribution of 1,3-butadiene and its reactive metabolites. None of the studies measuring blood and tissue concentrations of DEB in vivo have quantitated meso-DEB, only the enantiomeric forms (±)DEB. This section does not include the concentration reported in rodents as measured by Bond et al. (1986) by the technique of cryogenic trapping because of inaccuracies of the technique in comparison with GC-MS. Unfortunately, in monkeys cryogenic trapping was the only technique employed for quantitation of 1,3-butadiene and metabolites following inhalation exposures to 1,3-butadiene (Dahl et al., 1991). These results are included; however, the concentrations should not be compared with concentrations from rodents measured by a different technique. The analysis of data in tables from Dahl et al. (1990, 1991) is not included in this summary because of possible inaccuracies in the data. The authors, when comparing distribution of volatile and nonvolatile metabolites in blood, did not include the amount of CO_2 as a metabolite of 1,3-butadiene. In addition, as seen from Thornton-Manning et al. (1995a,b), the blood concentrations of metabolites may not be at steady state at 2 h, and therefore the comparison of blood concentrations at different exposure times may reflect differences in distribution as well as metabolism.

3.4.1. Blood and Tissue Concentrations

3.4.1.1. *Species Differences*

To determine if differences in distribution between rats and mice existed following inhalation exposures to 1,3-butadiene, Bond et al. (1987) measured the distribution of ^{14}C at 1, 2, 4, 8, 18, 27, 43, 51, and 67 h and 6, 10, and 13 days postexposure in male Sprague-Dawley rats following a 3.4-h nose-only inhalation exposure to 670 ppm 1,3-[1-^{14}C]butadiene and in male $B6C3F_1$ mice following a 3.4 h nose-only inhalation exposure to 65 ppm 1,3-[1-^{14}C]butadiene.

The radioactivity that remained at the time of tissue sampling was distributed widely throughout the body in both species. In both species at 1 h postexposure the highest concentration of radioactivity (expressed as nmol ^{14}C-butadiene equivalents/g tissue) was found in the bladder. However, none of the tissues appeared to accumulate 1,3-butadiene or its metabolites. At all timepoints following exposure, rats had higher concentrations of radioactivity in most tissues; however, when the tissue concentrations were normalized to the total amount of radioactivity that was inhaled, the normalized concentration in mice exceeded the rats. Except for the fat at 1, 2, and 4 h postexposure, the majority of the radioactivity that was measured in the tissues was due to nonvolatile metabolites. The study does not reveal if there were distributional differences in the concentrations of reactive metabolites because the radioactivity was not speciated. However, the study does show that 1,3-butadiene and its metabolites do not accumulate in the body to a significant extent, and that mice have a higher rate of metabolism than rats for conversion of 1,3-butadiene to other metabolites because the tissue concentrations of total radioactivity per amount of inhaled 1,3-butadiene were greater in mice than rats.

The blood and tissue concentrations of the reactive metabolites EB and DEB have been quantitated in mice and rats by several investigators (Bechtold et al., 1995; Himmelstein et al., 1994, 1995; Thornton-Manning et al., 1995a,b; 1997; 1998). None of the studies have quantitated specific enantiomers of EB and have quantitated only the racemic form of DEB, not the meso form. In all studies the blood and tissue concentrations of the reactive metabolites in mice exceeded the concentrations measured in rats. Figures 3-2 and 3-3 summarize the blood concentrations in male B6C3F$_1$ mice and Sprague-Dawley rats at 3, 4, and 6 h of inhalation exposure to concentrations of 1,3-butadiene ranging from 62.5 to 8,000 ppm. Tables 3-11 to 3-18 contain information related to blood and tissue concentrations of EB and DEB from the experiments described in more detail below.

Himmelstein et al. (1994) measured blood concentrations of 1,3-butadiene and metabolites during and up to 30 min following exposure in mice and rats exposed by nose-only inhalation to 62.5, 625, and 1,250 ppm 1,3-butadiene for 6 h. Steady-state blood concentrations of 1,3-butadiene were achieved in both species by 2 h. The blood concentrations in mice were approximately twice the blood concentrations in rats because of higher alveolar ventilation per gram of body weight of mice versus rats. In addition, steady-state blood concentrations of EB were attained in both species by 2 h of exposure. The blood concentrations of EB in mice were approximately 4–8 times higher than blood concentrations in rats. The blood concentrations of EB (mean ± s.d.) ranged from 0.56 ± 0.04 to 8.6 ± 0.6 μM and 0.07 ± 0.01 to 1.3 ± 0.09 μM in mice and rats, respectively. DEB was detected only in the blood of mice at concentrations ranging from 0.65 ± 0.10 to 2.5 ± 0.4 μM. The blood concentrations of DEB appeared to reach steady state during the exposure for 1,250 pm 1,3-butadiene exposure only.

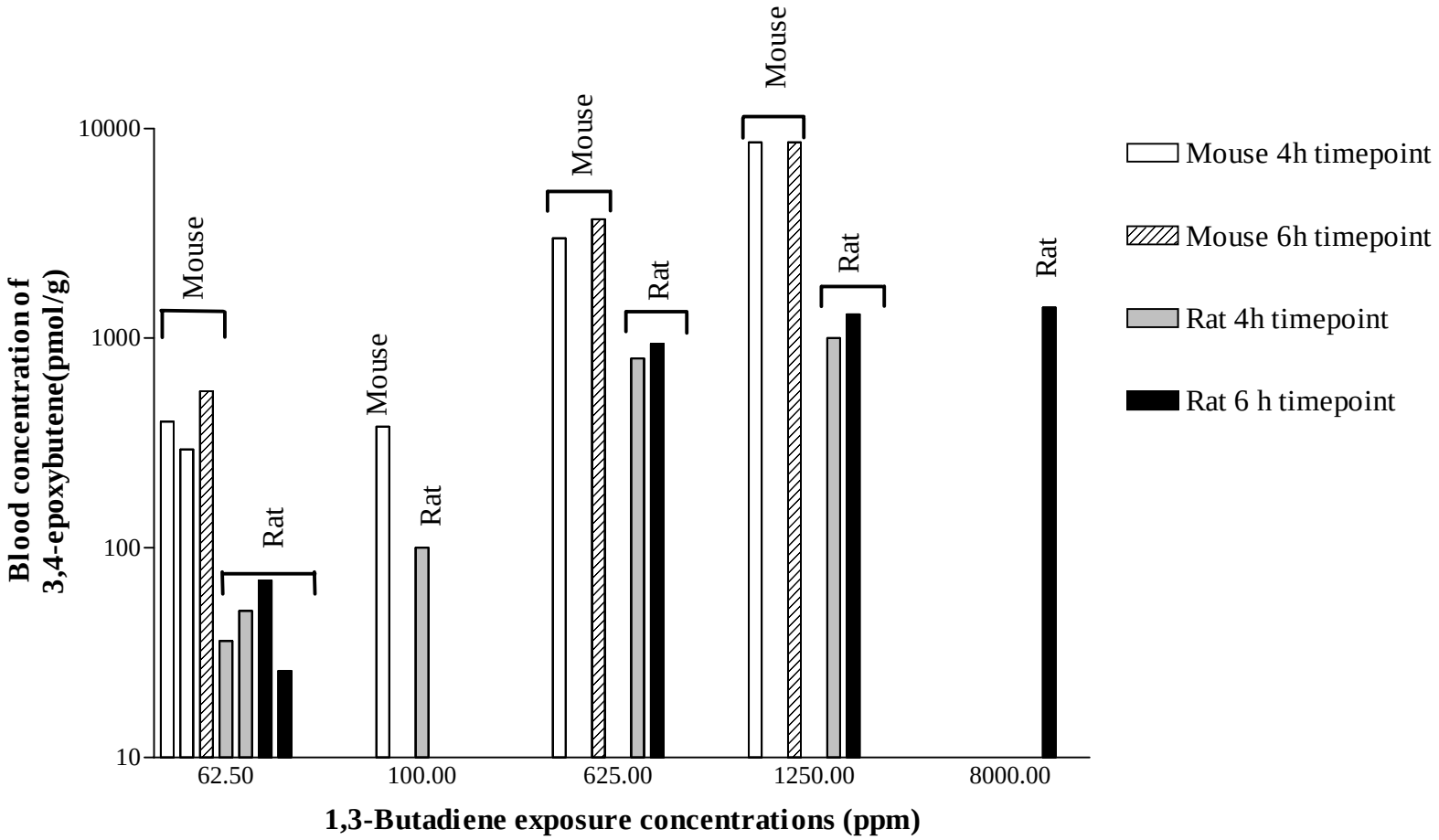

Figure 3-2. Comparison of blood concentrations of EB in male mice and rats[a].

[a]The data were obtained from Bechold et al. (1995), Himmelstein et al. (1994), Himmelstein et al. (1995), Thornton-Manning et al. (1995a), and Thornton-Manning et al. (1995b).

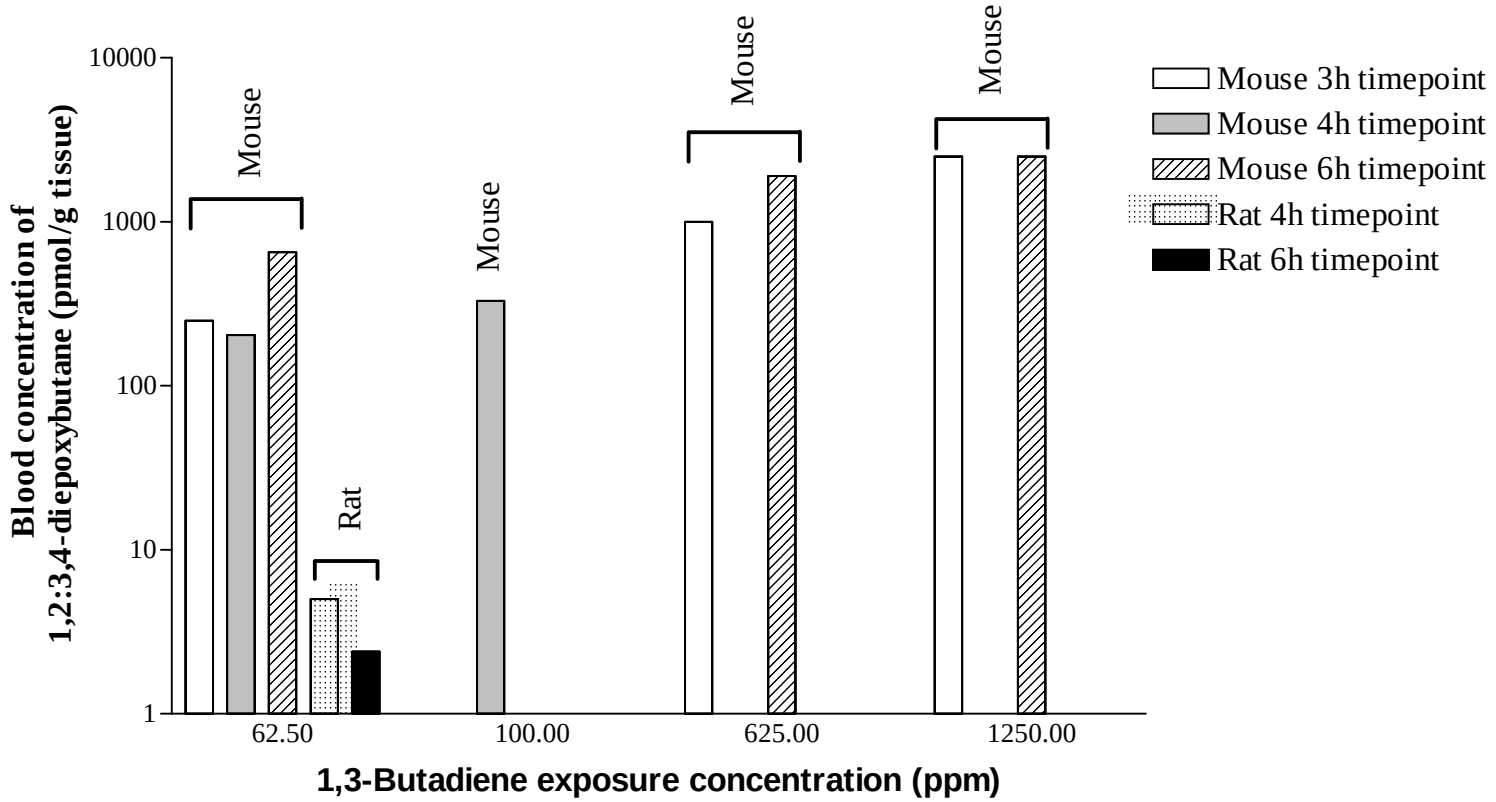

Figure 3-3. Comparison of blood concentrations of DEB in male mice and rats[a].

[a]The data were obtained from Bechtold et al. (1995), Himmelstein et al. (1994), Himmelstein et al. (1995), Thornton-Manning et al. (1995a), and Thornton-Manning et al. (1995b).

Another study by Bechtold et al. (1995) measured blood concentrations of EB and DEB in the blood of mice and rats exposed by nose-only inhalation to 100 ppm 1,3-butadiene. This study supported higher concentration of EB and DEB in the blood of mice compared with rats, consistent with the results of Himmelstein et al. (1994). However, this research used cryogenic isolation and gas chromatography–gas chromatography mass spectrometry (GC-GCMS) to achieve better sensitivity than Himmelstein (limits of detection twofold lower).

Himmelstein et al. (1995) also measured the concentrations of EB and DEB in lungs and livers of male B6C3F_1 mice exposed to 625 or 1,250 ppm 1,3-butadiene and in lungs and livers of male Sprague-Dawley rats exposed to 625, 1,250, or 8,000 ppm 1,3-butadiene by nose-only inhalation exposures. Limitations of this data include inaccuracy in reported concentrations as a result of the time lag from euthanasia to collection of tissue, which will result in loss of some of the parent and metabolites from the tissue. Since the rates of metabolism differ between mice and rats, the amount removed during the period of collection will differ, and therefore the difference in concentrations measured may not represent the true difference in concentrations during exposure. Maximum concentrations (mean ± s.d.) that were measured in the lungs of mice during the 625 ppm or 1,250 ppm exposures were 2,600 ± 200 and 3,700 ± 1,200 pmol/g tissue for EB, respectively, and 710 ± 60 and 1,500 ± 100 pmol/g tissue for DEB, respectively. EB, but not DEB, was detected in the lungs of rats, and the concentrations were approximately 11–16 times lower than in mice, 160 ± 30, 310 ± 70, and 1,300 ± 200 pmol/g tissue, for 625, 1,250, and 8,000 ppm 1,3-butadiene, respectively. In addition, EB, but not DEB, was detected in the livers from both mice and rats. Maximum concentrations measured in the livers of mice during the 625 ppm or 1,250 ppm exposures were 580 ± 120 and 930 ± 190 pmol /g tissue, respectively. Concentrations of EB in the livers of rats were approximately 9–11 times lower than mice, 60 ± 10, 160 ± 60, and 1,200 ± 100 pmol/g tissue, for 625, 1,250, and 8,000 ppm 1,3-butadiene, respectively.

Thornton-Manning et al. (1995a) also tried to address differences among species by measuring the concentrations of EB and DEB in blood and various tissues during and following nose-only inhalation exposure of male Sprague-Dawley rats and male B6C3F_1 mice to 62.5 ppm 1,3-butadiene for 4 h. The technique for blood collection was the same as that of Himmelstein et al. (1994); however, the method for tissue collection was different in that Thornton-Manning (1995a) froze the tissues in liquid nitrogen after collection and prepared them for analysis by GC-GCMS with a modification of a vacuum line-cryogenic distillation method (Dahl et al., 1984). In addition the method of analysis for 1,3-butadiene, EB, and DEB by GC-GCMS differed from Himmelstein et al. (1994 and 1995), resulting in a lower limit of detection for DEB (reported detection limits for tissues were 11–1,300 times lower than limits reported by Himmelstein and co-workers). This lower limit of detection allowed for detection of DEB in the blood of rats

exposed to 1,3-butadiene. However, the paper reported two different limits of detection for EB and DEB and did not explain the difference. Some of the values (mouse lung EB concentrations, and rat heart and lung DEB concentrations) reported by Thornton-Manning were below the reported limits of detection conducted via the EPA-approved procedure. The same limitations for interpretation of the data also apply with this research because of loss of compound from time of euthanasia to tissue collection. Thornton-Manning et al. quantitated EB and DEB in blood, heart, lung liver, fat, spleen, thymus, and bone marrow. Some tissues had undetectable amounts of EB (rat lung and liver) and DEB (rat liver and bone marrow). Table 3-11 reports measured concentrations. The concentrations were used to calculate tissue:blood concentration ratios to illustrate relative to blood which tissues had the greatest accumulation of reactive metabolites (Table 3-12). For EB the highest concentrations were in fat in both species, and for DEB the highest concentrations were reported in blood in both species. Consistent with Himmelstein et al. (1995), mice had higher tissue concentrations of reactive metabolites compared with rats. The ratio of tissue concentrations of EB in mice versus rats ranged from 3 to 11.5, with the highest concentration difference occurring in bone marrow. The ratio of tissue concentrations of DEB in mice versus rats ranged from 4 to 163, with the highest concentration difference occurring in lung (Table 3-13).

Although the higher concentrations of DEB in mouse blood compared with rat blood are consistent with Himmelstein et al. (1994), the mouse blood DEB concentrations reported by Thornton-Manning (1995a) appear to be approximately half the concentrations reported by Himmelstein et al. (1994). Thornton-Manning (1995a) reported blood concentrations of DEB at 2 h and 4 h of exposure of 100 and 204 pmol/g versus reported concentrations at 3 h and 6 h by Himmelstein (1994) of 200 and 695 pmol/g. If the increase of DEB versus time were linear, a blood concentration of 365 pmol/g would be predicted for 4 h exposure from Himmelstein's data. Part of the difference may result from differences in the actual exposure concentrations, approximately 2.1 versus 2.8 μmol/L 1,3-butadiene, for Thornton-Manning and Himmelstein, respectively. These concentrations were calculated from actual exposure concentrations expressed in ppm and the estimated molar volume for each laboratory.

The tissue:blood concentration ratios in mice (Table 3-12) were less than the reported partition coefficients (Table 3-1) for EB in the liver and lung and for DEB in the liver and fat. The tissue:blood concentration ratio of EB in fat was greater than the reported partition coefficient. Other tissues could not be compared because the partition coefficients have not been reported. Concentrations of EB have been shown to reach steady state in the body within 4 h; therefore, the ratios in the liver and lung are lower than the partition coefficients, probably

Table 3-11. Tissue:blood concentrations (pmol/g tissue)[a].

	3,4-Epoxybutene		1,2,3,4-Diepoxybutane	
Tissue	**Rats**	**Mice**	**Rats**	**Mice**
Blood	36 ± 7	295 ± 27	5 ± 1	204 ± 15
Heart	40 ± 16	120 ± 15	3 ± 0.4	144 ± 16
Lung	ND[b]	33 ± 9	0.7 ± 0.2	114 ± 37
Liver	ND	8 ± 4	ND	20 ± 4
Fat	267 ± 14	1,302 ± 213	2.6 ± 0.4	98 ± 15
Spleen	7 ± 6	40 ± 19	1.7 ± 0.5	95 ± 12
Thymus	12.5 ± 3.2	104 ± 55	2.7 ± 0.7	109 ± 19
Bone marrow	0.2 ± 0.1	2.3 ± 1.5	ND	1.4 ± 0.3

[a]The concentrations of EB and DEB (mean ± SE; n=3 or 4) in each of the tissues of male Sprague-Dawley rats and male B6C3F$_1$ mice following a 4 h exposure to 62.5 ppm 1,3-butadiene were taken from Thornton-Manning et al. (1995a).
[b]ND is reported for tissues where the concentration of the analyte was below the limit of detection.

Table 3-12. Tissue:blood concentration ratios[a].

	3,4-Epoxybutene		1,2,3,4-Diepoxybutane	
Tissue	**Rats**	**Mice**	**Rats**	**Mice**
Heart	1.1	0.41	0.6	0.71
Lung	ND[b]	0.11	0.14	0.56
Liver	ND	0.027	ND	0.098
Fat	7.4	4.4	0.52	0.48
Spleen	0.19	0.14	0.34	0.47
Thymus	0.35	0.35	0.54	0.54
Bone Marrow	0.006	0.008	ND	0.007

[a]The ratios were determined from the corresponding tissue and blood concentrations of EB and DEB in each of the tissues of male Sprague-Dawley rats and male B6C3F$_1$ mice following a 4 h exposure to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1995a).
[b]ND is reported for tissues where the concentration of the analyte was below the limit of detection.

Table 3-13. Mouse:rat concentration ratios[a].

Tissue	3,4-Epoxybutene	1,2,3,4-Diepoxybutane
Blood	8.2	41
Heart	3	48
Lung	ND[b]	163
Liver	ND	ND
Fat	4.9	38
Spleen	5.7	56
Thymus	8.3	4
Bone marrow	11.5	ND

[a]The ratios were determined from the corresponding tissue and blood concentrations of EB and DEB in each of the tissues of male Sprague-Dawley rats and male B6C3F$_1$ mice following a 4-h exposure to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1995a).
[b]ND is reported for tissues where the concentration was not available for the rat.

because of metabolism of the compound in these tissues. For DEB, steady state was not achieved during the 4-h exposure; therefore, the lower ratios could be caused by distribution of the compound through the body or because of metabolism in these tissues.

The more accurate methodology of GC-MS has not been performed for the blood and tissues of monkeys exposed to 1,3-butadiene by inhalation. The blood and tissue of monkeys have been analyzed by a technique called cryogenic trapping to quantitate CO_2, 1,3-butadiene, EB, and DEB and BDdiol (Dahl et al., 1991). The technique has limitations in that the trapping of compounds may not be specific; therefore, the concentrations cannot be compared with rodent data quantitated by GC-MS. Dahl et al. (1991) exposed monkeys for 2 h by inhalation to 1,3-[1-^{14}C]butadiene at concentrations of 10.1, 310, or 7,760 ppm. The fraction of radioactivity attributed to nonvolatile metabolites represented the majority of metabolites quantitated in blood, which was 87% and 54% of total radioactivity in blood at 10 and 310 ppm 1,3-butadiene, respectively. However, at 7,760 ppm 1,3-butadiene, the fraction attributed to 1,3-butadiene represented the majority of material quantitated in blood, 68% of total radioactivity. The fraction attributed to CO_2 was a larger percentage of the total radioactivity in blood than either EB or DEB. The fraction attributed to EB was 1.6 ± 0.5, 500 ± 280, and 1,100 ± 500 pmol 1,3-[^{14}C]butadiene equivalents/mL blood (mean ± SE, n = 3) for the exposures to 10.1, 310, or 7,760 ppm 1,3-butadiene, respectively. The fraction attributed to a combination of DEB and BDdiol

was 1.9 ± 0.6, 290 ± 170, and 590 ± 240 pmol 1,3-[^{14}C]butadiene equivalents/mL blood (mean ± SE, *n* = 3) for the exposures to 10.1, 310, or 7,760 ppm 1,3-butadiene, respectively. These two fractions combined represented a total of 1%, 17.3%, and 4.7% of the radioactivity quantitated in the blood for the 10.1, 310, and 7,760 ppm 1,3-butadiene, respectively.

3.4.1.2. *Gender Differences*

The potential gender differences in the kinetics of 1,3-butadiene has been researched to a limited extent. Thornton-Manning et al. (1995b) examined gender differences in the distribution of EB and DEB by determining tissue (blood, femur, lung, and liver) concentrations of EB and DEB in male and female Sprague-Dawley rats exposed by nose-only inhalation to 62.5 ppm of 1,3-butadiene for 6 h. For EB only the lung concentration differed significantly between male and female rats (Table 3-14). The concentration of EB in the lungs of male rats was five times higher than in female rats. The highest concentrations of EB were observed in the adipose tissues of both sexes. In contrast, all tissue concentrations of the DEB were greater in females than in males. The concentrations of DEB in blood from female rats were 4.75-fold greater than in blood from male rats. The greatest gender difference was seen in the concentration of DEB in adipose tissue, with females having a sevenfold greater tissue concentration than males. The mammary tissue of females also contained relatively high concentrations of DEB. However, the data in this study are not consistent with results from previous studies. The concentrations of DEB in blood, femur, and fat from male rats in this study following a 6-h exposure were approximately 50% of the concentrations from a previously reported 4-h exposure (Thornton-Manning et al., 1995a), and the lung concentrations of DEB were approximately twofold higher for the 6-h exposure compared with the 4-h exposure.

Thornton-Manning et al. (1995b) suggest that the greater production of the highly mutagenic DEB in females may play a role in the increased incidence of mammary tumors observed in a chronic carcinogenicity study with rats (Owen et al., 1987). However, in contrast with the rats, the blood concentrations of EB and DEB in female B6C3F$_1$ mice are only 50% of the concentrations in male mice following nose-only inhalation exposure for 6 h to 62.5 ppm 1,3-butadiene (data obtained from Himmelstein et al., 1994, and Thornton-Manning et al., 1997). Therefore, the gender differences noted from the rats cannot be generalized to all species.

3.4.1.3. *Chronic Exposures*

An important issue concerning distribution of 1,3-butadiene and its metabolites is whether distribution is altered by chronic exposures. This is important because the cancer bioassays are repeated daily exposures. Bond et al. (1988) showed reduced metabolism of

Table 3-14. Female:male concentration ratio[a].

	3,4-Epoxybutene		1,2,3,4-Diepoxybutane	
Tissue	**Rats[b]**	**Mice[c]**	**Rats[b]**	**Mice[c]**
Blood	1.1	0.43	4.8	0.49
Femur	1.1	ND[d]	6.4	ND
Lung	0.21	ND	3.4	ND
Fat	1.1	ND	7	ND

[a]Ratios of the tissue concentrations of EB and DEB in female and male Sprague-Dawley rats and $B6C3F_1$ mice exposed for 6 h by nose-only inhalation to 62.5 ppm 1,3-butadiene.
[b]The tissue concentrations for both male and female rats were obtained from Thornton-Manning et al. (1995b).
[c]The blood concentration was obtained from Himmelstein et al. (1994) for male mice and from Thornton-Manning et al. (1995b) for female mice.
[d]ND is reported for tissues where data were not available for one of the genders.

1,3-butadiene in vitro in lung microsomal incubations from rats and mice repeatedly exposed to 1,3-butadiene by inhalation.

To address the effects of chronic exposures, Thornton-Manning et al. (1997) exposed female Sprague-Dawley rats and $B6C3F_1$ mice by nose-only inhalation exposure to 62.5 ppm 1,3-butadiene either for a single 6-h exposure or for 6 h/day for 10 days. Concentrations of EB and DEB were analyzed in the blood, femur, mammary gland, fat, and ovary immediately following exposure as described previously (Thornton-Manning et al., 1995a). Table 3-15 shows the concentrations of EB and DEB in the blood of both species following either a single or repeated exposure to 1,3-butadiene (In the table, the blood concentrations for rats following a single exposure are the values reported in Thornton-Manning et al. [1995b] because the authors stated that this was where the data were obtained. However, the values reported in Thornton-Manning et al. [1997] for blood for the rat were greater than in the original citation, 44 versus 29.4 pmol/g for EB and 14 versus 11.4 pmol/g for DEB. The authors gave no explanation for the discrepancy.) Although blood concentrations of EB increased in both species following repeated exposures, the increase was not statistically significant. Again, although not statistically significant, blood concentrations of DEB in the rat increased following repeated exposures, whereas blood concentrations of DEB in the mouse decreased. The authors found statistically significant increases in concentration of EB in mammary tissue and fat in both mice and rats and in femurs of rats. The authors found statistically significant increases in concentration of DEB in

Table 3-15. Blood concentrations of reactive metabolites (pmol/g tissue)[a].

Species	3,4-Epoxybutene		1,2,3,4-Diepoxybutane	
	Single exposure	Repeated exposure	Single exposure	Repeated exposure
Rat	29.4 ± 2.0[b]	64 ± 8	11.4 ± 2[b]	17 ± 2
Mouse	239 ± 24	317 ± 19	345 ± 33	247 ± 32

[a]Blood concentrations (mean ± SE, *n* = 3–6) of EB or DEB in female Sprague-Dawley rats and B6C3F$_1$ mice following a single 6-h nose-only inhalation exposure or following repeated 6-h nose-only inhalation exposures for 10 days to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1997).
[b]Original value that was reported in Thornton-Manning et al. (1995b).

the mammary gland of rats and statistically significant decreases in concentration of DEB in the lung, fat, and mammary glands of mice. Tissue:blood concentration ratios calculated from the concentrations of EB and DEB illustrate that distribution of EB and DEB changes from single and repeated exposures (Tables 3-16 and 3-17). However, the experiments conducted in Thornton-Manning et al. (1997) cannot address whether these differences resulted from changes in the rates of formation or degradation of EB and DEB following repeated exposures or reflected the accumulation of the compounds in tissues as the analytes approached steady-state following repeated exposures. In addition, as seen in Table 3-18, the female mouse:rat concentration ratios of EB and DEB decrease with repeated inhalation exposure to 1,3-butadiene

Another study (Thornton-Manning et al., 1998) showed different results in female rats exposed to higher concentrations of 1,3-butadiene. The study reported blood concentrations of EB and DEB from female Sprague-Dawley rats exposed by nose-only inhalation to 8,000 ppm 1,3-butadiene for 8 h or repeatedly for 8 h/day for 10 days. In contrast to the 62.5 ppm 1,3-butadiene exposures, the blood concentration of EB decreased, 4,030 ± 190 versus 3,310 ± 260 pmol/g tissue, for single and repeated exposures to 8,000 ppm 1,3-butadiene, respectively, but the decrease was not statistically significant. Consistent with the 62.5 ppm 1,3-butadiene exposures, blood concentration of DEB increased significantly, 11 ± 1 versus 17 ± 1 pmol/g tissue, for single and repeated exposures to 8,000 ppm, respectively.

An interesting result from the two previously described chronic studies by Thornton-Manning et al. (1997, 1998) is the comparison of blood and tissue concentrations of reactive metabolites of 1,3-butadiene. In both studies, concentrations of EB and DEB were measured in blood, femur, lung, mammary gland, fat, heart, uterus, and ovary. Tissue and blood concentrations of EB were significantly greater in female rats exposed repeatedly to 8,000 ppm

Table 3-16. 3,4-Epoxybutene tissue:blood concentration ratios[a].

Tissue	Rats		Mice	
	Single exposure	Repeated exposure	Single exposure	Repeated exposure
Femur	0.34	0.23	0.24	NR[b]
Lung	0.11	ND[c]	0.11	0.47
Mammary gland	1.95	4.8	3.1	3.8
Fat	7.1	7	5	5.3

[a]The ratios were calculated from blood and tissue concentrations in female Sprague-Dawley rats and B6C3F$_1$ mice following a single 6-h nose-only inhalation exposure or following repeated 6-h nose-only inhalation exposures for 10 days to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1997). The blood concentrations used in the calculations are given in Table 3-14.
[b]NR is reported for the tissues in which EB was not reported.
[c]ND is reported for the tissues in which EB was not detected.

Table 3-17. 1,2,3,4-Diepoxybutane tissue:blood concentration ratios[a].

Tissue	Rats		Mice	
	Single exposure	Repeated exposure	Single exposure	Repeated exposure
Femur	0.64	0.53	0.62	0.74
Lung	0.43	0.25	0.63	0.58
Mammary gland	0.95	0.87	0.77	0.77
Ovary	0.56	0.56	0.49	0.61
Fat	0.7	0.72	0.59	0.7

[a]The ratios were calculated from blood and tissue concentrations in female Sprague-Dawley rats and B6C3F$_1$ mice following a single 6-h nose-only inhalation exposure or following repeated 6-h nose-only inhalation exposures for 10 days to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1997). The blood concentrations used in the calculations are given in Table 3-14.

Table 3-18. Mouse:rat concentration ratios.

Tissue	3,4-Epoxybutene[a]		1,2,3,4-Diepoxybutane[a]	
	Single exposure	Repeated exposure	Single exposure	Repeated exposure
Blood	8.1[b]	5	30[b]	15
Femur	5.7	NR[c]	31	20
Lung	8	ND[d]	43	36
Mammary	13	3.9	24	13
Ovary			28	15
Fat	5.6	3.7	25	13

[a]Mouse:rat ratios were determined from tissue concentrations of EB and DEB in female Sprague-Dawley rats and female $B6C3F_1$ mice following a single 6-h nose-only inhalation exposure or following repeated 6-h nose-only inhalation exposures for 10 days to 62.5 ppm 1,3-butadiene (Thornton-Manning et al., 1997).
[b]Although Thornton-Manning et al. (1997) reported blood concentrations of DEB in rats as 14 pmol/g respectively following a single 6-h exposure to 62.5 ppm 1,3-butadiene, the blood concentration (11.4 pmol/g) from the original work (Thornton-Manning et al., 1995b) from which the concentration was originally reported is used to generate this table. The reason for the discrepancy between the two papers is not clear.
[c]NR is reported for the tissues in which EB was not reported.
[d]ND is reported for the tissues in which EB was not detected.

compared with female rats exposed repeatedly to 62.5 ppm 1,3-butadiene. However, the blood and fat concentrations of DEB were not significantly different, the mammary gland and femur concentrations of DEB decreased significantly, and the lung concentration increased significantly in female rats repeatedly exposed to 8,000 ppm 1,3-butadiene compared with female rats exposed repeatedly to 62.5 ppm 1,3-butadiene.

3.4.2. Molecular Dosimetry

Several investigators have examined binding of the metabolites of 1,3-butadiene to hemoglobin, DNA, and nucleoproteins to correlate with the varying toxicity noted among animal species and to develop an internal marker for use as a dosimeter to measure 1,3-butadiene exposure in humans. Early work focused mainly on quantitation of total radioactivity associated with hemoglobin, DNA, or nucleoproteins in tissues following in vivo exposures to 1,3-butadiene, and more recent work has focused on identification and quantification of specific adducts of hemoglobin and DNA. The studies note species differences in concentration of the

molecular markers, with the differences most pronounced at higher concentrations and more similarity at lower concentrations. The differences are not as significant as the species differences in toxic responses. A marker that is specific for the internal dose of DEB has not been identified. This section includes only in vivo research that studied the association between the exposure concentration of 1,3-butadiene and specific biomarker concentrations as well as species differences in concentrations of the biomarkers for a given 1,3-butadiene exposure concentration.

3.4.2.1. *Hemoglobin Adducts*

Several investigators have studied the use of hemoglobin adducts as biomarkers of exposure to 1,3-butadiene. Most of the studies to date have quantitated adducts formed by EB, although some recent studies have quantitated hemoglobin adducts resulting from either EBD or DEB conjugation with hemoglobin. Several investigators have noted species differences in hemoglobin adduct formation from metabolites of 1,3-butadiene.

Sun et al. (1989b) injected male B6C3F$_1$ mice and male Sprague-Dawley rats intraperitoneally with 1,3-[^{14}C]-BD at doses of 1, 10, 100, or 1,000 μmol/kg, and hemoglobin adduct formation was quantitated at 24 h after exposure. Hb adduct concentrations were quantitated by measuring total ^{14}C associated with globin isolated from the collected blood samples and was not metabolite specific. Hb adduct formation was linearly related to doses up to 100 μmol/kg for both species. The Hb adducts accumulated linearly after repeated injections of 100 μmol/kg for 3 days. The BD-derived Hb adducts showed lifetimes of approximately 24 and 65 days in mice and rats, respectively, which correlates with the lifetimes of red blood cells. In this study rats were four to five times more efficient in forming hemoglobin adducts compared with mice; however, this may be because of the route of administration and may not reflect the dosimetry that would be expected following inhalation exposures.

Osterman-Golkar et al. (1991) investigated binding of EB to hemoglobin as a potential indicator of 1,3-butadiene exposure. Osterman-Golkar and co-workers exposed male Wistar rats by inhalation to 1,3-butadiene at concentrations of 250, 500, or 1,000 ppm for 6 h/day, 5 days/week for 2 weeks. Adducts of EB at the N-terminal valine in hemoglobin were analyzed by the N-alkyl Edman procedure followed by quantitation with GC-MS of the thiohydantoin derivatives. The investigators only quantitated adducts formed from binding at carbon 1 of the enantiomers of EB. In this study, the hemoglobin adduct formation was stable and increased linearly with exposure concentration, proving to be a useful dosimeter for long-term exposure to 1,3-butadiene. However, the authors noted that the quantitation method used in the study lacks the sensitivity to detect the low levels of adducts that would be expected in humans from occupational exposure to 1,3-butadiene.

Albrecht et al. (1993) also studied the formation of adducts from EB in female Wistar rats and CB6F1 mice exposed to 0, 50, 200, 500 or 1,300 ppm 1,3-butadiene by inhalation for 6 h/day for 5 days. Hemoglobin was isolated 18 h following the last exposure, and N-terminal valine adducts were isolated by a modified Edman degradation procedure. The two regioisomers formed from binding of EB at either carbon 1 or 2 to valine were quantitated, but the diastereomers for each regioisomer were not resolved by the method used. The hemoglobin adducts quantitated in mice were approximately three to six times greater than in rats. The rats exhibited a linear dose-dependent increase in adduct concentration up to exposure concentrations of 1,300 ppm 1,3-butadiene, whereas the mice exhibited a nonlinear dose-dependent increase in adduct concentration.

Osterman-Golkar et al. (1998) measured N-terminal valine adducts of EB in male B6C3F_1 mice and Sprague-Dawley rats exposed by inhalation to 0, 2, 10, or 100 ppm 1,3-butadiene 6 h/day, 5 days/week for 1, 2, 3, or 4 weeks. In the study, the diastereomers formed from binding at carbon 1 of EB with valine, N-(2-hydroxy-3-butenyl)valine, were quantitated, and the amount of adducts from carbon 2 was based on the ratio of adducts that had been measured from other in vitro and in vivo experiments. In contrast to Albrecht et al. (1993), the concentrations of adducts increased linearly with 1,3-butadiene exposure concentration in mice but not in rats. The adduct levels were approximately four times greater in mice compared with rats at 100 ppm 1,3-butadiene, but at lower 1,3-butadiene exposure concentrations the adducts were similar. The authors reported that blood concentrations of EB estimated from the hemoglobin adduct levels agreed well with reported concentrations of EB in BD-exposed mice and rats (Himmelstein et al., 1994; Thornton-Manning et al., 1995a).

Swenberg et al. (2000) reported hemoglobin adducts derived from EB. The investigators measured diastereomers formed from conjugation at carbon 1 and carbon 2 of EB that were approximately two times greater in female and male B6C3F_1 mice compared with Crl:CDBR rats exposed by inhalation to 1,000 ppm 1,3-butadiene 6 h/day, 5 days/week for 13 weeks.

Recent work has focused on developing methods to measure hemoglobin adducts from DEB and EBD. Pérez et al. (1997) developed a method to measure N-(2,3,4-trihydroxybutyl)valine adducts, which can be formed from reaction of either DEB or EBD with N-terminal valine. In male Wistar rats exposed to 0, 50, 200, or 500 ppm 1,3-butadiene by inhalation 6 h/day for 5 days, the concentrations of two of the stereoisomeric forms of N-(2,3,4-trihydroxybutyl)valine were approximately 3–32 times greater than the hemoglobin adducts formed by EB at carbon 1. The ratio was greatest at the lowest 1,3-butadiene exposure concentration. This suggests that the majority of the trihydroxyvaline adducts were formed from EBD, because EB blood concentrations in rodents exceed DEB concentrations.

Gender differences have not been noted in studies with hemoglobin adducts from EB or trihydroxybutyl adducts from EBD or DEB. In the previously described study of Albrecht et al. (1993), the investigators also compared adduct formation in male and female C3HX101/EL mice exposed by inhalation to 0–500 ppm 1,3-butadiene (specifics of exposures were not given) and did not find a gender difference in adduct formation. The results for female Wistar rats reported by Albrecht et al. (1993) cannot be compared with those of Osterman-Golkar et al. (1991) for male Wistar rats to examine gender differences in rats for several reasons: (a) only one exposure concentration was comparable, (b) Albrecht quantitated both regioisomers of EB whereas Osteman-Golkar quantitated only one, and (c) the duration of the exposures differed between studies. Tretyakova et al. (1996) reported greater concentrations of N-terminal valine adducts from EB in female $B6C3F_1$ mice compared with male mice exposed to 1,000 ppm 1,3-butadiene 6 h/day, 5 days/week for 13 weeks; however, the authors did not state whether the difference was statistically significant and the variation in the data was as high as 64%. In a Health Effects Institute (HEI) report from Swenberg et al. (2000), male and female $B6C3F_1$ mice and Sprague-Dawley rats were exposed by inhalation to 1,000 ppm 1,3-butadiene 6 h/day, 5 days/week for 13 weeks. There was not a statistically significant difference in the concentration of hemoglobin adducts from EB (total included both regioisomers) or of trihydroxybutyl adducts formed from either DEB or EBD between genders of either species.

Studies of molecular dosimetry in exposed workers are limited. Osterman-Golkar et al. (1996) studied hemoglobin adducts in 17 workers exposed to 1,3-butadiene in a petrochemical plant and nine referents employed at the same factory but not exposed to 1,3-butadiene. With stationary and personal monitoring devices, the ambient 1,3-butadiene concentrations for workers handling 1,3-butadiene containers was 11.2 ± 18.6 mg/m^3 (approximately 5 ppm) versus ≤ 1.2 mg/m^3 (approximately 0.5 ppm) for maintenance and laboratory workers. The Hb adduct measured was 2-hydroxy-3-butylvaline, formed by reaction of N-terminal valine with carbon 1 in EB. Higher concentrations of Hb adducts (0.16 ± 0.099 pmol/g) were recorded in the workers handling 1,3-butadiene containers compared with maintenance workers, laboratory workers, and nine unexposed controls (approximately 0.05 pmol/g).

Pérez et al. (1997) detected the trihydroxybutylvaline adducts in occupationally exposed humans. Concentrations of trihydroxyvaline adducts in two humans occupationally exposed to approximately 1 ppm 1,3-butadiene were 10 and 14 pmol/g globin compared with two human control values of 1.8 and 3.3 pmol/g globin. An adduct of EB, N-(2-hydroxy-3-butenyl)valine, was measured in the same individuals; 0.15 and 0.2 pmol/g for exposed versus 0.05 and 0.02 pmol/g for controls. Consistent with rodents, trihydroxybutylvaline adducts were greater than EB adducts by 70-fold.

In an HEI report by Swenberg et al. (2000), N-(2,3,4-trihydroxybutyl)valine adducts were measured in exposed Chinese workers and compared with concentrations in nonexposed U.S. laboratory workers. The mean total amount of trihydroxybutylvaline adducts in the U.S. laboratory workers was 36 ± 23 pmol/g globin for nonsmokers (n=7) and 40 ± 9 for smokers (n=4) compared with 39 ± 13 in a control set of Chinese workers (n=25). Swenberg et al. reported that the control values were overestimations because 15/51 controls had concentrations below the method's limit of detection. The BD-exposed Chinese workers had concentrations of trihydroxybutylvaline adducts of 88 ± 59 pmol/g globin. The difference between control and exposed Chinese workers was highly significant ($p<0.001$).

3.4.2.2. *DNA Adducts*

Research has also focused on the use of DNA adducts as biomarkers of 1,3-butadiene exposure. Investigators have attempted to quantitate adducts from EB, EBD, and DEB. More recent studies have shown that the guanine adducts formed from either EBD or DEB predominate over the guanine adducts formed from EB, indicating that EBD may be a major metabolite in vivo. As with the hemoglobin adducts, an adduct specific for DEB has not been quantitated in vivo in animals exposed by inhalation to 1,3-butadiene.

Kreiling et al. (1986a) reported the radioactivity associated with liver nucleoproteins and DNA after exposure of male B6C3F$_1$ mice and Wistar rats in a closed inhalation system to 1,3-[1,4-^{14}C]butadiene until the animals took up 0.21 mmol of 1,3-butadiene (4 h and 6 h, for mice and rats, respectively). The livers were collected 30 h after exposure for analysis of radioactivity associated with hepatic nucleoproteins and DNA. The mice had approximately twice as much radioactivity associated with nucleoproteins as rats, but the amount of radioactivity associated with DNA was similar between species. The nature of the chemical species associated with the two fractions was not characterized.

Jelitto et al. (1989) reported species-dependent differences in the in vivo formation of DNA adducts by male B6C3F$_1$ mice and male Wistar rats exposed in a closed inhalation system to initial concentrations of 500 ppm 1,3-[1,4-^{14}C]butadiene until 98% of total radioactivity was eliminated from the headspace. Liver DNA was isolated and qualitatively analyzed for N7 guanine adducts of EB and DEB (although the adduct monitored could be formed from EBD also) by alkaline elution and comparison of HPLC profiles with synthesized adduct standards. Jelitto and co-workers identified 7-N-(2-hydroxy-3-1-yl)guanine (adduct of EB at carbon 1) and 7-N-(2,3,4-trihydroxybutyl)guanine (adduct of either EBD or DEB) in livers from mice but not rats. Jelitto also exposed male Sprague-Dawley rats and B6C3F$_1$ mice for 7 h to 250, 500, and 1,000 ppm 1,3-butadiene (although not stated, the exposure is assumed to be a constant concentration) and immediately after exposure isolated cell nuclei from liver and lung tissue to

analyze by alkaline elution. The tissue from mice but not rats showed protein-DNA and DNA-DNA cross-links for all exposures.

Two studies have investigated the enantioselective formation of EB-derived DNA adducts. Koivisto et al. (1997) exposed male Sprague-Dawley rats to 200 ppm 1,3-butadiene by inhalation 6 h/day for 5 days and isolated DNA from liver to quantitate the specific enantio- and regioisomeric adduct formation at the N-7 position of guanine. The adducts were quantitated by means of a ^{32}P-postlabeling/HPLC assay. The major adduct formed was the carbon 2 isomer derived from the R enantiomer of EB (47% of all N-7 guanine adducts). The other three adducts were the carbon 1 isomer from the R enantiomer, the carbon 2 isomer from the S enantiomer, and the carbon 1 isomer from the S enantiomer; the contributions to the total N-7 guanine adducts were 22%, 18%, and 14%, respectively.

Koivisto et al. (1998) extended the work to study differences in adduct concentrations in the lung and testis of male mice exposed by inhalation to 0, 50, 200, or 500 ppm 1,3-butadiene 6 h/day for 5 days. DNA was isolated from the lung and testis and analyzed for EB-derived, N-7 guanine adducts. Both organs had a dose-dependent increase in N-7 adducts, and at the highest concentration the adduct concentration in the testis was approximately twice that in the lung. In addition, the isomeric distribution of the adducts differed between tissues. In the lung, the predominant form of the adduct was the carbon-1 isomer from the S enantiomer of EB (49% of the total N-7 guanine adducts), whereas only two of the four isomeric forms were detected in the testis. The carbon-2 isomer from the S enantiomer was the predominant form of the N-7 guanine adducts (71% of total) in the testis. The overall S/R ratio of the adducts was similar between tissues, 77/23 for the lung and 71/29 for the testis. Koivisto et al. (1998) hypothesize that the tissue differences in enantiomeric adduct distribution may be due to differences in DNA repair.

More recent studies have quantitated guanine adducts formed from either DEB or EBD in rats. Tretyakova et al. (1998) developed a method for detection of DNA adducts with liquid chromatography electrospray ionization tandem mass spectrometry. Using the developed technique, Tretyakova et al. reported the concentrations of hydroxybutenyl guanine adducts of EB and trihydroxybutyl guanine adduct from either EBD or DEB in the liver of B6C3F$_1$ mice and F344 rats exposed by inhalation to 1,250 ppm 1,3-butadiene 6 h/day for 10 days. The guanine adducts were approximately twice as high in mice compared with rats. The trihydroxybutyl guanine adduct was approximately 1.5-fold higher than the hydroxybutenyl guanine adducts from EB in both species, consistent with results for hemoglobin adducts.

A recent adduct study by Koc et al. (1999) addressed the relationship between exposure dose and molecular dosimetry of guanine adducts in mice and rats. The results of the research indicated that EBD may be a major metabolite in the blood and tissues of animals exposed to 1,3-butadiene and a continued need for a unique marker for DEB binding to tissue DNA. Koc et al.

exposed female B6C3F$_1$ mice and F344 rats to 0, 20, 62.5, or 625 ppm 1,3-butadiene by inhalation for 6 h/day, 5 days/week for 4 weeks and quantitated the molecular dosimetry of N-7 guanine adduct formation by EB, DEB, and EBD in the liver, lung, and kidney. The adducts measured included the racemic and meso forms of N-7-(2,3,4-trihydroxybut-1-yl)-guaninie (THB-Gua), –7-(2-hydroxy-3-buten-1-yl)-guanine (EB-Gua I), and N-7-(1-hydroxy-3-buten-2-yl)-guanine (EB-Gua II). THB-Gua can be formed from the various stereoisomers of either DEB or EBD, and EB-Gua I and EB-Gua II are formed from EB. The adducts were quantitated by liquid chromatography/positive ion electrospray ionization/mass spectrometry/mass spectrometry. The number of adducts was similar in the three tissues of both species. EB-Gua displayed a linear increase with 1,3-butadiene exposure concentration in both species, whereas THB-Gua plateaued after 62.5 ppm in rats but continued to increase in mice, although not linearly. This correlates with the results of Thornton-Manning et al. (1998), which reported no increase in blood concentrations in rats exposed to 8,000 ppm 1,3-butadiene compared with rats exposed to 62.5 ppm 1,3-butadiene by inhalation. In this study, interspecies differences in the number of N-7 adducts were lower than interspecies differences in concentrations of metabolites from previous pharmacokinetic research. In the various tissues, the number of adducts per 10^6 normal guanine bases was higher in mice than in rats at 625 ppm 1,3-butadiene, but similar at lower concentrations. THB-Gua adducts were more abundant than EB-Gua adducts (6–27 times higher); the difference was greatest at higher exposure concentrations of 1,3-butadiene. Assuming that DNA repair is the same for the various adducts, THB-Gua would be expected to be lower than EB-Gua if formed solely from DEB. Based on previously published blood concentrations of EB and DEB at similar exposure concentrations and exposure conditions, Koc and co-workers hypothesize that the majority of THB-Gua adducts arise from EBD and that mice have more efficient repair of DNA than rats.

3.5. EXCRETION

1,3-Butadiene may be excreted via the respiratory tract as the parent compound or as a metabolite, or in the urine and feces as a metabolite. Several studies have quantitated the amount of radioactivity from inhaled 1,3-butadiene eliminated by rats, mice, and monkeys by various routes of excretion following inhalation studies (Bond et al., 1986; Dahl et al., 1991). Unfortunately, none of the inhalation studies have done a mass balance to completely quantitate the amount of inhaled 1,3-butadiene that was exhaled as parent or metabolite, excreted via urine or feces, or retained in the body. The studies estimated a total inhaled dose based on measured respiratory rates and the exposure concentrations used in the experiments. However, the exhaled concentrations of 1,3-butadiene during or following exposure were not reported in any of the studies, the retained dose was not measured in monkeys, and the exhaled CO_2 concentration

during exposure was not measured for rodents. Results from the studies were reported as percentages of total inhaled dose or as a percentage of the total retained dose, which was measured at euthanasia following exposure for mice and rats or as the total amount recovered in 96 h after exposure by all routes of excretion in monkeys. Because only one terminal carbon was labeled, the specific activities of the recovered metabolites are not known for CO_2 or the three-carbon metabolites. Therefore, the total amount of metabolite formed cannot be quantitated, and assessment of the percentage dose eliminated by each pathway is uncertain because of lack of knowledge of the amount of unlabeled metabolite that may be present in the sample. Because of the different protocols used for the studies, comparisons among the species with the data that have been gathered and reported are not adequate to quantitatively assess species differences in pharmacokinetics of excretion. The information reported here summarizes the pathways that are responsible for excreting 1,3-butadiene and the identification of the metabolites excreted via each route.

3.5.1. Pathways of Excretion

The major pathways for excretion of inhaled 1,3-butadiene by rodents are exhaled breath and urine (Bond et al., 1986), and although the absolute amounts of 1,3-[^{14}C]butadiene equivalents differ, the percentage of retained dose excreted by each pathway is similar between species. The exhaled air after exposure (note that the amount of 1,3-butadiene that was exhaled as parent material during exposure was not quantified) accounted for 27–77% of the amount of ^{14}C retained at the end of a 6-h inhalation exposure for rats to 80–7,100 ppm 1,3-[1-^{14}C]butadiene and 23–48% of the amount of ^{14}C retained in mice exposed to 8–1,000 ppm 1,3-[1-^{14}C]butadiene. At the same exposure concentrations of 1,3-butadiene, the total percentage of retained dose that was exhaled was similar between rats and mice; however, the rats had slightly higher percentages of retained 1,3-[^{14}C]butadiene equivalents exhaled as CO_2 and slightly lower percentages exhaled as 1,3-butadiene and metabolites when compared with mice. Mice and rats excreted similar percentages (approximately 30–50%) of the retained ^{14}C in urine following exposure to the same concentrations of 1,3-butadiene.

The rate of 1,3-butadiene excretion by rats and mice was not affected by exposure concentration (0.08–7,100 ppm 1,3-butadiene). Half-lives for urinary excretion of radioactivity were similar for both rats and mice (5.6 and 4.6 h, respectively), and although fecal excretion was somewhat greater in rats (22 h) than in mice (8.6 h), the error was large for rats so the difference was not significant (Bond et al., 1986). Elimination of radioactivity from the blood and tissues of rats and mice after inhalation exposure to 1,3-[^{14}C]-BD was biphasic; half-lives for initial removal were 2–10 h and for slower elimination were 5–60 days.

Similar to rodents, in monkeys the important routes of excretion appeared to be exhalation of 1,3-butadiene, metabolites of 1,3-butadiene, and CO_2, and urinary excretion of metabolites (Dahl et al., 1991). Dahl and co-workers quantitated the excretion of ^{14}C by various pathways from monkeys exposed for 2 h to 1,3-[1-^{14}C]butadiene at concentrations ranging from 10 to 8,000 ppm. Dahl and co-workers measured ^{14}C during inhalation and up to 96 h after exposure in exhaled breath, urine, and feces. Although exhaled breath was collected, the investigators only report ^{14}C exhaled as CO_2 or metabolites but not as parent compound. The ^{14}C recovered via the various routes of excretion accounted for only 1.40–2.88% of the estimated total amount of inhaled 1,3-butadiene. The majority of the amount inhaled would be assumed to be exhaled 1,3-butadiene; however, neither exhaled 1,3-butadiene nor the amount of ^{14}C retained in the monkeys was quantitated in the experiments, therefore the percentage of 1,3-butadiene exhaled is not known. Of the excretion routes quantitated, CO_2 represented the major route of excretion at 10.1 ppm 1,3-butadiene; at 310 ppm, urinary excretion represented the major route of excretion; and at 7,760 ppm, exhalation of other volatile metabolites represented the major route of excretion of the pathways that were quantitated. The percentage of the inhaled amount of 1,3-butadiene that was excreted as CO_2 or in urine and feces decreased with increasing exposure concentration of 1,3-butadiene, indicating saturation of metabolism of 1,3-butadiene. The volatile metabolites excluding 1,3-butadiene and CO_2 that were exhaled increased as a percentage of the inhaled amount of 1,3-butadiene at the highest exposure concentration.

Biliary excretion has been shown to be a minor pathway for elimination of 1,3-butadiene and its metabolites in vivo. Following ip injection of 14.3 or 143 μmol/kg of EB, two glutathione conjugates, *S*-(2-hydroxy-3-buten-1-yl)glutathione (I) and *S*-(1-hydroxy-3-buten-1-yl)glutathione (II), were detected in the bile of rats (Sharer and Elfarra, 1992). Total conjugates excreted in 60 min averaged 7.6% ± 4.2% of the administered dose with approximately a 3:1 ratio of conjugates I:II. At either dose, the amount of conjugates excreted in 30 min was at least 85% of that excreted in 120 min. When the dose of EB was varied between 14.3 and 286 μmol/kg and the combined amounts of conjugates I and II excreted in 60 min were determined, an apparent linear dose-relationship was obtained. Saturation was not observed at these dose levels. Although the study showed that glutathione conjugates of EB are formed in vivo after administration of EB, biliary excretion of glutathione conjugates account for only a small portion of the administered dose.

Urinary excretion was a major route of elimination in Sprague-Dawley rats and B6C3F_1 mice administered [4-^{14}C]EB by intraperitoneal injection (Richardson et al., 1998). Richardson and co-workers administered 1, 5, 20, and 50 mg EB/kg body weight to rats and mice and at 48 h after exposure recovered 53–58% and 46–67% of the administered radioactivity in urine, feces and cagewash from rats and mice, respectively. The unrecovered percentage could have been

exhaled as parent or metabolite or still retained in the body. Urine contained the greatest amount of radioactivity with 47–52% and 39–62% of the administered radioactivity in rats and mice, respectively. There were no significant differences among doses or between species.

3.5.2. Identification of Metabolites

Studies on urinary excretion of 1,3-butadiene metabolites in mice, rats, hamsters, monkeys, and humans have shown that all these species produce predominantly urinary metabolites resulting from hydrolysis and glutathione conjugation of the reactive 1,3-butadiene metabolites. The metabolites have been characterized in urine following inhalation exposures to 1,3-butadiene and ip injections of EB. Clear species differences exist as to which metabolite is formed predominantly as a result of differences in metabolic pathways responsible for detoxication of 1,3-butadiene metabolites. These differences correlate well with species differences in enzyme activity measured in vitro for detoxication of EB. In mice, rats, and hamsters, mercapturic acid metabolites resulting from glutathione conjugation at carbon 1 or carbon 2 of EB represent the largest percentage of the urinary metabolites that were formed. In humans and monkeys the largest percentage of metabolites result from initial hydrolysis of EB.

Several inhalation studies have identified and quantitated metabolites of 1,3-butadiene in urine of animals exposed to 1,3-butadiene (Nauhaus et al., 1996; Osterman-Golkar et al., 1991; Sabourin et al., 1992).

One of the first studies to report mercapturic acids derived from EB conjugation with glutathione was Osterman-Golkar et al. (1991), who exposed male Wistar rats to 0, 250, 500, and 1,000 ppm 1,3-butadiene by inhalation 6 h/day, 5 days/week, for 2 weeks. Urine from exposed animals was collected in the morning before exposure and in the evening after exposure. The urinary metabolite S-(2-hydroxy-3-butenyl-1-yl)-N-acetyl-L-cysteine was quantitated in urine following deacetylation, derivatization, and separation by HPLC. The work is limited in that only one urinary metabolite was quantitated; however, the work did show linear increase in the metabolite up to 1,000 ppm 1,3-butadiene and no accumulation of metabolites over time.

Sabourin et al. (1992) analyzed urinary metabolites from male F344/N rats administered 15 μmol 1,3-[1-^{14}C]butadiene intraperitoneally four times at 2-h intervals. Of the eight different peaks that were separated and identified by HPLC, the two predominant peaks were characterized further by GC-MS, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography-chemical ionization mass spectroscopy (GC-CIMS) following derivatization. One peak was identified as 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (MI) and the other as the –acetylcysteine conjugate of EB (MII). Sabourin et al. used HPLC to quantitate metabolites in urine from F344/N rats, Sprague-Dawley rats, B6C3F$_1$ mice, and Syrian hamsters (gender not specified) exposed by inhalation for 2 h to 7,600 ppm 1,3[1-^{14}C]butadiene and male cynomolous

monkeys exposed for 2 h to 10, 300, and 8,000 ppm 1,3[1-^{14}C]butadiene. At approximately 8,000 ppm 1,3-butadiene, the summation of MI and MII in all species constituted the largest percentage of the urinary metabolites that were quantitated. However, the ratio of MI to MI+MII varied among species and correlated (r^2=0.82) with epoxide hydrolase activities determined in vitro (the substrate used for the activities was benzo[a]pyrene-4,5-(K-region)-oxide). Concentration-dependent differences in the urinary profile of metabolites were not seen in monkeys at 300 and 8,000 ppm 1,3-butadiene. The investigators were unable to determine if the lowest concentration differed from the higher exposure concentrations because all the peaks except for MI were below the limit of detection.

In another study characterizing urinary metabolites in rodents (Nauhaus et al., 1996), male B6C3F_1 mice and Sprague-Dawley rats were exposed by nose-only inhalation to 800 ppm 1,3-[1,2,3,4-^{13}C]butadiene for 2 h. The urine was collected up to 20 h after exposure and qualitatively analyzed by NMR spectroscopy. The amount of metabolites was quantitated in the urine from one exposed rat and mouse. The total amount of urinary metabolites collected for the rat and mouse was 13 and 7 μmol, respectively, or 80 and 270 μmol/kg, respectively, when normalized to body weight. Nauhaus identified a total of 10 different metabolites, 9 of which were present in mouse urine and 5 in rat urine. Consistent with Sabourin et al. (1992), Nauhaus and co-workers found the majority of urinary metabolites in rodents following exposure to 1,3-butadiene resulted from glutathione conjugation of EB, 70.4% and 60.8% of total metabolites for the mouse and rat, respectively. The predominant metabolite in the mouse was N-acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine (43.9% of total metabolites), which results from glutathione conjugation at carbon 1 of EB, and the rat formed predominantly N-acetyl-S-(1-(hydroxymethyl)-2-propenyl)-L-cysteine (52.8% of total metabolites), which results from glutathione conjugation at carbon 2 of EB. The rat also had a higher percentage of urinary metabolites resulting from hydrolysis of EB compared with the mouse, 26.5% versus 7.1%, which is consistent with the reported epoxide hydrolase activities in each species (Sabourin et al., 1992). Metabolites that may have resulted from biotransformation of DEB differed between the mouse and rat. N-Acetyl-S-(1-(hydroxymethyl)-2,3-dihydroxypropyl)-L-cysteine, which could be formed from glutathione conjugation at carbon 2 of DEB, represented 4.6% of the total metabolites in the urine from the mouse but was not identified in urine from the rat. 1,2-Dihydroxypropanone, which the authors hypothesized could be derived from phosphorylation of DEB or the hydrolysis product erythritol, represented 5.5% of total metabolites in rat urine but was not detected in mouse urine. The amount of urinary metabolites that were hypothesized to be derived from DEB was four times greater in the mouse than in the rat when expressed as μmol/kg body weight. However, the hypothesized DEB-derived metabolites also could have been formed following biotransformation of BDdiol, which was detected in urine of both species, 2.9% and 5% of total

urinary metabolites in the mouse and rat, respectively. A metabolite of 3-butenal, N-acetyl-S-(1-hydroxy-3-butenyl)-L-cysteine, was detected in the urine of the mouse but not of the rat and represented 4% of the urinary metabolites. Nauhaus and co-workers also detected three-carbon metabolites that could not be assigned definitively to any one intermediate; these metabolites accounted for 10% and 5% of urinary metabolites in the mouse and rat, respectively.

Two studies have investigated the metabolites of EB in urine of rodents exposed ip to EB (Elfarra et al., 1995; Richardson et al., 1998). N-Acetylcysteine derivatives of the two glutathione conjugates of epoxybutene identified in the bile of rats by Sharer and Elfarra (1992) were detected in the urine of rats and mice administered EB ip (Elfarra et al., 1995). When rats were injected with EB at doses ranging from 71.5 to 285 μmol/kg, urinary excretion of S-(2-hydroxy-3-buten-1-yl)-N-acetyl-L-cysteine (I) and S-(1-hydroxy-3-buten-2-yl)-N-acetyl-L-cysteine (II) within 8 h after exposure increased linearly with dose; the total amount of the two mercapturic acids combined averaged 17 ± 4% of the administered dose at all exposure concentrations. However, in mice the percentage of dose excreted as mercapturic acids I and II was dose dependent. Mice excreted 9 ± 3%, 7 ± 3%, and 26 ± 13% of the administered dose at the 71.5, 143, and 285 μmol/kg dose of EB, respectively. Consistent with the research of Nauhaus et al. (1996), rats preferentially excreted mercapturic acid II over I (approximate ratio 3:1), whereas mice preferentially excreted mercapturic acid I over II (approximate ratio 1.85:1).

Richardson et al. (1998) administered 1, 5, 20, or 50 mg/kg [4-^{14}C]EB to male B6C3F$_1$ mice and Sprague-Dawley rats and collected urine up to 48 h after exposure. Richardson et al. identified nine metabolites, including diastereomeric pairs from two regioisomers, in urine of rats administered EB ip and nine metabolites in urine of mice, two of which were different from metabolites in urine from rats. Consistent with other studies that have investigated urinary metabolites resulting from 1,3-butadiene exposure, Richardson and co-workers found that the majority of urinary metabolites resulting from EB were glutathione conjugates at carbon 1 or 2, with mice forming slightly more mercapturic acids at carbon 1 than carbon 2 at all doses except 1 mg/kg. Rats formed approximately twice the mercapturic acids from glutathione conjugation at carbon 2 compared with carbon 1. In both species, the R stereoisomer predominated over the S stereoisomer for glutathione conjugation at both carbon 1 and carbon 2. Several novel metabolites were identified by Richardson et al. (1998), including two mercaptoacetic acids, which were most probably produced from deamination of EB-cysteine conjugates followed with catabolism by decarboxylases, in urine from mice, and (4-[N-acetyl-L-cystein-S-yl]-2-hydroxybutanoic acid, which was probably an oxidation product of 1,2-dihydroxy-4-[N-acetyl-L-cysteinyl]butane), in urine from rats. Notable was the absence of urinary metabolites derived from DEB biotransformation or from 3-butenal in the urine of both species. However, unlike Nauhaus et al. (1996) who detected the three-carbon metabolites 3-(N-acetyl-L-cystein-S-

yl)propan-1-ol and 3-(N-acetyl-L-cystein-S-yl)-3-propanoic acid in both species, Richardson et al. detected these three carbon metabolites only in the urine from rats administered EB ip. The difference in the profile of urinary metabolites following exposure of rodents to EB from ip injection versus inhalation to 1,3-butadiene could be due to differences in metabolism because of the kinetics by the different routes of administration or possibly because some the metabolites identified from 1,3-butadiene exposure do not proceed through the EB pathway.

Richardson et al. (1998) also found that the stereochemistry of EB appears to affect biotransformation of the compound. Selective stereoisomers were formed in both species administered racemic EB ip. In contrast to the results from Nieusma et al. (1997), both rats and mice had more urinary metabolites originating from the R-stereoisomer than the S-stereoisomer of EB. However, there were no stereoisomeric differences between the species.

In addition to identification of urinary metabolites in laboratory animals, two in vivo studies have reported data on the urinary excretion of 1,3-butadiene metabolites by humans. The studies have not identified all metabolites present in urine from humans exposed to 1,3-butadiene, but have focused on quantitation of metabolites that make up the largest percentage of metabolites in urine from laboratory animals exposed by inhalation to 1,3-butadiene.

In the first study, Bechtold et al. (1994) attempted to measure two metabolites of 1,3-butadiene, 1,2-dihydroxy-4-(N-acetylcysteinyl-S-)-butane (MI) and 1-hydroxy-2-(N-acetylcysteinyl-S-)-3-butene (MII), in urine of workers employed at the Texaco Chemical Co. in Port Neches, Texas, a 1,3-butadiene extraction plant. MI and MII were selected because they were the predominant metabolites identified in urine from laboratory animals exposed by inhalation to 1,3-butadiene. The study population included (a) exposed employees who worked in two areas (described as low- and high-exposure areas) with time-weighted average concentrations of 3 to 4 ppm 1,3-butadiene over the previous 6 months, (b) intermediate-exposed employees who worked variable time periods in low- and high-exposure areas, (c) nonexposed employees who worked in areas with historical time-weighted average concentrations of less than 0.1 ppm 1,3-butadiene, and (d) outside controls who had no known exposure to 1,3-butadiene. Urine samples were analyzed from 7 exposed employees, 3 intermediate-exposed employees, 10 nonexposed employees, and 9 outside controls. MI, but not MII, was detected in the urine samples. The average values of MI for exposed employees, intermediate-exposed employees, nonexposed employees, and outside controls were 3,200 ± 1,600, 1,390 ± 550, 630 ± 190, and 320 ± 70 ng/mL, respectively. Although the actual exposure to 1,3-butadiene for each individual was not known, urinary concentrations of MI for the exposed groups (combined) were significantly higher ($p<0.05$) compared with the outside control group.

In the second study, Ward et al. (1996b) reported increased concentrations of the urinary metabolite 1,2-dihydroxy-4-(N-acetyl-cysteinyl)-butane in workers at a styrene-butadiene rubber

plant. Exposure was assessed in workers from areas of higher exposure (reactor, recovery, tank farm, laboratory) and lower exposure (blend, coagulation, bailers, shipping, utilities, shops) with badge dosimeters. Concentration of the metabolite was measured in urine. The detection limit (0.25 ppm) was exceeded in 20/40 dosimeter readings in the high-exposure group and in 0/20 readings in the low-exposure group. Urine samples were analyzed from 16 high- and 9 low-exposure subjects. Expressed as ng/mg creatinine, metabolite concentrations were 1,690 ± 201.3 and 355 ± 250 in Study I (conducted in 10/91), and 761 ± 245 and 684 ± 176 in Study II (conducted in 6/92), respectively, for the high- and low-exposure groups.

3.6. PHARMACOKINETICS OF STRUCTURALLY RELATED COMPOUNDS

Studies with isoprene (2-methyl-butadiene) and limited studies with chloroprene (2-chloro-butadiene) have shown some similarities with the pharmacokinetics of 1,3-butadiene.

Similar to 1,3-butadiene, the initial step in biotransformation of isoprene and chloroprene is oxidation to monoepoxides (Bartsch et al., 1979; Bogaards et al., 1996; Del Monte et al., 1985; Gervasi and Longo, 1990; Wistuba et al., 1994). Isoprene can also form diepoxides (Bogaards et al., 1996; Bond et al., 1991; Dahl et al., 1987; Del Monte et al., 1985; Gervasi and Longo, 1990; Wistuba et al., 1994). The biotransformation of chloroprene to a diepoxide has not been studied. Epoxide hydrolase is involved in the biotransformation of isoprene (Bogaards et al., 1996; Bond et al., 1991; Dahl et al., 1987; Del Monte et al., 1985; Gervasi and Longo, 1990; Wistuba et al., 1994); however, glutathione transferase does not appear to be a major pathway for biotransformation of isoprene (Buckley et al., 1999). The role of glutathione transferase in the metabolism of chloroprene has been implicated because of depletion of nonprotein sulfhydryl in tissues from mice and rats exposed by inhalation to chloroprene (Melnick et al., 1996a; Plugge and Jaeger, 1979).

Del Monte et al. (1985) showed that mouse hepatic microsomal monooxygenases converted isoprene to two monoepoxides and a diepoxide. Specifically, 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene were major and minor metabolites, respectively, with the latter representing about 20% of the former. Of the two monoepoxides, only 3,4-epoxy-2-methyl-1-butene metabolite was metabolized further in microsomal incubations to a diepoxide. Addition of 1,2-epoxy-3,3,3-trichloropropene to microsomal incubations with isoprene increased the concentrations of 3,4-epoxy-3-methyl-1-butene in incubation media, indicating the role of epoxide hydrolase in the biotransformation of isoprene. Because of the rapid hydrolysis of the two monoepoxides in water, Del Monte et al. (1985) measured the diols of the epoxides to quantitate enzymatic activity. Biotransformation of isoprene to the diol of 3,4-epoxy-3-methyl-1-butene was O_2 and NADPH dependent and inhibited by cytochrome P450 inhibitors such as CO, SKF 525-A, and metyrapone. For production of the diol of 3,4-epoxy-3-methyl-1-butene from

isoprene, the K_m (mM) and V_{max} (nmol diol/mg protein/min) values were 0.09 and 1.8, 0.08 and 2.9, and 0.07 and 2.1, respectively, in hepatic microsomes from control, phenobarbital-induced, and 3-methylcholanthrene-induced mice. In addition, for the production of the diepoxide from 3,4-epoxy-2-methyl-1-butene, the K_m (mM) and V_{max} (nmol diepoxide/mg protein/min) values were 0.24 and 1.7, 0.29 and 5.1, and 0.22 and 2.0, respectively, in microsomes from control, phenobarbital-induced, and 3-methylcholanthrene-induced mice. The increase in V_{max} for the formation of the diepoxide was significant ($p<0.01$) in incubations with hepatic microsomes from phenobarbital-treated mice.

Gervasi and Longo (1990) provided species comparisons for the in vitro metabolism of isoprene by hepatic microsomal preparations from rats, mice, rabbits, and hamsters. Hepatic microsomal preparations from all these species metabolized isoprene to 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene. The former was the major metabolite in all species. For the production of the diol of 3,4-epoxy-3-methyl-1-butene from isoprene, the K_m (mM) and V_{max} (nmol diol/mg protein/h) were 0.08 and 0.24, 0.09 and 1.79, 0.2 and 0.66, and 0.06 and 1.20, respectively, in incubations with hepatic microsomes from rats, mice, rabbits, and hamsters. The V_{max} was almost an order of magnitude larger in the mouse compared with the rat. Microsomal preparations from all species further metabolized the 3,4-epoxy-2-methyl-1-butene isoprene dioxide (2-methyl-1,2,3,4-diepoxybutane).

In contrast to Del Monte et al. (1985) and Gervasi and Longo (1990), Wistuba et al. (1994) and Bogaards et al. (1996) found production of diepoxides of isoprene from both 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene.

Wistuba et al. (1994) studied the stereoselective metabolism of isoprene in liver microsomes from rats and mice. In addition to the two monoepoxides, the investigators also detected diepoxide formation from both monoepoxides. There was regioselectivity with the liver microsomes from mice, but not from rats. Wistuba et al. (1994) also found stereoselectivity in the hydrolysis by epoxide hydrolase and glutathione conjugation by glutathione transferase.

Bogaards et al. (1996) compared the metabolism of isoprene among liver microsomes from mice, rats, and humans. In all species, 3,4-epoxy-3-methyl-1-butene was the predominate monoepoxide of isoprene, and the enzymatic activities for the formation of both monoepoxides did not differ among species in microsomal incubations with the epoxide hydrolase inhibitor cyclohexene oxide. However, significantly less 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene were detected in rat and human microsomes, but not mouse microsomes, incubated without cyclohexene oxide. For microsomes from rats, the concentrations of 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene were reduced to 50% and 70% of concentrations detected in incubations with cyclohexene oxide. For microsomes from humans, concentrations of 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene were reduced

to 4% and 25% of concentrations detected in incubations with cyclohexene oxide. The enzymatic activities for the formation of the diepoxide from 3,4-epoxy-3-methyl-1-butene in hepatic microsomes ranged from 780 to 1,210 pmol diepoxide/min/nmol P450, with microsomes from humans exhibiting the lowest activity and microsomes from CD-1 mice exhibiting the highest activity. However, activity in microsomes from $B6C3F_1$ mice was approximately the same as humans, 806 vs. 780 pmol diepoxide/min/nmol P450. The enzymatic activities for the formation of the diepoxide from 3,4-epoxy-2-methyl-1-butene in hepatic microsomes ranged from 666 to 1,360 pmol diepoxide/min/nmol P450, with microsomes from humans exhibiting the lowest activity and microsomes from Wistar rats exhibiting the highest activity.

Bogaards et al. (1996) also determined the human cytochromes P450 responsible for oxidation of isoprene to the two monoepoxides and diepoxides. Of the cytochromes P450 examined, the highest activity for the formation of 3,4-epoxy-3-methyl-1-butene from isoprene was measured in microsomes prepared from cell lines expressing cytochrome P450 2E1 followed by cytochromes P450 2B6 > P450 2D6 > P450 2A6. 3,4-Epoxy-2-methyl-1-butene and the diepoxides were detected only in incubations with microsomes from cell lines expressing cytochrome P450 2E1.

Bogaards et al. (1999) studied the enzymatic conjugation of the two monoepoxides of isoprene, 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene, with glutathione. The studies used purified glutathione-S-transferases of the alpha, mu, pi, and theta class from rats and humans. 3,4-Epoxy-2-methyl-1-butene was conjugated more efficiently than 3,4-epoxy-3-methyl-1-butene. For both rat and human, the mu and theta class were more efficient than the alpha and pi class at conjugating the monoepoxides. The rat glutathione transferases were more efficient than the human transferases at metabolizing both monoepoxides; the theta class in rats had 2.1- to 6.5-fold higher activities than in humans, and the mu class had 5.2- to 14-fold higher activities than in humans. There were also differences in the site of conjugation between the two monoepoxides. 3,4-Epoxy-3-methyl-1-butene was almost exclusively conjugated with glutatione at carbon 3, while no selectivity was noted with 3,4-epoxy-2-methyl-1-butene. In S9 fractions from livers of mice, rats, and humans, mice and rats had similar rates of conjugation, while the rates in humans were 25- to 50-fold lower.

Similar to 1,3-butadiene, the pharmacokinetics of isoprene in vivo appear to differ among species.

In a study by Dahl et al. (1987), groups of 30 male F344 rats were exposed by nose-only inhalation to [^{14}C]isoprene at concentrations of 8.0, 266, 1,480, or 8,200 ppm for 6 h (5.5 h for the 8,200 ppm), and urine, feces, and exhalants were collected over a 66 h postexposure period. The retained ^{14}C (total amount remaining in animal at the end of exposure including isoprene) decreased as a percentage of inhaled ^{14}C with increasing exposure concentrations, suggesting

saturation of metabolism. The investigators defined metabolite (nonisoprene)-associated radioactivity as the total amount of ^{14}C measured in exhaled CO_2, urine, feces, and carcass during the 66 h postexposure period. The authors did not account for the metabolites including CO_2 exhaled during exposure or lost during sacrifice. The major pathway for excretion of isoprene in rats appeared to be the urine for all exposure concentrations. The authors noted, however, that this finding was tentative and may be the result of a labeling artifact because the terminal carbons of isoprene were not labeled. During the postexposure period, >75% of the metabolite-associated radioactivity was excreted in the urine. The radioactivity in the feces and exhaled CO_2 accounted for 1.7%–13.4% and 1.9%–5.3% of the metabolite-associated radioactivity, respectively. The half-life (mean ± SE) for urinary excretion of ^{14}C was 10.2 ± 1.0 h (range of 8.8 to 11.1 h). The investigators used cryogenic trapping to tentatively identify metabolites in the blood and tissues. Generally, the concentration of metabolites in the blood increased with exposure concentration and duration of exposure. The authors noted that 85% of the radioactivity in the blood was associated with material of low volatility, which probably represented covalently bound metabolites, conjugates of isoprene metabolites, or tetrols. The radioactivity trapped at -45°C, which was attributed to the diols and diepoxides of isoprene, accounted for the majority of the volatile metabolites at all exposure concentrations. The percentage of inhaled, isoprene-derived ^{14}C present as diepoxides or diols in the blood remained fairly constant with time but decreased with exposure concentration. Only at the two highest exposure concentrations were materials detected that possessed volatilities matching those of isoprene and isoprene monoepoxides. Assessing the distribution of isoprene and its metabolites in some animals of the 1,480 ppm exposure group revealed that fat, followed by liver and blood, contained the largest amounts of the radioactivity (concentrations were not reported).

Bond et al. (1991) conducted similar experiments as Dahl et al. (1987), but with male $B6C3F_1$ mice at exposure concentrations of 20, 200, and 2,000 ppm isoprene. Isoprene significantly reduced minute ventilation in mice exposed to 2,000 ppm isoprene compared with controls exposed to air only. This effect was not seen in rats (Dahl et al., 1987). Except for the lowest exposure concentrations, mice retained similar percentages of inhaled ^{14}C compared with rats. However, mice had lower percentages of metabolite-associated radioactivity excreted in the urine (52.4%–73.4%) but higher percentages excreted as CO_2 (2.1%–18.4%) and in feces (5.5%–35.5%) compared with rats exposed to similar concentrations of isoprene.

Peter et al. (1987) also noted differences in the pharmacokinetics of isoprene in male Wistar rats and male $B6C3F_1$ mice. Animals were exposed in closed inhalation systems to concentrations of isoprene as high as 4,000 ppm for up to 10 h. At concentrations <300 ppm, the rate of metabolism was found to be directly proportional to the isoprene concentration, but saturation of metabolism was detected at higher concentrations. Pharmacokinetic parameters

were estimated from a two-compartment model as described for similar experiments with 1,3-butadiene (Bolt et al., 1984; Kreiling et al., 1986b). The V_{max} for the metabolism of isoprene in rats and mice was 130 and 400 μmol/h/kg, respectively. The estimated half-lives were 4.4 and 6.6 min for mice and rats, respectively. Estimated metabolic clearance was approximately twice as high in mice compared with rats, 12,000 vs. 6,200 mL/h, respectively. The estimated clearance of uptake from the chamber was more than twice as high in mice compared with rats, 16,000 vs. 7,300 mL/h, respectively, and approximately equal to the estimated metabolic clearance, indicating that metabolism of isoprene was limited by uptake. The thermodynamic partition coefficient was approximately equivalent in mice and rats, 7.0 vs. 7.8, respectively, and the steady-state, whole body:air concentration ratio was also approximately the same in mice and rats, 1.7 vs. 1.2, respectively. The endogenous rate of isoprene production was approximately 4 times greater in rats than mice, 1.9 vs. 0.4 μmol/h/kg, respectively.

Buckley et al. (1999) compared the routes of excretion and the urinary metabolites in male B6C3F_1 mice and Fischer 344 rats after ip administration of 64 mg [^{14}C]isoprene/kg body weight. The disposition and time course of ^{14}C did not differ among species; there were no statistically significant differences in the percentage of administered ^{14}C excreted among the various routes of excretion, which included volatiles in breath, CO_2 in breath, urine, feces, and carcass and tissues. Exhaled volatiles accounted for the largest percentage (approximately 50%) of administered radioactivity in mice and rats, followed by urine, which accounted for approximately 30% of the administered radioactivity in mice and rats. CO_2 in breath and the carcass and tissues accounted for approximately 2% and 2–3% of administered radioactivity, respectively. Feces accounted for 0.2–7% of the administered radioactivity; however, feces from mice were thought to be contaminated by urine. The profile of urinary metabolites differed among species. In the rat, four major metabolite peaks were identified in the urine. The biotransformation of isoprene in mice appeared to be more complex because the urine from exposed mice contained several minor metabolite peaks in addition to four metabolite peaks that were detected in the urine from rats. The major metabolite peaks in urine from rats were identified as 2-hydroxy-2-methyl-3-butenoic acid (53% of the total urinary radioactivity), 2-methyl-3-buten-1,2-diol (23%), and the glucuronide conjugate of 2-methyl-3-buten-1,2-diol (23%). The other major metabolite peak in rat urine was not uniquely identified. In mouse urine, 2-hydroxy-2-methyl-3-butenoic acid, 2-methyl-3-buten-1,2-diol, and 2-methyl-3-buten-1,2-diol accounted for 15%, 3.5%, and 2.5% of the total urinary radioactivity, respectively. The unidentified metabolite peak in mouse urine, which was thought to be directly correlated with the peak in rat urine, accounted for 25% of the total urinary radioactivity. None of the metabolites in either species were derivatives of glutathione conjugates; therefore, in contrast to 1,3-butadiene,

glutathione conjugation does not appear to be a major pathway in the biotransformation of isoprene.

Chloroprene (2-chloro-butadiene) is also structurally similar to 1,3-butadiene, but its pharmacokinetics have not been studied extensively. Studies have shown that the biotransformation of chloroprene results in the formation of peroxides that may interact with tissue thiols (Haley, 1978). Furthermore, cytochrome P450 may form an epoxide intermediate similar to that formed during 1,3-butadiene metabolism (Bartsch et al., 1979). In contrast to isoprene, glutathione transferase may play a role in the biotransformation of chloroprene. Plugge and Jaeger (1979) found significant depletion of nonprotein sulfhydryl at 24 h postexposure in the lungs of male Sprague-Dawley rats that were fasted and exposed by inhalation to 100 or 300 ppm chloroprene for 4 h compared with rats that were fasted and exposed to air only. Melnick et al. (1996a) found significant depletion of nonprotein sulfhydryl in the livers from male and female B6C3F_1 mice exposed to 80 ppm chloroprene by inhalation for 12 weeks, and in livers from male and female Sprague-Dawley rats exposed by inhalation to 200 ppm for 12 weeks. Female but not male rats had significant depletion of nonprotein sulfhydryl following 1 day of exposure to 200 ppm chloroprene.

In summary, studies have shown that the structurally similar isoprene and chloroprene are metabolized to monoepoxide intermediates, and the isoprene monoepoxides are further metabolized to diepoxides. The monoepoxides and diepoxides from isoprene were detected with in vitro incubations with microsomes from all species investigated, including humans. Although the enzymatic activities for the formation of the monoepoxides and diepoxides did not differ among species, enzymatic hydrolysis of the monoepoxides is significantly greater in humans compared with rats and mice. In vivo inhalation studies with rats and mice exposed to isoprene showed some differences in the disposition of isoprene compared with 1,3-butadiene. As with 1,3-butadiene, the in vivo rate of isoprene metabolism was greater in mice compared with rats. However, the contributions of urinary excretion and CO_2 exhalation toward the elimination of 1,3-butadiene and isoprene differ between the two chemicals. In addition, glutathione transferase plays an important role in the biotransformation of 1,3-butadiene. Although metabolism of the isoprene monoepoxides by glutathione transferase has been measured in vitro, the lack of urinary metabolites of glutathione conjugates indicate it is not involved in the biotransformation of isoprene in vivo. Glutathione transferase may be involved in the biotransformation of chloroprene.

3.7. DISCUSSION

Current data indicate that toxicity of 1,3-butadiene depends on the metabolic activation to reactive intermediates. Because differences among species have been noted in toxic responses to

1,3-butadiene exposure, the pharmacokinetics are important in assessing the risk of adverse health effects from exposure to this compound. This chapter summarized research related to absorption, distribution, biotransformation, and excretion of 1,3-butadiene with emphasis on identifying species differences in the pharmacokinetics of 1,3-butadiene. The research supports the idea that species differences in the pharmacokinetics may contribute but not be solely responsible for differences in toxicity between mice and rats. However, data gaps still exist in the knowledge about 1,3-butadiene metabolism that limit the ability to accurately assess the risk to humans from exposure to 1,3-butadiene based on experimental animal data.

Qualitatively, metabolism of 1,3-butadiene is similar among species that have been studied, including humans. Oxidation of 1,3-butadiene and intermediate metabolites of 1,3-butadiene and detoxication of intermediate metabolites epoxide hydrolase and glutathione transferase are the major metabolic pathways in biotransformation of 1,3-butadiene. Several reactive intermediates have been identified in either in vivo or in vitro studies as shown in Figure 3-1, including 3-butenal, crotonaldehyde, EB, DEB, and EBD. However, the major metabolites that have been detected directly in vivo are EB and DEB. EBD has been detected indirectly in vivo by DNA and hemoglobin adducts and also may be a major metabolite.

However, although the pathways of biotransformation are qualitatively similar among species, there are quantitative differences in predominant pathways for detoxication and in the rates of metabolism by the various pathways. Although hepatic microsomes from mice, rats, and humans all oxidize 1,3-butadiene to EB and EB to DEB, based on the ratio of V_{max}/K_m in liver (estimated intrinsic clearance), which would be relevant at lower exposure concentrations of 1,3-butadiene, the rate of oxidation is greatest in mice with humans and rats approximately equivalent. For the detoxication pathways, mice and rats predominantly remove EB and DEB through conjugation by glutathione transferase, whereas epoxide hydrolase is more important in humans. Using the estimated intrinsic clearance (V_{max}/K_m) (Table 3-9) as a means of comparison among species, we find that 5.4–11% of the intrinsic clearance of EB is through oxidation to DEB in mice, compared with 0.13–2.4% and 2–3% for rats and humans, respectively.

The differences in the rates of metabolism noted in vitro have also been noted in vivo in rats and mice, and have been reflected in blood and tissue concentrations of reactive metabolites, in the profile of urinary metabolites, and in biomarkers such as hemoglobin adducts and DNA adducts. Inhalation studies have demonstrated that 1,3-butadiene is readily absorbed into the blood, distributed as parent compound and metabolites throughout the body, and eliminated in exhaled breath as parent and metabolites or in urine as metabolites. The inhalation uptake of 1,3-butadiene by mice is approximately twice the uptake of rats, partly because of higher ventilation per kg of body weight, higher tissue perfusion, and higher rates of metabolism. EB and DEB are the only two metabolites of 1,3-butadiene that have been measured directly in vivo in rats and

mice, with the blood and tissue concentrations in mice significantly greater than those reported in rats. Reported blood concentrations of EB are 4–8 times higher in mouse blood than rat blood, whereas reported tissue concentrations are 3–16 times greater in mice compared with rats. For DEB, the species differences are greater. The reported blood concentrations of DEB in mice are approximately 40-fold higher than in rats, whereas tissue concentrations of DEB are 4–163-fold higher in mice compared with rats. These differences are greater at higher exposure concentrations because blood concentrations of DEB were similar in female rats exposed to 8,000 ppm 1,3-butadiene compared with female rats exposed to 62.5 ppm 1,3-butadiene for 6 h. This plateau in DEB blood concentrations resulting from saturation of the oxidation of EB to DEB may explain in part the difference of carcinogenic response between mice and rats.

Profiles of urinary metabolites that have been detected among species following inhalation exposures to 1,3-butadiene show clear species differences. The profiles reflect the predominant clearance of EB in mice through initial conjugation with glutathione versus the predominant clearance of EB in humans through initial hydrolysis. The greater body burden of 1,3-butadiene and its metabolites in mice versus rats is also reflected in the recovery of total urinary metabolites normalized to the body weight of the animals, which was approximately three times greater in mice compared with rats exposed by inhalation to equal concentrations of 1,3-butadiene.

The effect of repeated exposures on 1,3-butadiene pharmacokinetics has been studied to a limited extent. One in vitro study showed reduced activities from microsomes prepared from the liver and lungs of mice and rats exposed repeatedly to 1,3-butadiene. In vivo, EB and DEB concentrations have been quantitated in mice and rats exposed repeatedly to 1,3-butadiene and compared with animals with a single exposure to 1,3-butadiene. Although the disposition of EB and DEB appeared to change in both species from single and repeated exposure, the design of the studies could not address whether the changes were from metabolic alterations or from continued accumulation as the metabolites approached steady-state during repeated exposures.

Molecular dosimetry studies also have shown species-related differences in the formation of various adducts. Hemoglobin adducts and DNA adducts formed from EB are approximately two- to sixfold higher in mice than in rats at 1,3-butadiene exposure concentrations exceeding approximately 100 ppm, but similar between mice and rats at lower exposure concentrations, and therefore do not reflect species differences in susceptibility to toxic response from exposure to 1,3-butadiene. Recently, trihydroxybutyl adducts of hemoglobin and DNA have been identified. These adducts are formed from either DEB or EBD. In rats, the trihydroxybutylvaline adducts of hemoglobin exceeds the concentration of EB-derived hemoglobin adducts by 3- to 32-fold, although blood concentrations of EB are higher than DEB in rats. In addition, the trihydroxybutylguanine adduct exceed the EB-derived guanine adducts in both species. Based on

blood concentrations of DEB and EB, the majority of the trihydroxybutyl adducts are thought to be derived from EBD, indicating that EBD may be a major metabolite of 1,3-butadiene in vivo. Currently, no data exist on species differences in concentrations of EBD in vivo, representing a major data gap in the research on 1,3-butadiene pharmacokinetics.

Other data gaps in the research on species differences in pharmacokinetics of 1,3-butadiene include lack of in vivo measurements of other potentially reactive metabolites such as 3-butenal and crotonaldehyde, although these metabolites would be expected to be minor in vivo based on in vitro data and urinary data. In addition, the stereochemistry of 1,3-butadiene pharmacokinetics may be important. Limited research has recently shown some species differences in stereometabolism as well as species and tissue differences in adduct formation. Data on interindividual variability in humans related to the pharmacokinetics of 1,3-butadiene is limited. The greatest variability reported was in enzyme activities for oxidation of EB to DEB measured in hepatic microsomes, which varied approximately 60-fold. However, variability for V_{max}/K_m reported for conjugation of DEB by glutathione transferase and hydrolysis by epoxide hydrolase varied by threefold. Although not specifically studied for 1,3-butadiene or its metabolites, polymorphisms in cytochromes P450, epoxide hydrolase, or glutathione transferases could be expected to affect the pharmacokinetics of 1,3-butadiene in vivo. For example, Bogaards et al. (1999) reported differing activities for the alpha, mu, pi, and theta classes of glutathione transferases for conjugation of isoprene monoepoxides, structurally similar compounds to EB. The mu and theta classes were more efficient at conjugating the epoxides, and since these classes are expressed in 50% and 40–90% of the population, respectively, individuals lacking these enzymes may have altered detoxification of the epoxides. In addition, limited data exist to address gender and age differences in the pharmacokinetics of 1,3-butadiene. Lack of knowledge in these areas limits the ability to make precise assessments of the risk to humans from exposure to 1,3-butadiene.

4. MUTAGENICITY

The mutagenic effects of 1,3-butadiene have been reviewed extensively (Rosenthal, 1985; de Meester, 1988; Arce et al., 1990; Norppa and Sorsa, 1993; Jacobson-Kram and Rosenthal, 1995; Pacchierotti et al., 1998a; IARC, 1999). There is extensive evidence that 1,3-butadiene induces genotoxic effects in a variety of in vitro and in vivo test systems through its metabolism to several DNA-reactive intermediates (primarily the epoxide metabolites EB, DEB, and EBD). Most of the in vivo studies discussed in the cited reviews were assays in mice and rats using cytogenetic endpoints, and the results generally support the dichotomy in carcinogenic response in which mice are more responsive than rats. Table 4-1 summarizes much of the earlier data on 1,3-butadiene and its metabolites. This review will focus on recently published studies performed in vivo (both somatic and germ cell effects), with an emphasis on those studies providing information relative to the mode of action of 1,3-butadiene metabolites. Tables 4-2 through 4-5 present a tabular display of the studies discussed in the following sections.

4.1. 1,3-BUTADIENE

4.1.1. Gene Mutations

Most of the earlier in vivo genotoxicity studies used cytogenetic endpoints (aberrations, micronuclei [MN], or sister chromatid exchange [SCE]). This reflected the dearth of available in vivo assays for measuring gene mutations. The ability to detect mutations at the *hprt* locus obtained from T lymphocytes from exposed mammals including mice, rats, monkeys, and humans provides an important step in developing an understanding of chemically induced mutational processes. Cochrane and Skopek (1994a) used $B6C3F_1$ mice to evaluate the mutagenic potential of 1,3-butadiene. Mice were exposed for 6 h/day, 5 days/week for 2 weeks to 1,3-butadiene at 625 ppm. The induced *hprt* mutant frequency was 6.2×10^{-6} compared with 1.2×10^{-6} from unexposed controls. They reported that about half of the mutations were frameshift, and of the base-pair mutations induced by 1,3-butadiene mutations at AT base pairs predominated.

Tates et al. (1994, 1998) reported mixed results in two strains of mice exposed to 1,3-butadiene. In the first experiments male (102/E1 × C3H/E1)F1 mice were exposed to 0, 200, 500, or 1,300 ppm of 1,3-butadiene 6 h/day for 5 days. 1,3-Butadiene treatment resulted in a linear dose-related increase in *hprt* mutant frequency; only the high dose increase was significantly different from controls (Tates et al., 1994). In a second experiment using the same strain, mice were exposed at 500 and 1,300 ppm (Tates et al., 1998). Because of technical problems there were no data from the 1,300 ppm exposed group and the authors reported a ten-fold increase in *hprt* mutant frequency in the 500 ppm group. However, when four control and

Table 4-1. Summary of earlier mutagenicity testing of 1,3-butadiene, 1,3-butadiene monoepoxide and 1,2:3,4-diepoxybutane (derived from tables in IARC, 1999).

Test system	Results	Dose (LEC or HIC)[a]	Comments
1,3-butadiene			
S. typhimurium TA100, reverse mutation	positive	1,080 ppm	requires metabolic activation
S. typhimurium TA1535, reverse mutation	positive	86 ppm	requires metabolic activation
S. typhimurium TA1537, reverse mutation	negative	1,080 ppm	
S. typhimurium TA98, reverse mutation	negative	1,800 ppm	
S. typhimurium TA97, reverse mutation	negative	1,800 ppm	
E. coli WP2 , reverse mutation	negative	1,080 ppm	
Drosophila melanogaster, sex-linked recessive lethal	negative	500 ppm	inhalation exposure
Mouse lymphoma L5178Y, gene mutation	negative	650 ppm	
Chinese hamster ovary cells in vitro, SCE	positive	1.35	requires activation
Human lymphocytes, in vitro, SCE	negative	2,160 ppm	
Human lymphocytes, in vitro, SCE	positive	108	
$B6C3F_1$ mouse bone marrow in vivo, SCE	positive	116 ppm	6 h inhalation
Sprague-Dawley rat bone marrow in vivo, SCE	negative	4,000 ppm	6 h inhalation
$B6C3F_1$ mouse bone marrow in vivo micronucleus	positive	116 ppm	6 h inhalation
$B6C3F_1$ mouse peripheral blood in vivo, micronucleus	positive	7 ppm 70 ppm	13 wk inhalation 6 h/day, 5d/wk 2 wk inhalation 6 h/day, 5d/wk
Sprague-Dawley rat bone marrow in vivo, micronucleus	negative	500 ppm	1 wk inhalation 6 h/day, 5d/wk

Table 4-1. Summary of earlier mutagenicity testing of 1,3-butadiene, 1,3-butadiene monoepoxide and 1,2:3,4-diepoxybutane (derived from tables in IARC, 1999) (continued).

Test system	Results	Dose (LEC or HIC)[a]	Comments
1,2-epoxy-3-butene			
S. typhimurium TA100, reverse mutation	positive	26	without metabolic activation
S. typhimurium TA1535 reverse mutation	positive	1,750	without metabolic activation
S. typhimurium TA1537, TA 1538, TA 98, reverse mutation	negative	8,750	not tested with metabolic activation
*E.coli*WP2, reverse mutation	positive	not reported	
human TK6 cells in vitro, gene mutations *tk* locus	positive	17.5	without metabolic activation
human TK6 cells in vitro, gene mutations *hprt* locus	positive	10.5	without metabolic activation
Chinese hamster cells in vitro, SCE	positive	0.07	with and without metabolic activation
1,2:3,4-diepoxybutane			
S. typhimurium TA100, TA 1535, reverse mutation	positive	20, 5	without metabolic activation
S. typhimurium TA1537, TA 1538, TA 98, reverse mutation	negative	167	with or without metabolic activation
E. coli WP2, reverse mutation	positive	167	
Saccharomyces cerevisiae D4, gene conversion	positive	430	without metabolic activation
Saccharomyces cerevisiae D3, mitotic recombination	positive	400	without metabolic activation
Saccharomyces cerevisiae D7, reverse mutation	positive	130	without metabolic activation
Neurospora crassa, reverse mutation	positive	4,300	without metabolic activation
Drosophila melanogaster, somatic mutation	positive	430	in feed
Drosophila melanogaster, sex-linked recessive lethal	positive	100	injection

Table 4-1. Summary of earlier mutagenicity testing of 1,3-butadiene, 1,3-butadiene monoepoxide and 1,2:3,4-diepoxybutane (derived from tables in IARC, 1999) (continued).

Test system	Results	Dose (LEC or HIC)[a]	Comments
1,2:3,4-diepoxybutane (continued)			
Drosophila melanogaster, sex-linked recessive lethal	positive	175	in feed
Chinese hamster ovary cells in vitro, gene mutations, *hprt* locus	positive	2.15	without metabolic activation
Mouse lymphoma L5178Y cells in vitro, gene mutations, *tk* locus	positive	0.3	without metabolic activation
Chinese hamster ovary cells in vitro, SCE	positive	0.025	without metabolic activation
Swiss-Webster mouse bone marrow in vivo, SCE	positive	1	single ip injection
NMRI mouse bone marrow in vivo, SCE	positive	22	2 h inhalation
Chinese hamster bone marrow in vivo, SCE	positive	34	2 h inhalation

[a] LEC, lowest effective concentration; HIC, highest ineffective concentration; in vitro tests, μg/ml; in vivo tests, mg/kg bw.

Table 4-2. Summary table of genetic effects of 1,3-butadiene (details in text).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Gene mutations			
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo	positive	490	Cochrane & Skopek, 1994a
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo	positive	1,000	Tates et al., 1994
(102 × C3H)F1 mouse T lymphocytes, *hprt* locus, in vivo	positive	390	Tates et al., 1998
CD1 mouse T lymphocytes , *hprt* locus, in vivo	negative	1,000	Tates et al., 1998
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo	positive	970	Meng et al., 1998
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo	positive	50	Meng et al., 1999a
B6C3F$_1$ mouse, bone marrow, *lacI* locus, in vivo	positive	490	Recio & Goldsworthy, 1995
T-stock female mice, spot test, in vivo	positive	390	Adler et al., 1994
F344 rat T lymphocytes, *hprt* locus, in vivo	positive	580	Meng et al., 1998
F344 rat T lymphocytes, *hprt* locus, in vivo	positive	290	Meng et al., 1999a
Human T lymphocytes, *hprt* locus, in vivo (autoradiographic assay)	positive	1.2 (avg)	Ward et al., 1994
Human T lymphocytes, *hprt* locus, in vivo (autoradiographic assay)	positive	0.1 (avg)	Ward et al., 1996b
Human T lymphocytes, *hprt* locus, in vivo (cloning assay)	negative	1.2 (avg)	Hayes et al, 1996
Human T lymphocytes, *hprt* locus, in vivo (cloning assay)	negative	0.3 (avg)	Tates et al., 1996
Cytogenetic effects-Somatic cells			
(102 × C3H)F1 mouse bone marrow & peripheral blood MN, in vivo	positive	40	Adler et al., 1994
(102 × C3H)F1 mouse spleen & peripheral blood MN, in vivo	positive	100	Stephanou et al., 1998
Human peripheral blood, chromosome aberrations, in vivo	negative	0.1 (avg)	Au et al., 1995

Table 4-2. Summary table of genetic effects of 1,3-butadiene (details in text) (continued).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Cytogenetic effects-Somatic cells (continued)			
Human peripheral blood, chromosome aberrations, in vivo	negative	0.8 (avg)	Hallberg et al., 1997
Human peripheral blood, chromosome aberrations & SCE, in vivo	negative	0.3 (avg)	Sram et al., 1998
Human peripheral blood, chromosome aberrations, MN, & SCE, in vivo	negative	0.6 (avg)	Sorsa et al., 1994
Cytogenetic effects-Germ cells			
CD-1 male mice, dominant lethal, in vivo	positive	155	Hackett et al., 1988a
CD-1 male mice, dominant lethal, in vivo	positive	980 (acute)	Anderson et al., 1993
CD-1 male mice, dominant lethal, in vivo	positive	10 (subchronic)	Anderson et al., 1993
CD-1 male mice, dominant lethal, in vivo	positive	98 (subchronic)	Brinkworth et al., 1998
CD-1 male mice, dominant lethal, in vivo	positive	50 (subchronic)	Anderson et al., 1998
C3H male mice, heritable translocation, in vivo	positive	390 (5days)	Adler et al., 1995
C3H male mice, heritable translocation, in vivo	positive	1,000 (5days)	Adler et al., 1998
Sprague-Dawley rats, dominant lethal, in vivo	negative	580 (subchronic)	Anderson et al., 1998

[a] LEC, lowest effective concentration; HIC, highest ineffective concentration; in vitro tests, μg/ml; in vivo tests, mg/kg bw.

Table 4-3. Summary table of genetic effects of 1,2-epoxy-3-butene (EB) (details in text).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Gene mutations			
$B6C3F_1$ mouse T lymphocytes, *hprt* locus, in vivo, ip	positive	100	Cochrane & Skopek, 1994a
(102 × C3H)F1 mouse T lymphocytes, *hprt* locus, in vivo, ip	positive	3 × 33	Tates et al., 1998
(102 × C3H)F1 mouse T lymphocytes, *hprt* locus, in vivo, drinking water	negative	17	Tates et al., 1998
$B6C3F_1$ mouse T lymphocytes, *hprt* locus, in vivo, inh	positive	9	Meng et al., 1999b
Lewis rat T lymphocytes, *hprt* locus, in vivo, ip	negative	40	Tates et al., 1998
Lewis rat T lymphocytes, *hprt* locus, in vivo, drinking water	negative	7	Tates et al., 1998
Fisher 344 rat T lymphocytes, *hprt* locus, in vivo, inh	negative	8	Meng et al., 1999b
Human TK6 cells, *hprt* locus, in vitro	positive	10.5	Cochrane & Skopek, 1994b
Cytogenetic effects-Somatic cells			
(102 × C3H)F1 mouse spleen MN, in vivo, ip	positive	40	Xiao & Tates, 1995
(102 × C3H)F1 mouse bone marrow MN, in vivo, ip	positive	20	Adler et al., 1997
CD-1 mouse bone marrow MN, in vivo, ip	positive	40	Anderson et al., 1997
BALB/c mouse spleen MN & SCE, in vivo, ip	positive	48.8	Stephanou et al., 1997
BALB/c mouse peripheral blood MN, in vivo, ip	positive	22	Russo et al., 1997
CD-1 mouse splenocyte SCE, in vitro	negative	70	Kligerman et al., 1999
CD rat splenocyte SCE, in vitro	negative	70	Kligerman et al., 1999
Lewis rat spleen MN, in vivo, ip	positive	80	Xiao & Tates, 1995
Sprague-Dawley rat bone marrow MN, in vivo, ip	positive	120	Anderson et al., 1997
Human peripheral blood SCE, in vitro	positive	3.5	Uuskula et al., 1995

Table 4-3. Summary table of genetic effects of 1,2-epoxy-3-butene (EB) (details in text) (continued).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Cytogenetic effects-Somatic cells (continued)			
Human peripheral blood SCE, in vitro	positive	8.75	Bernardini et al., 1998
Human peripheral blood SCE, in vitro	negative	70	Kligerman et al., 1999
Cytogenetic effects-Germ cells			
(102 × C3H)F1 mouse sperm. MN, in vivo, ip	positive	40	Xiao & Tates, 1995
BALB/c mouse sperm. MN, in vivo, ip	positive	55	Russo et al., 1997
(102 × C3H)F1male mouse dominant lethal, in vivo, ip	negative	120	Adler et al., 1995
Sprague-Dawley rat testes, MN, in vitro	negative	70	Sjöblom & Lähdetie, 1996
Sprague-Dawley rat spermatids, MN, in vivo	positive	13	Lähdetie et al., 1997

[a] LEC, lowest effective concentration; HIC, highest ineffective concentration; in vitro tests, μg/ml; in vivo tests, mg/kg bw.

Table 4-4. Summary of genetic effects of 1,2:3,4-diepoxybutane (DEB) (details in text).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Gene mutations			
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo, ip	positive	7	Cochrane & Skopek, 1994a
(102 × C3H)F1 mouse T lymphocytes, *hprt* locus, in vivo, ip	negative	40	Tates et al., 1998
(102 × C3H)F1 mouse T lymphocytes, *hprt* locus, in vivo, drinking water	negative	21	Tates et al., 1998
B6C3F$_1$ mouse T lymphocytes, *hprt* locus, in vivo, inh	positive	9	Meng et al., 1999b
Lewis rat T lymphocytes, *hprt* locus, in vivo, ip	negative	100	Tates et al., 1998
Lewis rat T lymphocytes, *hprt* locus, in vivo, drinking water	negative	8.5	Tates et al., 1998
Fisher 344 rat T lymphocytes, *hprt* locus, in vivo, inh	positive	2	Meng et al., 1999b
Human TK6 cells, *hprt* locus, in vitro	positive	0.2	Cochrane & Skopek, 1994b
Cytogenetic effects-Somatic cells			
(102 × C3H)F1 mouse spleen MN, in vivo, ip	positive	15	Xiao & Tates, 1995
(102 × C3H)F1 mouse bone marrow MN, in vivo, ip	positive	9	Adler et al., 1995
CD-1 mouse bone marrow MN, in vivo, ip	positive	30	Anderson et al., 1997
BALB/c mouse peripheral blood MN, in vivo, ip	positive	9	Russo et al., 1997
CD-1 mouse splenocyte SCE, in vitro	positive	1.7	Kligerman et al., 1999
CD rat splenocyte SCE, in vitro	positive	1.7	Kligerman et al., 1999
Lewis rat spleen MN, in vivo, ip	positive	15	Xiao & Tates, 1995
Sprague-Dawley rat bone marrow MN, in vivo, ip	positive	25	Anderson et al., 1997
Human peripheral blood SCE, in vitro	positive	0.17	Norppa et al., 1995
Human peripheral blood SCE, in vitro	positive	1.7	Kligerman et al., 1999

Table 4-4. Summary of genetic effects of 1,2:3,4-diepoxybutane (DEB) (details in text) (continued).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Cytogenetic effects-Germ cells			
(102 × C3H)F1 mouse sperm. MN, in vivo, ip	positive	30	Xiao & Tates, 1995
BALB/c mouse sperm. MN, in vivo, ip	positive	9	Russo et al., 1997
(102 × C3H)F1male mouse dominant lethal, in vivo, ip	positive	18	Adler et al., 1995
Sprague-Dawley rat testes, MN, in vitro	positive	0.4	Sjöblom & Lähdetie, 1996
Sprague-Dawley rat spermatids, MN, in vivo	positive	16.7	Lähdetie et al., 1997

[a] LEC, lowest effective concentration; HIC, highest ineffective concentration; in vitro tests, μg/ml; in vivo tests, mg/kg bw.

Table 4-5. Summary table of genetic effects of 3,4-epoxy-1,2-butanediol(EBD) (details in text).

Test system	Results	Dose (LEC or HIC)[a]	Reference
Gene mutations			
Human TK6 cells, *hprt* locus, in vitro	positive	47	Cochrane & Skopek, 1994b
Cytogenetic effects-Somatic cells			
(102 × C3H)F1 mouse bone marrow MN, in vivo, ip	positive	120	Adler et al., 1997
(102 × C3H)F1male mouse dominant lethal, in vivo, ip	positive	26	Adler et al., 1995
Cytogenetic effects-Germ cells			
Sprague-Dawley rat testes, MN, in vitro	negative	102	Sjöblom & Lähdetie, 1996
Sprague-Dawley rat spermatids, MN, in vivo	positive	30	Lähdetie et al., 1997

[a] LEC, lowest effective concentration; HIC, highest ineffective concentration; in vitro tests, μg/ml; in vivo tests, mg/kg bw

two treated mice with zero mutants were excluded from the analysis, the difference was not significant (initial group size was five animals). In the third experiment with this strain using 500 and 1,300 ppm 1,3-butadiene, an additional shorter expression time was incorporated into the design. The *hprt* mutant frequencies at 1,300 ppm were higher than the 500 ppm, but neither were significantly different from controls. There were no differences in mutant frequencies between the 35- and 70-day expression times in the 1,300 ppm group, but the mutant frequency at 70 days was twice that at 35 days expression time. The authors could offer no apparent reason for the failure to detect a mutagenic response in this third experiment. The mice for all of the above experiments were bred and treated in Dr. Adler's laboratory in Neuherberg, Germany.

A single experiment used CD-1 male mice exposed to 1,3-butadiene concentrations of 12.5, 65, or 130 ppm in Dr. Anderson's laboratory at BIBRA. The authors stated that statistical analysis indicated no trace of a mutagenic effect (Tates et al., 1998), as would be expected with the concentrations used.

Meng et al. (1998) reported on a study in which female $B6C3F_1$ mice and F344 rats were exposed by inhalation to 1,250 ppm 1,3-butadiene for 1 or 2 weeks (6 h/day, 5 days/week). Groups of animals were necropsied before exposure (controls) and weekly up to 10 weeks after the last exposure. The researchers measured *hprt* mutants in both spleen and thymus using the T-cell cloning assay. Mutant frequencies (mf) in both tissues of both species increased for several weeks and then declined. Maximal frequencies were: in thymus, 11.3×10^{-6} in mice (2 weeks) and 4.9×10^{-6} in rats (3 weeks); in spleen, 19.7×10^{-6} in mice (5 weeks) and 8.4×10^{-6} in rats (4 weeks). They determined a relative mutagenic potency (RMP) as the ratio of cumulative increase in mutant frequency in treated versus controls. For the spleen the RMP was about fivefold greater in mice than rats, which is similar to differences in metabolism. In a more recent report (Meng et al., 1999a), female mice and rats were exposed to 1,3-butadiene at 0, 20, 62.5, and 625 ppm, 6 h/day, 5 days/wk for 2 or 4 weeks. Animals were sacrificed at 2, 4, 5, 6, and 8 weeks after exposure for *hprt* mutant analysis in spleen T-cells. Mf increased linearly in both species at 625 ppm. Mf in mice was about fivefold and tenfold above controls after 2 and 4 weeks' exposure, and in rats, though linear, was only twofold above controls after 4 weeks' exposure. In the time-course experiments, the patterns were very similar to those observed in the previous study with 1,250 ppm 1,3-butadiene. Calculated RMPs were as follows:

	62.5 ppm	625 ppm
$B6C3F_1$ mice	11	61
F344 rats	not significant	7

Molecular analysis of mutants from control and 1,3-butadiene-treated mice indicated a significant decrease in base substitution mutations. Of base substitutions, approximately 60% were at AT sites in controls, whereas about 55% were at GC sites in the treated groups (most in the nontranscribed strand). The decrease in base substitutions was reflected by a significant increase in the frequency of large deletions in 1,3-butadiene-derived mutants.

Several recent studies have measured in vivo mutations using the phage *lacI* or *lacZ* genes incorporated into a rodent genome. Recio and Goldsworthy (1995) summarized experiments in which male B6C3F_1 *lacI* transgenic mice were exposed to 62.5, 625, and 1,250 ppm 1,3-butadiene (6 h/day, 5 days/week) for 4 weeks. Two weeks after the last exposure, animals were euthanized and DNA was extracted from bone marrow to be examined for *lacI* mutagenesis. Mutant frequencies increased in a dose-response manner, reaching an apparent plateau at 625 ppm (about a fourfold increase above controls). Sequence analysis of *lacI* mutant colonies from the 625 and 1,250 ppm groups indicated an increased frequency of point mutations at A:T base pairs. A second group of animals was exposed to 1,3-butadiene at 625 ppm for only 5 days (Recio et al., 1996a). The observed *lacI* mutant frequency was 12.6×10^{-5} compared to 15.3×10^{-5} after the 4-week exposure at 625 ppm. Subsequently, *lacI* mutagenesis was examined in spleen samples from the same animals (Recio et al., 1998). Although mutant frequencies were significantly increased at all concentrations, the maximum response (also fourfold above controls) was reached at 62.5 ppm. Mutants from the air controls and the 1,250 ppm exposed groups were amplified and sequenced to identify mutational sites. As in the bone marrow mutants, the frequency of mutations at A:T sites was increased. But more striking was an increase in G:C $\rightarrow$ A:T at non-CpG sites (21% of mutations in the 1,250 ppm group compared with 5% in controls). The authors stated that this increase in G:C bases was not observed in bone marrow. These findings are consistent with those observed in 1,3-butadiene-induced *hprt* mutant T lymphocytes from B6C3F_1 mice (Cochrane and Skopek, 1994b).

The mouse spot test, although seldom used, was developed to detect mutations in seven recessive genes (expressed as visible spot changes in coat color) in exposures of pregnant female mice. Adler et al. (1994) exposed pregnant females to 0 and 500 ppm 1,3-butadiene on days 8-12 of pregnancy. The incidence of visible spots in the exposed group was significantly above controls. Other endpoints indicative of maternal or fetal toxicity, litter size, number weaned, and malformations were not different from controls.

Several studies of genetic effects in exposed workers have recently been reported. Ward et al. (1994) measured the frequency of *hprt* mutations in lymphocytes of workers in a 1,3-butadiene production plant (two studies) and in a styrene-butadiene rubber (SBR) plant. In the first study exposure estimates were based on 8-h samples in two production areas and in a central control area. Mean 1,3-butadiene concentration in the production areas was 3.5 ppm, but the

majority of samples showed concentrations below 1 ppm; mean 1,3-butadiene concentration in the control area was 0.03 ppm. Variant frequencies at the *hprt* locus in PHA-stimulated peripheral blood T-cells of a high-exposure group were increased more than threefold compared with the low-exposure and nonexposed groups. The eight individuals in the high-exposure group had *hprt* variant frequencies varying from 0.94×10^{-6} to 8.98×10^{-6} and the variant frequency generally correlated with the level of the metabolite dihydroxybutane in the urine. Whether the difference was due to differences in exposure or genetic differences in metabolism cannot be ascertained from the data. A second study was conducted in the same plant about 1 year later (Ward et al., 1996). Measured 1,3-butadiene concentrations in personal samplers were markedly lower, 0.30 ± 0.59, 0.21 ± 0.21, and 0.12 ± 0.27 ppm in areas defined as high, medium, and low exposure (no controls were reported for the second study). The corresponding *hprt* variant frequencies were 5.33 ± 3.76, 2.27 ± 0.99, and $2.14 \pm 0.97 \times 10^{-6}$, respectively. Individual data were not reported for this study, but again there is a high standard deviation in the highly exposed group. The Ward et al. (1996) paper also reported preliminary results from workers in a styrene-butadiene rubber plant. Workers were assigned to high (20 of 40 personal samplers exceeded the 0.25 ppm detection limit and 11 had a concentration over 1 ppm) and low (none of 26 exceeded the detection limit) exposure groups. In nonsmokers, the *hprt* variant frequencies were 7.47 ± 5.69 and $1.68 \pm 0.85 \times 10^{-6}$ for the high and low groups, respectively. Although the variant frequency for smokers in the high-exposure group (6.24 ± 4.37) was not different from nonsmokers, the frequency for smokers in the low-exposure group was about twice that of the nonsmoker group (3.42 ± 1.57). These preliminary findings with small sample sizes and no detail about smoking history or other confounding factors raise several unanswerable questions regarding the contribution of 1,3-butadiene exposure to the observed differences in *hprt* variants.

Hayes et al. (1996) employed the T-cell cloning assay to detect mutant frequencies in lymphocytes of workers in a rubber production factory. 1,3-Butadiene levels were measured using personal samplers during the 6-h work shift and expressed as a 6-h time-weighted average (TWA). These were supplemented with several grab samples. Three different work areas were identified: initial distillation and recovery from dimethyl formamide, polymerization, and recovery, with median air levels of 3.5, 1.0, and 1.1 ppm, respectively. The T-cell cloning assay was performed from postshift blood samples. Unexposed subjects were age- and gender-matched and a brief questionnaire was administered. Tabular *hprt* mutant frequencies were presented grouped only by gender and exposed versus unexposed. Mean mutant frequencies were somewhat higher in females than males. Smoking (in males only) was not different in either group, but mutant frequency did significantly increase with age. Mean mutant frequencies, raw and adjusted for age, sex, cloning efficiency, and exposure status were similar in exposed

and nonexposed workers. Adjusted mean frequency for total exposed workers was 18.0×10^{-6} compared with 13.6×10^{-6} for nonexposed workers.

In a third study, Tates et al. (1996) used the T-cell cloning assay on blood samples collected from workers in a 1,3-butadiene plant in the Czech Republic. Workers were sampled in 1993 and 1994, but most of the blood samples from 1993 were lost to technical errors. A detailed analysis was conducted on the later group of 19 exposed and 19 nonexposed workers from other parts of the same plant. Personal samplers indicated a mean 1,3-butadiene concentration of 1.76 ppm, with individual samples ranging from 0.012 ppm to 19.77 ppm. The geometric mean *hprt* mutant frequencies (adjusted for age, smoking, and cloning efficiency) were 7.10×10^{-6} for exposed and 10.59×10^{-6} for the controls. The range of mutant frequencies among individuals was similar for both groups and individual mutant frequencies in the exposed group were not correlated with concentrations of 1,3-butadiene detected in the personal samplers.

The results in both the T-cell cloning assay groups are in apparent conflict with the Ward et al. (1994, 1996) findings both for exposed versus nonexposed and for smokers versus nonsmokers. There are a number of differences in the protocols of the two procedures that could account for the differences reported. Also, the study populations differed. Ward et al. (1994, 1996) conducted studies at a monomer production facility and an SBR production facility in Texas, Tates et al. (1996) conducted the study in a monomer facility in the Czech Republic, and Hayes et al. (1996) conducted the study in a polybutadiene rubber production plant in China. Hence, these differences as well as the lack of precision of exposure determinations preclude any direct comparison of these reports.

4.1.2. Cytogenetic Effects

4.1.2.1. *Somatic Cells*

Most of the rodent in vivo cytogenetic studies on 1,3-butadiene—especially in somatic cells—have been thoroughly treated in the reviews cited in the introduction of this chapter. In those studies, positive results were reported for all cytogenetic endpoints studied in mice and negative results were consistently reported in rats.

Adler et al. (1994) exposed both male and female (102 × C3H)F1 mice to 1,3-butadiene at 50, 200, 500, and 1,300 ppm for 6 h/day for 5 days and measured micronuclei in bone marrow and peripheral blood sampled 18-24 h after exposure. Significant increases were seen at all dose levels in both tissues, with the slope of the response decreasing after 200 ppm and essentially leveling at 500 ppm. At 50 ppm the responses were similar in both sexes, but at higher concentrations the incidence of MN was significantly greater in males (19.2 vs. 14.1 at 500 ppm). In a later study Stephanou et al. (1998) exposed (102 × C3H)F1 males to 0, 130, 250, or 500 ppm 1,3-butadiene 6 h/day for 5 days. Animals were sacrificed 2, 5, and 13-14 days after the end of

exposure, spleens were dissected, and splenocytes were collected for micronucleus analysis. Blood samples were obtained from the 13–14 day group for peripheral blood micronucleus analysis. Although MN frequencies were elevated in all groups analyzed, again the slope decreased with increased concentration. Toxicity in the spleen precluded obtaining sufficient cells for measurement on day 2 in the 500 ppm group. Significantly fewer MN were recorded at the latest sampling at all concentrations. CREST antibody analysis used with splenocytes showed only a weak aneugenic response in day 2 samples from the 130 and 250 ppm groups.

Anderson et al. (1997) reported negative results using the Comet assay in liver and bone marrow in CD-1 male mice after exposure to 1,3-butadiene at 0, 12.5, 65, or 130 ppm, 6 h/day, 5 days/wk for 4 weeks. (This assay detects DNA migrating out of the cell nucleus during electrophoresis. Damaged DNA, with strand breaks migrates further than intact DNA and measurement of length, area, and density of the "tail" provides an index of DNA damage.)

There have been several studies evaluating cytogenetic effects of exposed workers. Au et al. (1995) measured chromosome aberration frequencies in blood samples of 10 exposed workers and 10 matched controls from the same population used in the Ward et al. (1996b) study cited above. They reported measurable, but not significant (p>0.1), increases in chromosome aberrations and chromatid breaks. Also, cells were exposed to gamma rays in G1 and aberrations were measured in the subsequent metaphase. With this indirect measure of DNA repair, chromatid breaks, deletions, and dicentrics were all significantly higher in cells from 1,3-butadiene-exposed workers. A second study by the same group exhibited similar results (Hallberg et al., 1997). Male workers in a 1,3-butadiene production plant wore personal film badges during a work shift, at the end of which they donated a blood and urine sample. Mean exposure was 2.4 ppm (TWA) among 24 exposed workers and 0.3 ppm among 19 control workers. Chromosome aberration frequencies in standard assays and in gamma ray challenge assays were higher in exposed workers, but in neither case was the increase statistically significant. Recently, Sram et al. (1998) examined cytogenetic damage in peripheral lymphocytes of workers in a 1,3-butadiene monomer production plant in the Czech Republic. Nineteen exposed workers and 19 matched controls from other parts of the same plant wore personal monitors for one work shift, after which they contributed blood samples. Both chromosome aberrations and SCE were significantly higher in exposed workers, even when corrected for smoking. Micronuclei were higher in exposed than in controls, but the difference was not significant. This negative finding is questionable because of the surprisingly high incidence of micronuclei in the nonsmoking control group (17.45 vs. 10.75 for control smokers). DNA damage measured by the Comet assay also was not elevated in the exposed workers.

Sorsa et al. (1994) investigated chromosomal damage in blood lymphocytes sampled in 1993 from workers in the factories described by Tates et al. (1996) above. Chromosome aberrations, MN, and SCE frequencies were not elevated above samples from unexposed persons. They did note that smoking had a slight effect in MN formation and SCE but not in chromosome aberrations. Preliminary data measuring chromosome aberrations and micronuclei in blood samples from the 1994 group of workers were reported by Tates et al. (1996). The percentage of aberrant cells was significantly increased ($p<0.01$) in exposed subjects; however, the frequency of micronuclei in lymphocytes was similar in exposed and unexposed subjects. Evaluation of data for each subject would be required to determine the basis for the apparent discrepancy of the results between the two years.

4.1.2.2. *Germ Cells*

The question of whether 1,3-butadiene has the capacity to induce stable genetic damage has been addressed in dominant lethal and heritable translocation experiments. Hackett et al. (1988a) exposed CD-1 mice by whole-body inhalation to 200, 1,000, and 5,000 ppm 1,3-butadiene for 6 h/day on 5 consecutive days. A significant increase in the number of early deaths of implants per pregnant female was observed during the first 2 weeks after exposure, but the results were not dose related. Males exposed to 200 ppm and 1,000 ppm sired litters with increased numbers of early deaths. This effect was not seen in the group exposed to 5,000 ppm. In addition, in the first week post-exposure the number of dams showing two or more dead implants per pregnancy was increased for all dose groups. Because these effects were noted in the first week or two following exposure, the authors suggested that spermatozoa and spermatids were the susceptible germinal stage.

In a second dominant lethal study, Anderson et al. (1993) treated male CD-1 mice with acute and subchronic inhalation exposures to 1,3-butadiene and mated the males to untreated females. In the acute study, the male mice were exposed to 0, 1,250, or 6,250 ppm for 6 h. The females were examined for live fetuses, postimplantation deaths, and gross malformations. The only statistically significant result was a small decrease in implantations at 1,250 ppm. In the subchronic study, the males were exposed to 0, 12.5, or 1,250 ppm for 6 h/day, 5 days/week for 10 weeks. Statistically significant effects were observed at both exposure levels. An increase in late deaths and abnormal fetuses was observed at 12.5 ppm. A decrease in the number of implantations and an increase in the number of dominant lethal mutations were found at 1,250 ppm, as well as an increase in fetal malformations. Further evaluation of the fetuses as well as evaluation of animals allowed to deliver their young was published in a report and a paper by Anderson et al. (1995, 1996). In fetuses recovered on gestation day 17, skeletal changes were evaluated in the small number of malformed fetuses and their normal litter mates and controls,

and karyotypes were examined from the livers of these fetuses. A few minor skeletal changes were seen in the skeleton, particularly in skull bones of fetuses with exencephaly. Pups from the other half of the litters were raised to 37 weeks of age for control and high-exposure animals in the acute study, and for 75 weeks for all animals in the subchronic study. There was no obvious increase in tumor incidence above control values, but a histologic examination had not been completed.

Adler and Anderson (1994) used male (102/E1 × C3H/E1)F1 mice to assess the stage at which male germ cells are affected by 1,3-butadiene. Adult males were exposed by inhalation to 0 or 1,300 ppm, 6 h/day for 5 consecutive days. Four hours after the end of exposure, each male was mated at a ratio of 1:2 to untreated virgin females. Females judged bred by the presence of a vaginal plug were replaced with new females, and mating continued for 4 consecutive weeks. Females were killed on gd 14 to 16 and examined for numbers of live and dead implants. Exposure of male mice to 1,300 ppm resulted in an increase in dead implants during the first to the third weeks of mating; however, statistical significance ($p \leq 0.01$) was reached only in the second week. When expressed as a percentage of dominant lethals, a significant increase was seen in the second (12.4%, $p \leq 0.01$) and third (5.5%, $p \leq 0.05$) weeks. Because of the time course for dominant lethal mutations to manifest as dead implantations, 1,3-butadiene again appears to induce dominant lethality in spermatozoa and late spermatids.

Brinkworth et al. (1998) exposed CD-1 mice, aged 8-10 weeks to either 0, 12.5, or 125 ppm 1,3-butadiene. The purpose of this study was to undertake a dominant lethal study in mice at sub-chronic low dose (6 h/day, 5 days/wk, 10 weeks) exposure to 1,3-butadiene. CD-1 mice, aged 8-10 weeks were assigned to 3 groups and exposed to either 0, 12.5, or 125 ppm 1,3-butadiene and then mated to untreated virgin females. Incidence of early deaths were significantly elevated only in the high (125 ppm) dose group relative to the control group ($p<0.01$). The incidence of late deaths and of dead fetuses was clearly higher in both dose groups than in the control, but the difference was not statistically significant.

Anderson et al. (1998) reported on a comparative study testing the hypothesis that genetic effects could be transmitted by males to their offspring following subchronic inhalation exposure to 1,3-butadiene in mice and rats. Because it had been previously shown that mice require only 4 weeks of exposure (Adler and Anderson, 1994), male CD-1 mice were exposed to 0 ppm, 12.5 ppm, 65 ppm, or 130 ppm for 6 h/day, 5 days/wk, for 4 weeks. In the rat study, Sprague-Dawley males were exposed to 0 ppm, 65 ppm, 400 ppm, or 1,250 ppm for 6 h/day, 5 days/wk, for 10 weeks. Each exposed male was mated with two untreated virgin females. Females were killed the day before parturition (day 17 after finding a vaginal plug in mice and on day 19 after finding a sperm-positive vaginal smear in rats). Females were examined for the number of live fetuses, the number of fetuses with gross malformations, and the number of postimplantation early and

late deaths. In mice there were no significant increases in implants between the three dose groups and controls. However, there was a significant increase in the mean number of early embryonic deaths in the 65 and 130 ppm dose groups, with a NOAEL of 12.5 ppm. There was no significant increase in mean number of late deaths or dead or abnormal fetuses from control. In rats, there was a statistically significant ($p<0.05$) reduction in implants in the 65 ppm exposure group, but not in the 400 and 1,250 ppm dose groups. There was no significant increase in the mean number of early or late deaths or congenital malformations between treated and control rat groups. In both rodent studies neither mating frequency nor pregnancy rate was significantly reduced as a result of exposure.

Pacchierotti et al. (1998a) conducted a cytogenetic analysis of chromosome aberrations in first-cleavage embryos derived from 1,3-butadiene exposed male mice. Male (102/E1 × C3H/E1) mice were exposed for 5 consecutive days (6 h/day) to 0, 130, 500, or 1,300 ppm of 1,3-butadiene in air. On the last day of inhalation exposure, mice were mated during a 3-week period with untreated females for evaluation of transmissible chromosome damage. First-cleavage fertilized oocytes following paired matings were analyzed for chromosome and chromatid break and exchange aberrations for each dose group. Statistically significant increases over controls were observed in the first mating week of mice exposed to 500 and 1,300 ppm doses and in the second mating week of animals treated at the 1,300 ppm dose. There was no evidence of increased frequency of zygote aberrations obtained from animals in third week matings. Aberration induction was 2.5 times higher in the first than in the second mating week, suggesting that late developmental stages of spermatozoa were again more sensitive to butadiene than earlier stages. Of 72 aberrations observed, 96% of them were chromosomal breaks or exchanges.

To determine whether the cytogenetic damage induced by 1,3-butadiene was transmissible, heritable translocation studies were conducted (Adler et al., 1995, 1998). Male C3H/ E1 mice were exposed by inhalation to 1,3-butadiene at 500 ppm (Adler et al., 1998) or 1,300 ppm (Adler et al., 1995) for 5 days for 6 h/day and mated to 102/E1 virgin females for 7 days (days 8-14 post-exposure). Offspring were tested for translocations as revealed by reduced litter size and confirmed by cytogenetic analysis of meiotic and somatic cells. The translocation frequency from treated males was 1.15% and 2.7% for the 500 and 1,300 ppm groups compared with 0.05% for historical controls. The authors stated that the data best fit a linear equation: $y = 0.05 + 6.9 \times 10^{-5}x$. Using this equation, the authors calculated the doubling dose (that dose which induces as many translocation mutations as occur spontaneously per generation) of 725 ppm·h, or a concentration of approximately 24 ppm over the 5 days of 6 h/day exposure.

The results of the dominant lethal studies suggest that only postmeiotic stages are affected by 1,3-butadiene exposure. Xiao and Tates (1995) measured micronuclei in germ cells of male

(102 × C3H)F1 mice after inhalation exposure to 1,3-butadiene at 500 or 1,300 ppm 6 h/day for 5 days. Mice were sacrificed at 2, 5, 11, and 15 days after exposure to sample various stages of spermatogenesis. Significant increases were observed only at the 15-day sampling, indicative of damage to early spermatocytes. In a second experiment, males were exposed at 200, 500, and 1,300 ppm under the same conditions and only early spermatocytes were analyzed. The incidence of MN was 2.80, 3.40, and 2.60, respectively, for the three concentrations (control incidence was 0.33). Both body weight and testis weight were noticeably reduced at the highest concentration. These results were confirmed by Tommasi et al. (1998) using the same strain of mice and 1,3-butadiene concentrations of 130, 250, and 500 ppm. The frequency of MN again was increased only in early spermatocytes, with values significantly higher than controls only at 250 ppm 1,3-butadiene.

Anderson et al. (1997) also used the Comet assay on haploid cells from the testis of the males in the study cited earlier, which used a 4-wk exposure and observed no indication of a response. Later, Brinkworth et al. (1998) exposed CD-1 males to 1,3-butadiene at 12.5 and 1,250 ppm, 6 h/day for 1 day or for 10 wk (5 day/wk). After exposure one testis was taken for the Comet assay and the other was injected with ^{3}H-thymidine for later determination of unscheduled DNA synthesis (UDS). A weak but significant positive response was seen with the Comet assay after 1,250 ppm 1,3-butadiene in the 1-day but not the 10-wk exposure. For the UDS assay mice were sacrificed 17 days after ^{3}H-thymidine injection so that cells that were early spermatids could mature to spermatozoa. Although the mean counts were higher than controls in both groups for the 1-day exposure, high variability between animals meant that the differences were not significant. The authors also reported that the recovery of sperm from the *cauda epididymis* was at least 100-fold lower than expected, indicating toxicity.

4.2. METABOLITES

A search of the mutagenicity literature databases in Toxnet revealed a surprising number of more than 400 citations to DEB; about three-fourths of these appeared prior to 1991. These papers account for two-thirds of the total number of papers citing 1,3-butadiene or either of the two more common epoxide metabolites (EB and DEB). DEB was one of the mutagens of choice of investigators exploring various aspects of chemically induced mutations in a wide variety of biological systems. It is noteworthy that the stereoisomers of DEB were recognized to exhibit differential mutagenic activity as early as 1963 (Moutschen et al., 1963). The focus of the following sections is on those systems and endpoints that might provide information relevant to an understanding of the mode of action of 1,3-butadiene.

4.2.1. Gene Mutations

In the same paper cited earlier, Cochrane and Skopek (1994a) reported on the mutagenic activity of EB and DEB in the mouse *hprt* assay. Mice received three daily intraperitoneal (ip) injections of 60, 80, or 100 mg/kg of EB or 7, 14, or 21 mg/kg of DEB. Mutant frequencies in *hprt* from splenic T-cells were dose related for both metabolites, with maximal responses of 8.6 $\times 10^{-6}$ and 13 $\times 10^{-6}$ for EB and DEB, respectively. In a study using human TK6 cells, Cochrane and Skopek (1994b) showed that all three epoxide-forming metabolites were effective at inducing mutations at both the *hprt* and *tk* loci. Concentrations inducing approximately twofold increases in mutant frequency at the *hprt* locus were 3.5 μM, 150 μM, and 450 μM for DEB, EB, and 3,4-epoxy-1,2-butanediol, respectively. Later, Steen et al. (1997a,b) characterized the mutants induced by EB and DEB in TK6 cells. Although both metabolites induced significant increases in A:T to T:A transversions, only DEB resulted in a higher incidence of deletions.

In a series of experiments, Tates et al. (1998) measured *hprt* mutations in both mice and rats after exposure to either EB or DEB. A clear dose related increase was seen only in male (102 × C3H)F1 mice that had received three ip injections of 33 or 100 mg/kg EB (48 h between injections). Mice injected with single ip injections of 20 or 40 mg/kg of DEB or 3 injections of 7 or 14 mg/kg DEB showed no evidence of a mutational response. Likewise negative responses were reported in Lewis rats injected three times with 33 or 100 mg/kg EB or singly with 20 or 40 mg/kg DEB. Neither EB nor DEB was mutagenic in rats exposed to concentrations of 0.1, 0.3, or 1.0 mM of the chemicals in drinking water for 30 days.

In addition to the 1,3-butadiene experiments with mice and rats, Meng et al. (1999b) exposed females of both species to both EB and DEB. Using published data and PBPK models, they calculated concentrations of the metabolites that, using inhalation exposures, would give blood-level concentrations of the metabolites similar to those seen after exposure to 62.5 or 625 ppm of 1,3-butadiene. Animals were exposed to EB at 2.5 and 25 ppm, 6 h/day, 5 days/wk for 4 weeks. A preliminary 6-h exposure of female mice at 25 ppm to measure blood levels confirmed the similarity to 625 ppm 1,3-butadiene. For DEB, researchers selected concentrations of 2 and 4 ppm. Calculations indicated that 4 ppm would result in blood concentrations of DEB similar to 62.5 ppm of 1,3-butadiene, but because of concerns of nasal toxicity to the highly reactive DEB, researchers chose to use 4 ppm as the high concentration and used 2 ppm as a backup level. As with the 1,3-butadiene experiments, animals were sacrificed at various times after exposure (up to 9 weeks) for measuring *hprt* mutants in spleen T-cells. With EB, *hprt* mutant frequencies were significantly elevated only at 7 weeks post-exposure in mice. In rats, neither concentration caused a significant increase, but there was a significant upward trend also at 7 weeks post-exposure. Results with DEB were strikingly different. Mutant frequencies were significantly above controls even at 1 week after exposure in both species. In mice, mutant frequencies

increased only marginally at 2, 4, and 6 weeks post-exposure, reaching a maximum of 2.8-fold above controls, before declining to control level by 9 weeks. In rats, the increase was greater, with a maximum of 3.3-fold above controls by week 4 followed by a steeper decline, again reaching control levels by 9 weeks post-exposure.

4.2.2. Cytogenetic Effects

4.2.2.1. *Somatic Cells*

Adler et al. (1995) injected (102 × C3H)F1 males and females with DEB at 0, 4.5, 9, 18, and 36 mg/kg and sampled bone marrow 24 h later. A clear linear dose-response of MN was observed, with no differences between sexes, unlike the greater response in males seen previously with 1,3-butadiene exposure (Adler et al., 1994). In a later study (Adler et al., 1997), mice were injected with 20, 40, and 80 mg/kg of EB (both sexes) or 60, 120, and 240 mg/kg of EBD (males only). Bone marrow MN increased with dose after EB injection, with all doses significantly above untreated controls. Even though the MN response for EBD was also dose related, only the higher two doses were significantly higher than controls, and the response at 240 mg/kg was about the same as the response after 40 mg/kg of EB.

Xiao and Tates (1995) evaluated the cytogenetic effects of EB and DEB in both (102 × C3H)F1 mice and Lewis rats. Male animals of both species received single ip injections of 40 or 80 mg/kg of EB. Animals were sacrificed at various time intervals after treatment and spleens were processed for scoring of micronuclei. EB was almost four times more effective in the mouse as in the rat. For DEB, mice were injected with 15 or 30 mg/kg and rats received single ip injections of 20, 30, or 40 mg/kg. In a separate experiment, rats received 3 daily injections of 10 mg/kg. The response in splenocytes was similar in both mice and rats at 30 mg/kg. Using selected slides from this study, the investigators employed centromeric probes and fluorescent in situ hybridization (FISH) to investigate the origin of the induced micronuclei. They reported (Xiao et al., 1996) that in both mice and rats a clear clastogenic response was observed for the metabolites, and that only mice exhibited a weak anuegenic response. A positive response was also reported by Anderson et al. (1997) for both metabolites in CD-1 mice and in Sprague-Dawley rats. In that study, there seemed to be little if any difference between the species in response to either metabolite, though differences in control levels complicate the comparison.

Stephanou et al. (1997) injected BALB/C mice with EB at 24.4, 48.8, and 73.2 mg/kg and measured micronuclei and SCE at 2 and 16 days after treatment. Although induction of micronuclei reached a maximum after 48.8 mg/kg, SCE induction continued to increase up to the highest dose used. That the two endpoints reflect different genetic lesions was further indicated in that the residual level of SCE at 16 days of the two higher concentrations was only marginally higher than the response of the lowest dose, whereas for micronuclei the response at 16 days was

only slightly lower than after 2 days and the dose-response curves were essentially identical in pattern.

Russo et al. (1997) also detected increased incidence of MN in peripheral blood from BALB/C mice injected with EB. They also found DEB to be more than twice as effective an MN inducer as EB.

Kligerman et al. (1999) recently reported on a series of experiments providing additional evidence that the species differences in response to 1,3-butadiene exposure reflect differences in formation of active metabolites rather than differences in processing genetic lesions to observed toxic responses. They examined SCE and aberrations in mouse, rat, and human lymphocytes treated in G_0 with EB or DEB. One-hour treatments with EB at concentrations up to 1,000 μM were without effect in all three species. Longer treatments of mouse lymphocytes resulted in slight increase in SCE (2 h) and toxicity with few second-division cells (4 h). Conversely, DEB was an effective inducer of both SCE and aberrations in all three species, exhibiting linear responses up to 40 μM (the highest concentration tested). Responses from whole-blood or splenic lymphocytes were consistently lower than from isolated lymphocytes in both rats and mice, reflecting the absence of glutathione transferase activity in lymphocytes.

The role of glutathione S-transferase (GST) genes GSTM1 and GSTT1 enzymes in the detoxification of butadiene metabolites has been evaluated by measuring the induction of SCE in cultured human lymphocytes. Uuskula et al. (1995) found that SCE induction in lymphocytes from GSTM1-null individuals was 31% higher than in lymphocytes from GSTM1-positive individuals treated with 50 or 250 mM EB. The same group (Norppa et al., 1995) reported no difference in SCE induction among GSTM1 nulls and GSTM1-positive lymphocytes when treated in vitro with DEB; however, they observed a 60% increase in SCE in lymphocytes from GSTT1-null individuals treated with 2 or 5 μM DEB. Subsequently, these investigators (Bernardini et al., 1998) observed increased SCE response to EB in human lymphocyte cultures from GSTT1-null donors when compared with cultures from GSTT1-positive donors. They also reexamined the GSTT1 phenotypes in the earlier GSTM1-null study and found that only two of the six GSTM1-null donors were also GSTT1-nulls and that the difference in SCE response was still 1.4 times higher in the GSTM1-null/GSTT1-positives than the GSTM1-positive donors. Neither GSTM1 nor GSTT1 deficiency affected the induction of SCE by 250 or 500 μM of EBD (Bernardini et al., 1996). In a separate study, Kelsey et al. (1995) found that GSTT1 deficiency significantly increased the frequency of SCE induced by DEB in lymphocyte cultures of workers exposed to 1,3-butadiene.

In the study cited above, Kligerman et al. (1999) reported that differences in response between whole blood and isolated lymphocytes were seen only between GSTT1 null and $GSTT1^+$ groups. Differences in phenotype of GSTM1 were not seen within the two GSTT1 groups. The

authors did note that variability in response within the groups or within individuals sampled at different times suggested that other enzyme polymorphisms or dietary factors might also be involved.

4.2.2.2. *Germ Cells*

Xiao and Tates (1995) measured micronuclei in germ cells from the mice and rats in the study cited above. In mouse germ cells, the incidence of micronuclei was similar to controls on days 1 and 3 after exposure, but was significantly increased on day 14. In rats, EB was equally effective on days 1 to 3 (late spermatids) and day 20 (early spermatocytes), and the frequency of micronuclei at 80 mg/kg was slightly higher than that observed on day 14 in the mouse. DEB increased the frequency of micronuclei only on day 3 after treatment in mouse germ cells, whereas significant increases of micronuclei were observed in rat germ cells at all doses and all time periods.

Russo et al. (1997) observed similar results in spermatids and spermatocytes in male BALB/C mice treated with EB and DEB; however, they reported significant increases in MN in spermatocytes as well as in spermatids. As with peripheral blood MN, DEB was the more effective inducer of MN.

Sjöblom and Lähdetie (1996) used an in vitro meiotic micronucleus assay to examine the effects of EB, DEB, and EBD in seminiferous tubule sections of male Sprague-Dawley rats. Tissue sections were cultured for 4 days with EB at 100, 500, or 1,000 μmol/L; DEB at 5, 10, or 20 μmol/L; or EBD at 10, 50, or 100 μmol/L. The frequency of micronuclei was increased only by DEB and the increase was clearly dose-related. The authors suggest that EB requires further metabolism by P450 enzymes, which they indicate do not occur in rat testes microsomes. That this was an in vitro "artefact" was confirmed in their subsequent report, in which the three metabolites were injected ip into male Sprague-Dawley rats and sampled for micronucleus formation at several time periods after injection (Lähdetie et al., 1997). All three metabolites were active in the induction of micronuclei measured in spermatids and representing meiotic stages from G1 onward. Although on face value the three metabolites show peak responses in different stages of meiotic prophase, detailed analysis indicates that the differences are not statistically significant. Also, because of metabolism there are certainly different concentrations of the various metabolites in the different stages of spermatogenesis.

Dominant lethal studies have also been conducted with all three epoxide metabolites. Adler et al. (1995) injected male $B6C3F_1$ mice ip with 18, 36, or 54 mg/kg DEB. Toxicity of the high dose resulted in reduced fertility, and the implantation rate was too low to measure dominant lethality in the first two mating periods. Increased incidence of dead implants was observed in the next mating period, corresponding to treatment of late spermatids. A dominant

lethal effect with the two lower doses was seen only in the early matings representing mature spermatozoa. Researchers also injected a second group of males with 17, 26, 34, 43, or 52 mg/kg EBD to be subsequently mated with virgin females 7, 14, 21, and 28 days post-treatment. In cytogenetic analysis of first-cleavage-division embryos from the 26 mg/kg group, they recorded increased chromosome aberrations in embryos from only the first mating, again representing effects on spermatozoa. Embryos derived from early spermatids (day 14 and 21 matings) and spermatocytes (day 28 mating) had aberration levels not significantly above controls. The three higher doses exhibited extreme toxicity, precluding cytogenetic analysis.

In a companion study, Tiveron et al. (1997) injected gonadotropin-treated B6C3HF1 females with 26, 34, 43, or 54 mg/kg of DEB. Chemical injections were 24 h after injection of PMS and 24 h before the injection of HCG. Matings to untreated males were 4 h after HCG and cytogenetic analysis conducted on one-cell zygotes. Although matings were reduced in all treatment groups, the effect was not dose related. Further, among the successful matings there was no reduction in the percent of oocytes fertilized or in the percentage of zygotes reaching first-cleavage metaphase. Significant increases in aberrations were observed at all dose levels, with the response best fitting to a linear quadratic curve. Comparing the response in this study with the treated male study cited above (Adler et al., 1995), they reported that oocytes transmit 4 to 8 times fewer aberrations than treated sperm. The authors cited the known presence of DNA repair capacity in preovulated oocytes and its absence in late spermatocytes and mature sperm as well as the fact that oocytes have significantly more cytoplasmic protein available for binding the DEB, thus reducing the effective nuclear dose of DEB.

In a later study, Adler et al. (1997) injected male (102 × C3H)F1 mice ip with either 120 mg/kg of EB or 240 mg/kg of DEB. In this study, males were mated to both (102 × C3H)F1 and NMRI females. Surprisingly, neither EB nor DEB showed a dominant lethal effect, despite the dose-related effect of both in the bone marrow micronucleus test discussed above.

Experiments detecting cytogenetic damage in male germ cells indicate that the dominant lethal effects detected only from late spermatids and spermatogonia are not due to differential sensitivity to damage of those cell types, but rather are a reflection of the biology of spermatogenesis. Genetic damage in spermatogonia or premeiotic spermatocytes, especially the type of chromosome damage resulting in micronuclei formation, is most likely to be cytotoxic. Also, DNA repair activity ceases by the mid-spermatid stage; hence damage (DNA adducts) would remain in the sperm and be first “processed” in the first cleavage division of the zygote.

4.3. DISCUSSION

The current database of more than 600 publications provides ample evidence of the breadth of mutagenic responses that result from exposure to 1,3-butadiene of a variety of

biological systems ranging from bacteria to human beings. It is also clear that the mutagenic and genotoxic responses require the metabolic activation of 1,3-butadiene to several DNA-reactive intermediates, especially DEB, EB, and possibly EBD (data on this metabolite are scant). Consistent with (more accurately, as a consequence of) the differences in metabolism described in Chapter 3, mutagenic responses in mice are consistently greater than the responses in rats when animals are exposed to 1,3-butadiene. That the species difference in *hprt* response is greater after 4 weeks exposure than after 2 weeks (Meng et al., 1999a) indicates that the differences continue to diverge with the length of exposure. That *lacI* mutation frequencies do not increase from 1 week to 4 weeks exposure (Recio et al., 1996b) is not a direct contradiction of the Meng finding because several differences in experimental design preclude a direct comparison of the two studies.

A range of studies, both in vitro and in vivo, on the two major metabolites indicate that EB primarily induces point mutations and small deletions, whereas DEB, as a bifunctional alkylating agent, results in larger deletions. Additionally, DEB is mutagenically active in in vitro systems at concentrations similar to those found in the blood of mice exposed to 1,3-butadiene by inhalation. EB requires concentrations in vitro 10- to 100-fold greater than those found in the blood of 1,3-butadiene-exposed mice. Studies with EB and DEB measuring *hprt* mutations in female mice and rats have revealed, at least initially, some unexpected results (Meng et al., 1999b). Although, as expected, EB was positive in the mouse and negative in the rat, at equivalent exposure concentrations DEB was about threefold more mutagenic in Fischer 344 rats than in C3B6F1 mice. Similarly, Xiao and Tates (1995) reported greater response in MN in bone marrow and male germ cells of Lewis rats than in (102 × C3H)F1 mice after ip exposure to DEB. Beyond differences in general metabolic rates, the rate of hepatic clearance in the rat is about two times slower than in the mouse, with possibly higher blood levels in the rat. Also, DEB seems to be less cytotoxic in rats, and as mutant yield is clearly a function of surviving cells, higher survival would result in higher observed mutant frequencies.

For alkylating agents, DNA adducts are the molecular precursors of the mutants observed in the various assay systems discussed above. Measurement of DNA adducts in target tissues can provide a direct and more accurate determination of the dose of carcinogen bound to DNA. As discussed in Chapter 3, a number of DNA adducts of 1,3-butadiene metabolites have been identified, mostly with guanine and adenine bases. The recent studies measuring specific DNA adducts have resolved some questions but have raised additional questions regarding the contribution of the different metabolites in both carcinogenic and mutagenic responses resulting from exposure to 1,3-butadiene. The report that EB-DNA adducts are not markedly different in rats and mice suggests that EB does not play a insignificant role in carcinogenesis of the two

species (Koc, et al., 1999). However, they also found that the THB-Gua adducts were predominantly EBD-derived adducts, rather than from DEB.

Many of the in vivo mutagenicity studies described above were part of a collaborative effort of several European laboratories in a project, "Multi-endpoint analysis of genetic damage induced by 1,3-butadiene and its major metabolites in somatic and germ cells of mice, rats and man: Genetic risk estimation by the parallelogram method." Mutations occurring in germ cells may be inherited by future generations and may contribute to genetic disease. In the weight-of-evidence scheme in the Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1986), 1,3-butadiene is in the highest category described based on animal data. As described in the Guidelines, there are a number of reasons that make it unlikely that there could ever be sufficient data relative to chemically induced genetic damage in humans.

The parallelogram approach was devised by Sobels (1977) as a way to estimate heritable genetic effects using data from somatic cells from both rodents and humans and data from rodent germ cell assays (measuring heritable effects) as three corners of a parallelogram from which the fourth (heritable effects in humans) could derived. Although the concept is simple in form, there are obviously many variables, both within and across species, that necessarily affect the confidence of any derived estimate. In the output of the European effort (Pacchierotti et al., 1998a), the results of the studies were summarized and genetic risk estimates were derived using the parallelogram concept and the more classical doubling dose concept. (The doubling dose can be traced back to the efforts to estimate the effects of ionizing radiation in the mid-1950s.) The doubling dose (DD) is simply that dose (concentration × time) that induces as many mutations per generation as occur spontaneously. When the dose-response is a linear function ($y = a + bx$), DD is simply the spontaneous incidence divided by the slope (a/b). Because the only heritable genetic endpoint data available are from the mouse heritable translocation test, the researchers used cytogenetic data in their calculations. Somatic cell data were from MN in mouse bone marrow (Adler et al., 1994) and chromosome aberrations in human lymphocytes (Sram et al., 1998). Using the linear portion of the curve of the mouse data researchers derived an estimated DD of 2,411 ppm·h. From the human data only an estimated concentration of 1.58 ppm for exposed workers was available. They used an exposure time of three years (lymphocyte lifespan) and assumed a work year of 46 weeks and 40 h/week. Combining these assumed figures with the observed frequencies of aberrant cells of 3.11% in exposed workers and 2.08% in controls, they calculated a DD of 16,394 ppm·h. In the second heritable translocation paper, Adler et al. (1998) calculated a DD of 725 ppm·h. Using simple proportions, a DD for heritable translocations in humans is calculated to be 4,930 ppm·h.

Alternatively, the parallelogram can be applied using the induced damage per unit dose. Using the same studies as above with slopes of 1.12×10^{-6} and 0.69×10^{-6} for MN and

translocations and the point estimate of 1.24×10^{-6} per ppm for aberrations in human lymphocytes, they calculated an estimated rate of heritable translocations in human germ cells of 0.76×10^{-6} per gamete per ppm of 1,3-butadiene.

Of course, application of the parallelogram method is overly simplistic and encompasses many assumptions, some a function of the chemical and some totally independent of any chemical exposure. Also, it must be recognized that the events measured in the cited studies are not truly toxic events. Although it is certainly true that translocations and other chromosome aberrations are critical and causative factors in many genetic diseases, their mere existence is not predictive of disease. This is because the impact of a mutation (in this case chromosome aberration) is a function of the physiological function of the gene (or genes) involved rather than the molecular nature of the genetic alteration.

The studies cited here, along with the many earlier genotoxicity studies discussed in the cited reviews, provide clear evidence that 1,3-butadiene is both mutagenic and clastogenic through its metabolism, primarily because of the mono- and diepoxide. For most endpoints, the difunctional DEB is clearly more effective than the monofunctional EB. Where both mice and rats have been studied, mice are more responsive than rats, except for the recent germ cell studies. Whether this exception is strain specific (among or between species) can only be answered with future work.

The role of glutathione S-transferase is also clearly established for the genotoxic effects of butadiene in human lymphocytes. That the induction of SCEs in human lymphocytes by the three metabolites is affected differently by GSTM1 and GSTT1 complicates risk estimation for human beings, assuming that SCE is a reliable biomarker for exposure (or effect).

5. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

5.1. REPRODUCTIVE EFFECTS

Several toxicity studies for 1,3-butadiene have examined reproductive effects in multiple species: one in rats, guinea pigs, rabbits, and dogs (Carpenter et al., 1944); three in rats (Owen et al., 1987; Owen and Glaister, 1990; Bevan et al., 1996); three in mice (NTP, 1984, 1993; Bevan et al., 1996); and an acute "sperm head morphology" study in B6C3F_1 mice (Hackett et al., 1988a). These studies are summarized in Table 5-1. In addition, several dominant lethal studies have been published that are related to reproductive toxicity; these are reviewed in Chapter 4. There are no data on reproductive or developmental effects in humans from exposure to 1,3-butadiene.

5.1.1. Carpenter et al. (1944)

Four groups, each consisting of 24 albino rats, 12 guinea pigs, 4 rabbits, and 1 dog, were exposed to 0, 600, 2,300, or 6,700 ppm 1,3-butadiene 7.5 h/day, 6 days/week for 8 months in 546-L chambers. Except for the dogs, which were all female (only one in each group), the animals were divided equally between the two sexes. Terminal body weights in rats were reduced to 90.5%, 86.3%, and 81.2% in the 600, 2,300, and 6,700 ppm groups, respectively, relative to controls. A similar trend was noted for male guinea pigs, while body weights for dogs and rabbits fluctuated. Microscopic lesions were observed solely in the liver, in which a mild, cloudy swelling was noted in 68% of the animals exposed to 6,700 ppm. Few results regarding fertility of rats, guinea pigs, and rabbits exposed to 1,3-butadiene were given in the report. Fertility, defined as the number of litters produced within a given time, was reduced in rats, with 3.3, 2.7, 2.5, and 2.6 litters being produced by animals exposed to 0, 600, 2,300, or 6,700 ppm, respectively. Because the results were not analyzed statistically and other details regarding the duration of the mating periods were not presented, it is not possible to conclude that 1,3-butadiene either did or did not have an effect on fertility in rats. Data presented on other species also were inadequate to conclude whether 1,3-butadiene had any effect on fertility.

5.1.2. Owen et al. (1987); Owen and Glaister (1990)

This 2-year chronic bioassay study is the same as the Hazleton Laboratories Europe, Ltd. (HLE, 1981b), study discussed previously by EPA (U.S. EPA, 1985). Male and female CD strain (Sprague-Dawley derived) rats (110 of each sex per group) were exposed by inhalation to 1,3-butadiene (99.2% purity) at target concentrations of 0, 1,000, or 8,000 ppm, 6 h/day, 5 days/week for 105 (females) or 111 (males) weeks. The average weekly concentration of the 1,3-butadiene dimer impurity (4-vinyl -1-cyclohexene) was 413 ± 219 ppm (v/v). Ten males and 10

Table 5-1. A summary of the reproductive and developmental effects of 1,3-butadiene.

Species	Dose, route, and time of exposure	Effects	LOAEL[a]	Reference
Reproductive Effects				
Male and female rats, guinea pigs, rabbits; female dogs	0, 600, 2,300, 6,700 ppm, 7.5 hr/day, 6 days/wk, 8 mos	↓ body wt in rats, guinea pigs; mild cloudy swelling in liver at 6,700 ppm in all species; ↓ fertility in rats	no statistical analysis	Carpenter et al., 1944
Male and female rats	0, 1,000, 8,000 ppm, 6h/day, 5 days/wk, 105 wks (females), 111 wks (males)	↑ mortality with exposure; benign and malignant mammary tumors, uterine sarcomas, thyroid follicular cell tumors (females); Leydig cell tumors, pancreatic exocrine adenomas (males)	1,000 ppm for 105 or 111 wks >8,000 ppm for non-neoplastic lesions	Owen et al., 1987; Owen and Glaister, 1990
Male and female $B6C3F_1$ mice	0, 625, 1,250 ppm, 6 hr/day, 5 days/wk, 60 or 61 wks	↑ mortality; numerous neoplasms including mammary gland; ovarian atrophy, uterine atrophy, (females); testicular atrophy (males)	625 ppm for 60 or 61 wks	NTP, 1984
Male and female $B6C3F_1$ mice	0, 6.25, 20, 62.5, 200, 625 ppm, 6 hr/day, 5 days/wk, 103 wks (some killed at 9 and 15 mos)	↑ mortality ($\geq$ 20 ppm); ovarian atrophy, germinal epithelial hyperplasia, angiectasis, granulosa cell tumors, uterine atrophy, mammary tumors (females); testicular atrophy (males)	6.25 ppm for 103 wks 62.5 ppm for 15 mos 200 ppm for 9 mos	NTP, 1993
Male and female rats and mice	0, 1,000 ppm, 6 hr/day, 5 days/wk, 13 wks	no effects on reproductive system (rats); ovarian atrophy (female mice); testicular atrophy (male mice)	1,000 ppm[b] for 13 wks in mice	Bevan et al., 1996

Table 5-1. A summary of the reproductive and developmental effects of 1,3-butadiene (continued).

Species	Dose, route, and time of exposure	Effects	LOAEL[a]	Reference
Reproductive Effects (continued)				
Male B6C3F$_1$ mice (sperm head morphology study)	0, 200, 1,000, 5,000 ppm, 6 hr/day, 5 days; killed at 5 wks after exposure	↓ % epididymal sperm, ↑ sperm head abnormalities	1,000 ppm for 5 days	Hackett et al., 1988a
Male 102/E1XC3H/E1 mice (flow cytometric analysis of spermatogonial cells)	0, 130, 500, 1,300 ppm, 6 hr/day, 5 days; killed 21 days after exposure and later	↓ testis weight, ↓ round and elongated spermatids	130 ppm for 5 days	Pacchierotti et al., 1998b
Developmental Effects				
Pregnant CD rats	0, 200, 1,000, 8,000 ppm, 6 hr/day, GD 6-15; killed GD 20	↑maternal toxicity, ↓ fetal body wt and CRL, ↑ skeletal and eye defects	1,000 ppm for 10 days	IISRP, 1982
Pregnant CD rats	0, 40, 200, 1,000 ppm, 6 hr/day, GD 6-15	↑maternal toxicity, no fetal effects	1,000 ppm for 10 days	Hackett et al., 1987a
Pregnant CD-1 mice	0, 40, 200, 1,000 ppm, 6 hr/day, GD 6-15	↑ maternal toxicity, ↓ fetal wt, ↑ skeletal variants	40 ppm for 10 days	Hackett et al., 1987b

[a] Lowest-observed-adverse-effect-level

[b] Frank effects on ovarian and testicular atrophy at this exposure level, not a LOAEL.

females were killed at 52 weeks. A comprehensive postmortem examination, including necropsy and histopathologic examination, was conducted on all gross lesions, all tissues from control and high-exposure groups, and selected tissues from low-exposure groups. Nonneoplastic lesions were not induced in reproductive organs in either male or female rats, although benign and malignant mammary tumors, uterine sarcomas, and thyroid follicular cell tumors were seen in females, while Leydig cell tumors and pancreatic exocrine adenomas were observed in males.

5.1.3. NTP (1984)

The first inhalation chronic bioassay study conducted by the National Toxicology Program (NTP, 1984) showed that, in addition to the numerous neoplasms induced by high concentrations of 1,3-butadiene in male and female B6C3F$_1$ mice, nonneoplastic lesions also were induced in reproductive organs. Male and female mice were exposed to 0, 625, or 1,250 ppm 1,3-butadiene 6 h/day, 5 days/week and then killed after 60 or 61 weeks of exposure. Among female mice, ovarian atrophy was seen in 40/45 (89%) mice exposed to 625 ppm and in 40/48 (83%) mice exposed to 1,250 ppm, compared with an incidence of only 2/49 (4%) in control mice. Involution of the uterus, which was considered a manifestation of ovarian atrophy, was seen in 7/46 (15%) and 14/49 (29%) mice exposed to 625 and 1,250 ppm, respectively, compared with 0/49 control mice. Uterine involution was characterized by fewer and less prominent endometrial glands. A low incidence of mammary gland neoplasms (acinar cell and adenosquamous carcinomas) was induced by 1,3-butadiene; nonneoplastic mammary lesions were not induced. Testicular atrophy was observed in 19/47 (40%) mice exposed to 625 ppm and in 11/48 (23%) mice exposed to 1,250 ppm compared with 0/50 control mice. Statistical analysis showed that the increased incidences of the lesions in male and female mice were significant (p<0.05) for all groups compared with their respective controls.

5.1.4. NTP (1993)

NTP (1993) conducted a second inhalation chronic bioassay study in male and female B6C3F$_1$ mice exposed to lower concentrations of 1,3-butadiene. Concentrations were 0, 6.25, 20, 62.5, 200, or 625 ppm 1,3-butadiene for 6 h/day, 5 days/week for 103 weeks, with interim evaluations at 9 and 15 months. Additional male mice were exposed to 200 ppm of 1,3-butadiene for 40 weeks or 625 ppm for 13 weeks (8,000 ppm·weeks), or 312 ppm for 52 weeks or 625 ppm for 26 weeks (16,000 ppm·weeks), followed by observation for the remainder of the 2 years (stop-exposure protocol). It should be emphasized that this study was designed to study neoplastic and general toxicological, rather than reproductive, endpoints. Further details are presented in Chapter 6.

The effects of 1,3-butadiene on reproductive organs in female mice are presented in Table 5-2. Ovarian atrophy was seen in the 200 ppm and 625 ppm exposure groups sacrificed for the 9-month interim evaluation. The atrophic ovaries were characterized by the absence of oocytes, follicles, and corpora lutea. No occurrences of this lesion were noted in the lower exposure groups. Hyperplasia of the germinal epithelium was observed in one animal exposed to 625 ppm for 9 months. Germinal epithelial hyperplasia was described as prominent down-growth of the mesothelial surface into the parenchyma of the ovary, forming tubular and glandlike structures. At the 15-month interim evaluation, ovarian atrophy was observed in mice exposed to 20 ppm or higher, with a significant increase in the incidence at 62.5 ppm or higher compared with concurrent controls. Hyperplasia of the germinal epithelium was seen at 200 and 625 ppm but was not significant. Angiectasis (dilation of blood vessels) was seen in one mouse in the control group, one exposed to 6.25 ppm, and two exposed to 200 ppm. The ovary, which was evaluated at 15 months in only two female mice exposed to 625 ppm, was atrophic in both. Among female mice exposed to 1,3-butadiene for 2 years, ovarian atrophy was observed in all exposure groups at incidences that were significantly elevated compared with controls. Therefore, using ovarian atrophy as an endpoint of reproductive toxicity, a no-observed-adverse-effect level (NOAEL) could not be defined in this mouse study. Although the functional integrity of the female reproductive system was not assessed, it can be assumed that animals without oocytes or follicles would be infertile and would express reduced estrogenic and progestin secretory capacities.

Uterine atrophy was seen at the two highest concentrations at 9 months, but was seen only at the highest concentration at the 15-month evaluation. After 2 years, the incidence of uterine atrophy among mice exposed to 200 and 625 ppm did not increase relative to that observed at 9 months.

Data regarding the effect of 1,3-butadiene on the reproductive organs of male B6C3F_1 mice are summarized in Table 5-3. The testes of a majority of males exposed to the highest concentration of 1,3-butadiene (625 ppm) were atrophic at the 9- and 15-month interim evaluations and at termination of the 2-year study. In the stop-exposure study, testicular atrophy was observed after 2 years in 5/50 mice exposed to 200 ppm 1,3-butadiene for 40 weeks and 3/50 exposed to 625 ppm for 13 weeks (8,000 ppm·weeks), and in 3/50 mice exposed to 312 ppm for 52 weeks and 5/50 exposed to 625 ppm for 26 weeks (16,000 ppm·weeks). The lack of a more prominent response in mice exposed to 625 ppm for 26 weeks may have been due to insufficient time for induction of testicular atrophy or, if atrophy was induced during exposure, the possibility of lesion repair following termination of the exposure and before the examination at 2 years.

Table 5-2. Reproductive tract lesions in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation.

Lesion	Concentration (ppm)					
	0	**6.25**	**20**	**62.5**	**200**	**625**
9-month interim evaluation						
Ovary[a]	10	—	—	10	10	8
Atrophy	0 (0%)[b]	—	—	0 (0%)	9 (90%)[c]	8 (100%)[c]
Germinal epithelial hyperplasia (NOS)	0 (0%)	—	—	0 (0%)	0 (0%)	1 (13%)
Uterus[a]	10	—	—	10	10	8
Atrophy[d]	0 (0%)	—	—	0 (0%)	3 (30%)	6 (75%)
15-month interim evaluation						
Ovary[a]	10	10	10	10	10	2
Atrophy	0 (0%)	0 (0%)	1 (10%)	9 (90%)[c]	7 (70%)[c]	2 (100%)[e]
Germinal epithelial hyperplasia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (30%)	1 (50%)
Angiectasis	1 (10%)	1 (10%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)
Uterus[a]	10	1	10	10	10	2
Atrophy[d]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
2-year study[f]						
Ovary[a]	49	49	48	50	50	79
Atrophy	4 (8%) $p<0.001$	19 (39%) $p<0.001$	32 (67%) $p<0.001$	42 (84%) $p<0.001$	43 (86%) $p<0.001$	69 (87%) $p<0.001$
Germinal epithelial hyperplasia	2 (4%) $p<0.001$	3 (6%) $p=0.460$	8 (17%) $p=0.017$	15 (30%) $p<0.001$	15 (30%) $p=0.010$	18 (23%) $p<0.001$

Table 5-2. Reproductive tract lesions in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation (continued).

Lesion	Concentration (ppm)					
	0	6.25	20	62.5	200	625
2-year study[f] (continued)						
Angiectasis	4 (8%) *p*=0.259	6 (12%) *p*=0.366	3 (6%) *p*=0.606	13 (26%) *p*=0.017	14 (28%) *p*=0.021	17 (22%) *p*=0.425
Uterus[a]	50	49	50	49	50	78
Atrophy[d]	1 (2%)	0 (0%)	1 (2%)	1 (2%)	8 (16%)	41 (53%)

[a]Number of animals for which this site was examined microscopically.
[b]Number of observations; percentage in parentheses.
[c]$p<0.01$, pairwise comparison with controls by Fisher's exact test.
[d]Statistical tests were not conducted for these lesions.
[e]$p<0.05$, pairwise comparison with controls by Fisher's exact test.
[f]*p* values for the statistical analysis (logistic regression test) for the 2-year study are presented; the value for the trend test is in the column for the control group, and the value for pairwise comparisons of individual exposed group with the corresponding control group is in the column for the exposed groups.

Source: NTP, 1993.

Table 5-3. Reproductive tract lesions in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation.

Lesion	Concentration (ppm)					
	0	**6.25**	**20**	**62.5**	**200**	**625**
9-month interim evaluation						
Testes[a]	10	10	10	10	10	10
Absolute weight (g)	0.117 ± 0.002	0.117 ± 0.003	0.114 ± 0.003	0.103 ± 0.004[b]	0.102 ± 0.002[b]	0.059 ± 0.003[b]
Relative weight (mg/g BW)[c]	2.89 ± 0.06	2.92 ± 0.09	2.76 ± 0.09	2.87 ± 0.12	2.54 ± 0.05[b]	1.57 ± 0.03[b]
Atrophy[d]	0 (0%)	—[e]	—	—	0 (0%)	6 (60%)
15-month interim evaluation						
Testes[a]	10	10	10	10	10	7
Absolute weight (g)	0.116 ± 0.003	0.113 ± 0.003	0.104 ± 0.004	0.112 ± 0.003	0.100 ± 0.003[b]	0.071 ± 0.004[b]
Relative weight (mg/g BW)	2.62 ± 0.07	2.79 ± 0.08	2.48 ± 0.04	2.66 ± 0.07	2.39 ± 0.05[f]	1.80 ± 0.05[b]
Atrophy[d]	0 (0%)	—	0 (0%)	—	0 (0%)	4 (57%)

Table 5-3. Reproductive tract lesions in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation (continued).

Lesion	Concentration (ppm)					
	0	6.25	20	62.5	200	625
2-year study						
Testes[a]	50	50	50	48	49	72
Atrophy[d]	1 (2%)	3 (6%)	4 (8%)	2 (4%)	6 (12%)	53 (74%)

[a]Number of animals for which this site was examined.
[b]$p \leq 0.01$, pairwise comparison with controls by Williams' or Dunnett's test.
[c]BW = body weight.
[d]Statistical tests were not conducted for these lesions.
[e]Testes were not examined microscopically at this concentration.
[f]$p<0.05$, pairwise comparison with controls by Williams' or Dunnett's test.

Source: NTP, 1993.

5.1.5. Bevan et al. (1996)

In a study on the subchronic effects of 4-vinylcyclohexene in rats and mice, groups of male and female rats and mice were exposed by inhalation to 1,000 ppm 1,3-butadiene for 6 hr/day, 5 days/wk for 13 weeks. Exposed male and female rats showed no effects on body weight or weight gain, but there was an increase in absolute and relative liver weights, and male rats had increased relative kidney weights with hyaline droplet accumulation in renal proximal tubules. No other microscopic effects were seen, and there were no clinical chemistry or hematologic effects in rats. In male and female mice exposed to 1,000 ppm 1,3-butadiene, there were no effects on body weight or weight gain. There was a significant decrease in circulating erythrocyte mass and an increase in reticulocyte counts in both males and females; male mice had mild macrocytic anemia. The most remarkable finding was ovarian atrophy in 6/10 female mice. Females also showed an increase in absolute and relative liver weight and a decrease in relative spleen weight and splenic atrophy in 3/10 mice. Males showed reduced testicular weights and testicular atrophy in 10/10 mice exposed to 1,000 ppm 1,3-butadiene. These data demonstrate that ovarian and testicular atrophy can develop as early as 13 weeks after exposure to a high concentration of 1,3-butadiene.

5.1.6. Hackett et al. (1988a)

This sperm-head morphology study was conducted in B6C3F_1 mice at Pacific Northwest Laboratories for NTP as part of a series of studies to investigate the effects of 1,3-butadiene on reproductive function. Adult male B6C3F_1 mice were exposed to 1,3-butadiene (99.88% purity; 174 ± 13 ppm mean headspace dimer [4-vinyl-1-cyclohexene] concentration) at concentrations of 0 (filtered air), 200, 1,000, or 5,000 ppm 6 h/day for 5 successive days. Measured concentrations (mean ± standard deviation [SD]) were 199 ± 6.12, 999 ± 22.6, and 4,980 ± 130 ppm. Positive controls received intraperitoneal injections of 167 mg/kg of ethyl methane sulfonate daily for 5 consecutive days. The mice were killed 5 weeks after exposure, weighed, and examined for gross lesions, with particular emphasis on the reproductive tract. Sperm collected from the right epididymis were examined for abnormal heads (blunt hook, banana, amorphous, pinhead, two heads/two tails, short) and other abnormalities (primarily midpiece abnormalities).

Piloerection and dyspnea were observed within the first 20 to 30 min after exposure in mice receiving 5,000 ppm; no clinical signs of toxicity were noted for the other groups. The percentages of epididymal sperm with normal morphology were 98.08%, 97.23% ($p<0.05$), and 96.34% ($p<0.05$) at 200, 1,000, and 5,000 ppm, respectively, compared with 98.40% for controls; these values also showed a significant exposure-related trend ($p\leq0.05$). The percentages of the following abnormalities were significantly elevated compared with controls ($p<0.05$): blunt hooks at 5,000 ppm, bananas at 1,000 and 5,000 ppm, and pinheads at 1,000 ppm. Amorphous,

two heads/two tails, and shorts were not significantly elevated at any dose. The predominant types of abnormalities were the banana followed by blunt hook and amorphous. The authors speculated that late spermatogonia or early primary spermatocytes were sensitive to 1,3-butadiene. The authors also stated that examining the sperm at only one time point following termination of exposure precluded a determination of the stage of spermatogenesis affected by the chemical.

5.1.7. Pacchierotti et al. (1998b)

Reproductive effects of 1,3-butadiene were studied in male mice by flow cytometric analysis of spermatogonial cells. Male (102/E1XC3H/E1) mice were exposed for 5 consecutive days (6 h/day) to 0, 130, 500 or 1,300 ppm of 1,3-butadiene in air. Results of the flow cytometric analysis of testis cell fractions at different butadiene concentrations and times after exposure revealed that testis weight was significantly decreased at all doses tested 21 days after exposure termination. At longer time intervals, progressive recovery was seen, which was dose-dependent. Testis weight loss paralleled the sequential depletion of round and elongated spermatid populations. These data, together with the dominant lethal studies (discussed in Chapter 4) confirm the toxicity of 1,3-butadiene on late developmental stages of spermatozoa in mice.

5.2. DEVELOPMENTAL EFFECTS

The developmental toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP, 1982) is the same as the Hazleton study discussed briefly in the 1985 EPA document (U.S. EPA, 1985). Hackett and co-workers also conducted two developmental toxicity studies, one using rats (Hackett et al., 1987a) and one using mice (Hackett et al., 1987b). The study using rats was conducted to confirm and extend the findings of the IISRP (1982) study in rats, and the mouse study was conducted for comparison of a rodent species more sensitive than the rat to the toxic effects of 1,3-butadiene. The acute and subchronic effects of 1,3-butadiene inhalation exposure in male mice on fetal abnormalities have already been alluded to in the dominant lethal studies of Anderson et al. (1993, 1998), Adler and Anderson (1994), Pacchierotti et al. (1998a), and Brinkworth et al. (1998).

5.2.1. IISRP (1982)

Pregnant Sprague-Dawley CD rats were exposed by inhalation to 1,3-butadiene at target concentrations of 0, 200, 1,000, or 8,000 ppm 6 h/day on gestation day (gd) 6 to 15 and killed on gd 20. Measured concentrations (mean ± SD) were 2.8 ± 1.2, 202 ± 14, 990 ± 24, and 7,647 ± 375 ppm for 0, 200, 1,000, and 8,000 ppm, respectively. The purity of the 1,3-butadiene was not

reported; the mean concentration of the dimer impurity, 4-vinyl-1-cyclohexene, was 108.6 ± 53.59 ppm, well below the target of 300 ppm.

Maternal body weight gain was markedly depressed in dams exposed to 1,000 and 8,000 ppm, especially during gd 6 to 9; a significant decrease was also noted during gd 9 to 12 in rats exposed to 8,000 ppm. During the later stages (gd 12 to 15 and 16 to 20), body weight gain was similar to controls. The gravid uterus and extragestational weights were similar to controls, but extragestational weight gain was significantly depressed by 17% ($p<0.05$) in dams exposed to 1,000 ppm and by 24% in dams exposed to 8,000 ppm ($p<0.05$). No effects were observed on other measures of maternal toxicity.

Fetal body weight and crown/rump length were significantly reduced at 8,000 ppm ($p<0.05$). The percentage of fetuses with major skeletal defects was significantly elevated at 1,000 and 8,000 ppm and minor skeletal defects were significantly elevated only at the lowest concentration. The percentage of fetuses showing minor external/visceral defects, predominantly subcutaneous hematomas, was significantly elevated only at 1,000 ppm, but the percentage was similar in all three experimental groups. The incidence of bilateral lens opacity was elevated at all concentrations but was significantly elevated only at 8,000 ppm. The incidence of marked-to-severe wavy ribs and the total number of abnormal ossifications and irregular ossification of the ribs were elevated at 8,000 ppm. The incidence of thoracic bipartite centers was elevated in all exposed groups; a dose-response relationship was not observed. Other malformations and variations occurred at incidences similar to those of controls or were not significantly elevated compared with controls.

5.2.2. Hackett et al. (1987a)

Pregnant rats were exposed to 0, 40, 200, or 1,000 ppm 1,3-butadiene (99.84% purity; 197 ± 6 ppm mean headspace dimer concentration) via inhalation on gd 6 to 15 (6 h/day). The measured concentrations (mean ± SD) were 40.1 ± 0.62 (mean ± SD), 199.8 ± 2.61, and 1,005 ± 11.9 ppm, respectively. No clinical signs of toxicity were observed. Final body weights were similar to those of controls; body weight gain, however, was reduced by about 30% ($p<0.05$) in the 1,000 ppm group during the first 5 days of exposure (gd 6 to 11). From gd 11 to 20, body weight gain was not significantly different from that of controls. The gravid uterine weights and extragestational weights (whole-body weight minus gravid uterine weight) were similar to those of controls, but extragestational weight gain was significantly lower (16%; $p<0.05$) in dams exposed to 1,000 ppm than in control dams.

The overall pregnancy rates were similar among all groups, ranging from 80% among dams exposed to 40 ppm to 93% among controls and dams exposed to 1,000 ppm (Table 5-4). Fetal measures, including the numbers of implantations/dam, resorptions/litter, dead

Table 5-4. Maternal toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation.

Parameter	Concentration (ppm)			
	0	**40**	**200**	**1,000**
No. dams assigned	30	30	30	30
No. of deaths	0	0	0	0
No. pregnant (%)	28 (93)	24 (80)	26 (87)	28 (93)
Whole-body weight (g)				
Day 0	242 ± 3.7[a]	239 ± 3.2	244 ± 3.0	242 ± 4.0
Day 20	362 ± 7.1	351 ± 5.9	369 ± 6.6	354 ± 6.1
Body weight gain (g)				
Days 0-6	21.4 ± 1.6	21.1 ± 1.6	22.9 ± 1.3	20.1 ± 1.5
Days 6-11	25.5 ± 1.3	23.6 ± 1.3	26.6 ± 1.5	17.5 ± 1.9[b]
Days 11-16	29.2 ± 1.4	30.9 ± 1.7	31.7 ± 1.9	31.2 ± 2.1
Days 16-20	44.5 ± 1.8	36.7 ± 2.5	43.6 ± 2.3	43.2 ± 2.9
Gravid uterine weight (g)	73.0 ± 2.9	69.5 ± 3.5	73.9 ± 2.8	71.2 ± 4.1
Extragestational weight[c] (g)	289 ± 5.7	282 ± 3.9	295 ± 5.8	283 ± 3.5
Extragestational weight gain[d] (g)	47.6 ± 2.8	42.7 ± 2.2	50.9 ± 3.0	39.9 ± 3.5[b]
Significant clinical signs	None reported	None reported	None reported	None reported

[a]Mean ± standard error.
[b]$p<0.05$, compared with corresponding control.
[c]Body weight on gd 20 minus gravid uterine weight.
[d]Extragestational weight minus body weight on gd 0.

Source: Hackett et al., 1987a.

fetuses/litter, fetal body weights, sex ratios, malformations, and variations, were not affected by exposure to 1,3-butadiene. Overall, no developmental toxicity was observed in rats exposed to 40 to 1,000 ppm during gd 6 to 15.

5.2.3. Hackett et al. (1987b)

Pregnant CD-1 mice were exposed to 0, 40, 200, or 1,000 ppm 1,3-butadiene (99.88% purity; 338 ± 72 ppm mean headspace dimer concentration), 6 h/day on gd 6 to 15. Measured concentrations were 39.9 ± 0.06, 199.8 ± 3.0, and 1,000 ± 13.1 ppm (mean ± SD).

The effects of 1,3-butadiene on maternal toxicity in CD-1 mice are summarized in Table 5-5. Three animals exposed to 1,000 ppm showed signs of dehydration: two died on gd 15, and early parturition occurred in the third. No other clinical signs of toxicity were observed. Exposure-related decreases in whole-body weights on gd 18, body weight gain during gd 11 to 16, gravid uterine weight, extragestational weight, and extragestational weight gain were significantly reduced in the 1,000 ppm exposure group compared with controls. Whole-body weight gain during gd 11 to 16 and extragestational weight gain were also reduced in the 200 ppm exposure group. None of these parameters were significantly affected in dams exposed to 40 ppm. The pregnancy rates in mice were uniformly low in all groups and unaffected by exposure to 1,3-butadiene.

The effects of 1,3-butadiene on various parameters of developmental toxicity in CD-1 mice are summarized in Tables 5-6 and 5-7. More resorptions per litter were observed among control dams than among exposed dams. Fetal body weights were reduced in all exposed groups compared with controls, and the reduction showed a significant exposure-related trend. The overall fetal body weights (males and females combined) were reduced by 4.5% at 40 ppm (not significant), 15.7% at 200 ppm ($p \leq 0.05$), and 22.4% at 1,000 ppm ($p \leq 0.05$) (Table 5-6). Significant differences from controls were seen at all treatment concentrations for fetal males and at the two higher concentrations for fetal females. Placental weights showed an effect similar to that of fetal body weights. Malformations occurred sporadically and at low frequencies in all exposure groups; increases were seen in several skeletal variations (Table 5-7). The frequency of supernumerary ribs was greatly elevated at 200 and 1,000 ppm; 6% of the fetuses/litter were affected at 200 ppm ($p<0.05$) and 9.9% at 1,000 ppm ($p<0.05$) compared with 1.7% in controls and 1.6% in the 40 ppm exposure group (not significant). There also was a marked increase in the total number of fetuses with supernumerary ribs at the 200 and 1,000 ppm exposure levels. A number of these were of normal length as opposed to those considered rudimentary ribs or ossification sites at lumbar 1. The frequency of reduced ossification of the sternebrae was elevated at 200 ($p<0.05$) and 1,000 ppm ($p<0.001$) (Fisher exact test); the litter incidence was elevated but not significantly. The percentages of reduced ossifications at all sites and the

Table 5-5. Maternal toxicity in pregnant CD-1 mice exposed to 1,3-butadiene by inhalation.

Parameter	Concentration (ppm)			
	0	**40**	**200**	**1,000**
No. dams assigned	32	33	31	33
No. of deaths	0	0	0	3
No. pregnant (%)	18 (56)	19 (57)	21 (68)	22 (67)
Whole-body weight (g)				
Day 0	28.4 ± 0.25[a]	28.3 ± 0.32	28.2 ± 0.32	28.4 ± 0.32
Day 18	54.9 ± 1.21[b]	55.4 ± 1.09	52.5 ± 1.01	50.8 ± 0.86[c]
Body weight gain (g)				
Days 0-6	2.7 ± 0.3	3.0 ± 0.3	2.5 ± 0.2	2.3 ± 0.2
Days 6-11	5.5 ± 0.4	5.8 ± 0.3	5.6 ± 0.3	4.8 ± 0.3
Days 11-16	13.3 ± 0.6[b]	12.7 ± 0.4	11.4 ± 0.5[c]	10.6 ± 0.4[c]
Days 16-18	5.5 ± 0.3[b]	5.7 ± 0.3	4.7 ± 0.4	4.8 ± 0.3
Gravid uterine weight (g)	19.3 ± 1.00[b]	20.3 ± 0.80	18.0 ± 0.87	16.8 ± 0.67[c]
Extragestational weight[d] (g)	35.5 ± 0.48[b]	35.1 ± 0.44	34.5 ± 0.46	34.1 ± 0.36[c]
Extragestational weight gain[e] (g)	7.60 ± 0.48[b]	6.99 ± 0.38	6.20 ± 0.38[c]	5.91 ± 0.28[c]
Significant clinical signs	None	None	None	Dehydration

[a]Mean ± standard error.
[b]$p \leq 0.05$, significant linear trend.
[c]$p \leq 0.05$, pairwise comparison with corresponding control parameter.
[d]Body weight on gd 18 minus gravid uterine weight.
[e]Extragestational weight minus body weight on gd 0.

Source: Hackett et al., 1987b.

Table 5-6. Developmental toxicity in CD-1 mice exposed to 1,3-butadiene by inhalation.

Parameter	Concentration (ppm)			
	0	**40**	**200**	**1,000**
No. pregnant (%)	18 (56)	19 (57)	21 (68)	22 (67)
No. litters with live fetuses	18	19	21	20
No. implantations/dam	12.7 ± 0.52	13.3 ± 0.44	13.0 ± 0.64	13.1 ± 0.43
No. resorptions/litter	1.06 ± 0.22	0.84 ± 0.22	0.67 ± 0.20	0.90 ± 0.19
Early resorptions	1.00 ± 0.23	0.58 ± 0.21	0.43 ± 0.13[a]	0.75 ± 0.16
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	11.7 ± 0.66	12.5 ± 0.52	12.3 ± 0.62	12.2 ± 0.51
Fetal body weight (g)	1.34 ± 0.03[b]	1.28 ± 0.01	1.13 ± 0.02[a]	1.04 ± 0.03[a]
Females	1.30 ± 0.03[b]	1.25 ± 0.01	1.10 ± 0.02[a]	1.06 ± 0.02[a]
Males	1.38 ± 0.03[b]	1.31 ± 0.02[a]	1.13 ± 0.02[a]	1.06 ± 0.02[a]
Placental weight (mg)	86.8 ± 2.99[b]	85.4 ± 2.29	78.6 ± 3.24[a]	72.6 ± 1.88[a]
Females	83.1 ± 3.03[b]	80.9 ± 2.46	74.7 ± 3.52[a]	70.1 ± 2.33[a]
Males	89.3 ± 3.03[b]	89.5 ± 2.27	80.1 ± 2.35[a]	74.5 ± 1.81[a]
Sex ratio (% males)	51.6 ± 3.91	49.8 ± 3.06	51.5 ± 3.68	51.8 ± 3.29

[a]$p \leq 0.05$, pairwise comparison with corresponding control.
[b]$p \leq 0.05$, significant linear trend.

Source: Hackett et al., 1987b.

Table 5-7. Malformations and variations in CD-1 mice exposed to 1,3-butadiene by inhalation.

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. fetuses/no. litters examined	211/18	237/19	259/21	244/20
No. fetal heads examined	105	120	130	120
Malformations[a]				
Exencephalus	1/1	—[b]	—	2/2
Open eye	1/1	—	—	1/1
Limb flexure	2/1	—	—	—
Fused sternebrae	—	—	—	2/2
Fused ribs	—	2/2	—	—
Variations				
Pale	2/2	—	—	—
Hydroureter	2/2	6/3	—	—
Abnormal sternebrae[c,d]	0.6 ± 0.9	0.4 ± 0.7	0.4 ± 0.8	0.8 ±1.3[e]
Misaligned sternebrae	10/6	3/3	9/8	10/8
Ossification site between sternebrae 5 and 6	—	1/1	1/1	3/3
Supernumerary ribs[c,d]	1.7 ± 2.3	1.6 ± 2.1	6.0 ± 3.6[e]	9.9 ± 3.0[e]
Supernumerary ribs (total number)	30/11	30/9	127/20	198/20
Normal length	6/5	5/1	29/9	68/10
Rudimentary	13/6	19/8	81/20	120/16
Ossification site at lumbar 1	11/5	6/4	17/10	10/7
Reduced ossification (all sites)[c]	1.7 ± 1.7	1.2 ± 1.5	2.7 ± 2.7	3.9 ± 2.6[e]
Skull	—	—	2/2	3/1
Sternebrae	31/13	20/9	57/16[f]	76/19[f]
Vertebrae (centra)	—	1/1	—	1/1
Phalanges	—	—	—	2/16

[a]Expressed as number of fetuses/number of litters; includes only those findings occurring in more than one fetus or at more than one concentration.
[b]—, no malformations or variations noted.
[c]Mean percentage per litter (mean ± SD).
[d]$p<0.05$, linear trend, orthogonal contrast test.
[e]$p<0.05$, Tukey's test.
[f]$p<0.05$, Fisher exact test (fetal incidence).
Source: Hackett et al., 1987b.

percentages of abnormal sternebrae (misaligned, scrambled, or cleft) per litter were also significantly elevated at 1,000 ppm ($p<0.05$). The percentages of supernumerary ribs and abnormal sternebrae also showed significant linear trends.

These studies show that inhalation exposure to 1,3-butadiene causes maternal toxicity, manifested as reduced body weight gain, in the mouse at 200 and 1,000 ppm; therefore, the NOAEL for maternal toxicity is 40 ppm. 1,3-Butadiene also caused developmental effects, manifested by reduced fetal body weight and increased frequency of skeletal variations at 200 and 1,000 ppm. In addition, inhalation exposure to 1,3-butadiene during gestation caused a significant reduction in body weight of male fetuses at 40 ppm. Therefore, a NOAEL for developmental toxicity in CD-1 mice could not be obtained. Although 1,3-butadiene did not induce a detectable increase in major malformations in the mouse fetus, the dose-related increases in supernumerary ribs and reduced ossifications, particularly of the sternebrae, indicate altered development and are a cause for concern.

5.3. STRUCTURE-ACTIVITY RELATIONSHIPS

A number of studies using 1,3-butadiene metabolites or chemically related agents have contributed to our understanding of the species susceptibility and reproductive organ toxicity of 1,3-butadiene. Data on structure-activity relationships and structural analogs of 1,3-butadiene are summarized in Table 5-8.

Studies using the mono- and diepoxide metabolites of 1,3-butadiene in mice and rats have contributed to a better understanding of species differences and of the responsible agent in ovarian toxicity (Doerr et al., 1995; 1996). Doerr et al. (1995) administered 1,2-epoxy-3-butene (EB) (1.43 mM/kg), 1,2:3,4-diepoxybutane (DEB) (0.14 mM/kg), or vehicle (sesame oil) to immature (28-day-old) female B6C3F_1 mice ip for 30 days. Follicle counts were reduced from controls as follows: EB caused a 98% reduction of small and 87% reduction of growing follicles, while, at a 10-fold lower dose, DEB caused an 85% reduction of small and 65% reduction of growing follicles. In a second study by Doerr et al. (1996), EB (0, 0.005, 0.02, 0.09, 0.36, or 1.43 mM/kg), DEB (0, 0.002, 0.009, 0.036, 0.14, or 0.29 mM/kg), or vehicle (sesame oil) was administered intraperitoneally once daily to immature female B6C3F_1 mice and Sprague-Dawley rats for 30 days. At the high dose, EB resulted in reduced ovarian and uterine weights ($p\leq0.05$) and decreased follicular counts in mice; rats, however, were unaffected. DEB caused decreased ovarian weights ($p\leq0.05$) in mice and rats at $\geq$0.14 mM/kg and decreased uterine weights ($p\leq0.05$) in mice at $\geq$0.14 mM/kg and in rats at 0.29 mM/kg. In mice, ED_{50} values for EB were 0.29 and 0.40 mM/kg and for DEB were 0.1 and 0.14 mM/kg for small and growing follicles, respectively. EB did not deplete the ovary of small or growing follicles in the rat, and significant depletion with DEB occurred only at 0.14 mM/kg (30%-40% of the follicular population

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene.

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
1,2-Epoxy-3-butene (EB) 1,2:3,4-Diepoxybutane (DEB)	Female $B6C3F_1$ mice (28-day)	1.43 mM/kg intraperitoneally for 30 days 0.14 mM/kg intraperitoneally for 30 days	Significant ↓ in small (98%) and growing (87%) follicles Significant ↓ in small (85%) and growing (63%) follicles	N/A	Doerr et al., 1995
1,2-Epoxy-3-butene (EB)	Female $B6C3F_1$ mice (28-day) or Female Sprague-Dawley rats (28-day)	0.005, 0.02, 0.09, 0.36, 1.43 mM/kg intraperitoneally once daily for 30 days	≥0.36 mM/kg (31.0 mg/kg): decreased follicle counts (ED_{50} = 0.29 and 0.40 mM/kg for small and growing follicles) 1.43 mM/kg (100.1 mg/kg): decreased ovarian and uterine weights None	0.36 mM/kg >1.43 mM/kg	Doerr et al., 1996
1,2:3,4-Diepoxybutane (DEB)	Female $B6C3F_1$ mice (28-day) or Female Sprague-Dawley rats (28-day)	0.002, 0.009, 0.036, 0.14, 0.29 mM/kg intraperitoneally once daily for 30 days	≥0.14 mM/kg (12.05 mg/kg): decreased ovarian and uterine weights; decreased follicular counts (ED_{50} = 0.1 and 0.14 mM/kg for small and growing follicles) ≥0.14 mM/kg (12.05 mg/kg): decreased ovarian weight, decreased follicular counts; 0.29 mM/kg (25 mg/kg): decreased uterine weight, severe general toxicity precluded complete evaluation	0.14 mM/kg 0.14 mM/kg	

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinyl-cyclohexene	Male and female F344 rats	0, 50, 100, 200, 400, 800 mg/kg by gavage, 5 days/wk, 13 wks	<u>Male and female rats and male mice</u> 13 weeks: no reproductive effects	>800 mg/kg for 13 wks	NTP, 1986
		0, 200, 400 mg/kg by gavage, 5 days/wk, 105 wks	105 weeks: no reproductive effects	>400 mg/kg for 105 wks	
	Male and female $B6C3F_1$ mice	0, 75, 150, 300, 600, 1,200 mg/kg by gavage, 5 days/wk, 13 wks	<u>Female mice</u> 13 weeks: 1,200 mg/kg, all had ↓ primary and mature Graafian follicles; ovaries in other groups not examined	N/A	
		0, 200, 400 mg/kg by gavage, 5 days/wk, 105 wks	105 weeks: 200 and 400 mg/kg, mixed benign tumors, hyperplasia, granulosa cell tumors, and granulosa cell carcinomas in ovaries; tubular cell hyperplasia	200 mg/kg for 105 wks	
4-Vinyl-cyclohexene	Male and female $B6C3F_1$ mice	0, 50, 250, 1,000 ppm by inhalation, 5 days/wk for 13 wks	1,000 ppm: 5/10 females showed ovarian atrophy	1,000 ppm for 13 wks	Bevan et al., 1996
	Male and female Sprague-Dawley rats	0, 250, 1,000, 1,500 ppm by inhalation, 5 days/wk for 13 wks	1,500 ppm: 2/10 females showed ovarian atrophy	1,500 ppm for 13 wks	

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinylcyclo-hexene	Female (28-day) $B6C3F_1$ mice	800 mg/kg/day intra-peritoneally for 30 days; ovaries collected at 30, 60, 120, 240, and 360 days from beginning of treatment	≥30 days: reduced ovarian weight, reduced number of small (11% of controls) and growing (22% of controls) follicles that were progressively decreased over time ≥240 days: increased serum FSH levels 360 days: complete ovarian atrophy, preneoplastic lesions, increased plasma androstenedione concentrations, no estrous cyclicity	800 mg/kg/day ip for 30 days	Hooser et al., 1994
4-Vinylcyclo-hexene	Swiss (CD-1) male and female mice, continuous breeding protocol	100, 250, 500 mg/kg/day by gavage for approximately 18 weeks	F1 females: 500 mg/kg--reduced primordial (33% decrease), growing (55% decrease), and antral follicles (33% decrease); no effect on other reproductive measures F1 males: 500 mg/kg--reduced testicular spermatid count (17% decrease); no effect on other reproductive measures	500 mg/kg/day 500 mg/kg/day	Grizzle et al., 1994

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinyl-cyclohexene	Female $B6C3F_1$ mice (28-day)	7.5 mM/kg intra-peritoneally for 30 days	Significant ↓ in small (86%-88%) and growing (72-83%) follicles	N/A	Doerr et al., 1995
Isoprene		7.34 mM/kg intra-peritoneally for 30 days	Significant ↓ in small (76%) and growing (46%) follicles		
Ethylcyclo-hexene		7.5 mM/kg intra-peritoneally for 30 days 1.43 mM/kg intra-peritoneally for 30 days	Significant ↓ in growing (37%) follicles		
Ethylcyclo-hexene mono-epoxide		7.5 mM/kg intra-peritoneally for 30 days 1.43 mM/kg intra-peritoneally for 30 days	No effect		
Vinylcyclo-hexane		7.5 mM/kg intra-peritoneally for 30 days	No effect		
Vinylcyclo-hexane mono-epoxide		1.43 mM/kg intra-peritoneally for 30 days	No effect		
Cyclohexene		1.43 mM/kg intra-peritoneally for 30 days	No effect		
Cyclohexene monoepoxide			No effect		
Epoxy butane			No effect		

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinylcyclo-hexene 4-Phenylcyclo-hexene Benzo[*a*]-pyrene (+ control)	Female B6C3F$_1$ mice (28-day)	6 mM/kg (650 mg/kg) intraperitoneally for 30 days 3 or 6 mM/kg (475 or 950 mg/kg) intra-peritoneally for 30 days 0.32 mM/kg (80 mg/kg) on 1st and 7th days of dosing	Significant reduction in number of small and growing follicles None Significant reduction in number of small and growing follicles	N/A	Hooser et al., 1993
4-Vinylcyclo-hexene diepoxide	Adult (58-day) and immature (28-day) female Fischer 344 rats	80 mg/kg (0.57 mM/kg) intraperi-toneally for 30 days	<u>Adults</u> Reduced uterine weight, reduced number of regular estrous cycles; reduced number of primordial (33% of control) and primary (38%) follicles <u>Immature</u> Reduced number of primordial (19%), primary (45%), and growing (54%) follicles;	N/A	Flaws et al., 1994a
4-Vinyl-1-cyclohexene diepoxide	Male and female B6C3F$_1$ mice Male and female Fischer 344 rats	0, 2.5, 5, 10 mg/animal topically 5 days/week for 13 or 105 weeks 0. 3.75, 7.5, 15, 30, 60 mg/animal topically 5 days/week for 13 weeks; 0, 15, or 30 mg/animal for 105 weeks	<u>Females</u> 13 weeks: ≥5 mg/animal--ovarian atrophy; 10 mg/animal--uterine atrophy 105 weeks: ≥2.5 mg/animal--ovarian atrophy <u>Males</u> 105 weeks: ≥5 mg/animal--inflammation of epididymis 13 weeks: None 105 weeks: None	5 mg/animal for 13 wks 2.5 mg/animal for 105 wks 5 mg/animal for 105 wks None	NTP, 1989

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinylcyclo-hexene diepoxide	Female (28-day) B6C3F$_1$ mice Female (28-day) Fischer 344 rats	80 mg/kg intraperi-toneally for 6, 8, 10, or 12 days	8 days: increased atretic primordial follicles; 12 days: increased atretic primary follicles, significant loss of follicles (64%) 10 days: increased atretic primordial follicles; 12 days: increased atretic primary follicles, significant loss of follicles (35%)	80 mg/kg ip for 8 days 80 mg/kg ip for 10 days	Kao et al., 1999
4-Vinylcyclo-hexene diepoxide	Female (28-day) Fischer 344 rats	80 mg/kg intraperi-toneally for 1 or 15 days	1 day: increased number of primary follicles, decreased *bax* expression 15 days: reduced number of primordial and primary follicles, increased *bax* expression	N/A	Borman et al., 1999

Table 5-8. A summary of reproductive and developmental toxicity effects of chemicals structurally similar to 1,3-butadiene (continued).

Chemical	Species	Dose, route, and time of exposure	Effects	LOAEL	Reference
4-Vinylcyclohexene diepoxide	Male (28-day) $B6C3F_1$ mice	320 mg/kg/day intraperitoneally for 5, 10, 15, 20, 25, or 30 days; two additional groups dosed for 30 days and allowed to recover for 30 or 60 days	≥5 days: decreased testis weight 5 days: loss of Type I and B spermatogonia in Stages II to VI and of preleptotene spermatocyte in Stages VI to VIII 30 days: no germ cells except spermatogonial stem cells in testis, reduced seminal vesicle weight ≥30 days recovery: 100% recovery in seminiferous tubule 60 days recovery: spermatozoa in epididymis	320 mg/kg ip for 5 days	Hooser et al., 1995
		40, 80, 160, 320 mg/kg intraperitoneally for 30 days	≥80 mg/kg: reduced testis weight ≥160 mg/kg: greatly reduced or absent epididymal sperm, scattered empty seminiferous tubules, some stages of spermatids and spermatocytes absent 320 mg/kg: reduced seminal vesicle weight	80 mg/kg ip for 30 days	
4-Vinylcyclohexene		800 mg/kg intraperitoneally for 30 days	None		
Vinylcyclohexene 1,2-monoepoxide		200 mg/kg intraperitoneally for 30 days	None		
Isoprene	Male $B6C3F_1$ mice Male F344 rats	0, 70, 220, 700, 2,200, 7,000 ppm inhalation 6 h/day, 5 days/week, 13 or 26 weeks	13 and 26 weeks: 7,000 ppm-- testicular atrophy 13 weeks: none 26 weeks: 7,000 ppm-- hyperplasia of interstitial cells	7,000 ppm for 13 weeks 7,000 ppm for 26 weeks	Melnick et al., 1994

depleted). Severe toxicity occurred in the highest DEB dose group in rats, which precluded complete evaluation. These results clearly show that mice are more sensitive than rats to the ovotoxic effects of the mono- and diepoxides of 1,3-butadiene, and that DEB is the more potent ovotoxicant in both species.

The 1,3-butadiene dimer, 4-vinylcyclohexene, has been tested in short- and long-term toxicological and carcinogenicity studies in rats and mice. Male and female F344 rats and male B6C3F$_1$ mice dosed by gavage with 0, 200, or 400 mg/kg of 4-vinylcyclohexene in corn oil 5 days/week for 105 weeks failed to develop neoplastic or nonneoplastic lesions in the reproductive organs (NTP, 1986). However, in female mice, the incidences of granulosa cell neoplasms, mixed benign tumors, granulosa cell hyperplasia, and tubular cell hyperplasia were increased, but no increase in the incidence of nonneoplastic lesions was reported. In the 13-week study, none of the male or female rats (0, 50, 100, 200, 400, or 800 mg/kg) or the male mice (0, 75, 150, 300, 600, or 1,200 mg/kg) showed reproductive effects, but all female mice treated with 1,200 mg/kg had reduced numbers of primary and mature Graafian follicles whether they survived until termination (5/10) or died before termination (5/10). Ovaries in other dose groups were not examined for numbers of follicles.

Bevan et al. (1996) reported on a study in which mice and rats were exposed by inhalation to 4-vinylcyclohexene for 6 h/day, 5 days/week for 13 weeks. Male and female B6C3F$_1$ mice were exposed to 0, 50, 250, or 1,000 ppm while rats were exposed to 0, 250, 1,000, or 1,500 ppm. In mice, 5/10 females exposed to 1,000 ppm showed ovarian atrophy, and in rats exposed to 1,500 ppm 2/10 females showed ovarian atrophy.

In a study to more carefully evaluate the ovarian toxicity of 4-vinylcyclohexene, Hooser et al. (1994) reported on mice exposed by intraperitoneal injection to 800 mg/kg/day for 30 days. Animals were killed at 30, 120, 240, and 360 days from the start of exposure and ovarian follicle counts were performed. At 30 days, there was a significant decrease in follicle counts (30% of control numbers), but not until 240 days were follicle counts reduced to <1% of control values. At the latter time point, there was also an increase in serum follicle stimulating hormone (FSH), indicating a lack of feedback inhibition by estradiol on the hypothalamus and pituitary. At 360 days, complete ovarian atrophy was reported, as well as a sustained increase in serum FSH levels, and preneoplastic changes in the ovary (blood-filled cystic structures lined with flattened cells in which there were small, irregularly shaped foci of hypertrophic cells).

The functional consequences of the germ-cell effects of 4-vinylcyclohexene were studied by Grizzle et al. (1994) in mice exposed to 100, 250, or 500 mg/kg/day in a continuous breeding study protocol. CD-1 male and female mice were dosed with 4-vinylcyclohexene in corn oil by gavage at doses of 0, 100, 250, or 500 mg/kg/day for 1 week, then cohabited in breeding pairs for 14 weeks and dosed throughout the mating period. Litters born were evaluated, then euthanized.

Litters born after week 15 were reared until postnatal day (PND) day 21 with continuous exposure to the dams, then control and high-dose F1 weanlings were raised and bred within dose groups. Testicular spermatid count was decreased in F1 males by 17%, whereas treated females had significantly reduced numbers of primordial (33% decrease), growing (55% decrease), and antral follicles (33% decrease) after treatment with 500 mg/kg compared to controls. However, no changes in testis weight, ovarian weight, or estrous cyclicity were seen, and no effect on reproductive competence was detected.

Doerr et al. (1995) studied structure-activity relationships for the ovarian toxicity of 4-vinylcyclohexene and several related olefins and their monoepoxides. 4-Vinylcyclohexene was administered intraperitoneally to female B6C3F_1 mice at 7.5 mmol/kg for 30 days as a positive control. The analogues of vinylcyclohexene (ethylcyclohexene, vinylcyclohexane, cyclohexene: all analogues with only a single unsaturated site) were similarly given at 7.5 mmol/kg, while their monoepoxides were given at 1.43 mmol/kg (maximum tolerated doses). Following day 30, mice were killed and the ovaries were removed and sectioned for histologic examination. Besides vinylcyclohexene, only the analogue ethylcyclohexene caused a significant reduction in growing (37%) but not small follicles, and not to the same extent as vinylcyclohexene (86%-88% for small and 72%-83% for growing follicles). None of the monoepoxides of the vinylcyclohexene analogues affected small or growing follicle counts. Additional groups of animals were given isoprene at 7.34 mmol/kg and epoxybutane (EB analogue) at 1.43 mmol/kg, while EB was given at 1.43 mmol/kg, and DEB at 0.14 mmol/kg. At a dose similar to that for vinylcyclohexene, isoprene reduced small follicles by 76% and growing follicles by 46%. EB almost completely depleted the population of both small and growing follicles at a dose more than fivefold below that for vinylcyclohexene, whereas epoxybutane (a monoepoxide analogue of EB that cannot form a diepoxide) did not affect follicle counts. DEB, at a dose tenfold lower than that for EB, depleted 85% and 63% of small and growing follicles, respectively. These structure-activity data indicate that the tested analogues of vinylcyclohexene that had only a single unsaturated site (and their monoepoxide metabolites) are not ovotoxic, and that only those related compounds that can be metabolized to a diepoxide or were a diepoxide were ovotoxic. In addition, the study showed a relationship between chemical reactivity, as measured by nicotinamide alkylation, and ovotoxicity; the diepoxide metabolites of vinylcyclohexene and DEB were approximately 3.5 to 10-fold more reactive than their monoepoxide precursors and other structurally related monoepoxides.

Similarly, Hooser et al. (1993) studied the effect of substituting a phenyl group for the vinyl group in 4-vinylcyclohexene. Mice were treated with either 4-vinylcyclohexene (6 mM/kg/day ip for 30 days) or 4-phenylcyclohexene (3 or 6 mM/kg/day ip for 30 days). A positive control group was treated with benzo[*a*]-pyrene (0.32 mM/kg) on the first day of dosing

and again 7 days later. Animals were killed shortly after exposure ended, and both 4-vinylcyclohexene and benzo[*a*]-pyrene caused dramatic reductions in small and growing follicles, but no treatment-related effects were seen with 4-phenylcyclohexene, possibly related to its inability to form a mono- or diepoxide.

The reproductive toxicity of 4-vinylcyclohexene diepoxide has also been reported in studies from several laboratories. When male and female F344 rats were treated dermally with 0, 3.75, 7.5, 15, 30, or 60 mg/animal or B6C3F_1 male and female mice were treated dermally with 0, 2.5, 5, or 10 mg/animal of 4-vinyl-1-cyclohexene diepoxide 5 days/week for 13 weeks, the only reproductive effects noted were diffuse ovarian atrophy in 10/10 female mice that received 10 mg/mouse and in 4/10 receiving 5 mg/mouse, and uterine atrophy in 2/10 female mice that received 10 mg/mouse (NTP, 1989). In a 2-year study, male and female rats were exposed dermally to 0, 15, or 30 mg/animal, and male and female mice were exposed to 0, 2.5, 5, or 10 mg/animal. No reproductive lesions were seen in rats, but ovarian atrophy occurred in almost all mice treated with 2.5, 5, and 10 mg/mouse (43/49, 42/49, and 47/50, respectively, compared with 12/50 for controls). Ovarian atrophy was similar to that observed in animals exposed to 1,3-butadiene and was characterized by a complete absence of follicles and corpora lutea. Tubular hyperplasia also occurred at a high incidence in all dose groups (35/49, 38/49, and 34/50, respectively, compared with 5/50 for controls). In addition, the combined incidence of luteomas, granulosa cell tumors, benign mixed tumors, and malignant granulosa cell tumors was increased in the mid- and high-dose groups (5 or 10 mg/animal, respectively). In male mice, subacute inflammation of the epididymis occurred in 0/50, 6/50, and 13/49, respectively, compared with 0/50 for controls. These data clearly show that mice are more susceptible to 4-vinylcyclohexene-induced reproductive toxicity than are rats regardless of the route of exposure (i.e., oral versus dermal).

Several studies have investigated the effects of 4-vinylcyclohexene diepoxide on the destruction of ovarian follicles. Flaws et al. (1994a) reported the effects of age at treatment of female rats on the ovarian toxicity of 4-vinylcyclohexene diepoxide. Adult (58-day) and immature (28-day) female Fischer 344 rats were treated with 80 mg/kg ip for 30 days. Adult rats showed significantly reduced uterine weight, number of regular estrous cycles, and number of primordial (33% of control) and primary (38% of control) follicles. In immature rats, the effects were reduced primordial (19%), primary (45%), and growing (54%) follicles, with no other signs of toxicity. These data indicate that 4-vinylcyclohexene causes ovarian toxicity in both adult and immature animals, and that the effects on uterine weight may be indirect as a consequence of the reduction in number of regular estrous cycles and estrogen production. Kao et al. (1999) reported on the differential response to sensitive indicators of ovarian toxicity in immature female mice and rats exposed to 4-vinylcyclohexene diepoxide (80 mg/kg ip for 6, 8, 10, or 12 days). An

increase in atretic follicles was detected in mice after 8 days, and in rats after 10 days, whereas a significant loss in number of follicles was detected in both species after 12 days. However, the reduction in number of follicles in mice (64%) was greater than that in rats (35%). Finally, Borman et al. (1999) reported that immature female Fischer 344 rats given a single ip dose of 80 mg/kg 4-vinylcyclohexene diepoxide showed an increase in the number of primary follicles along with decreased expression of the cell death gene *bax*, in contrast to a group of animals treated with the same dose for 15 days, which showed a reduced number of primordial and primary follicles and increased *bax* expression. These data suggest that a single dose of 4-vinylcyclohexene diepoxide may cause a reduction in apoptosis in favor of DNA repair, resulting in an apparent "protective" effect.

Studies to look more carefully at the effects of 4-vinylcyclohexene and its epoxides on male reproduction and fertility were reported by Hooser et al. (1995). Male (28 day) B6C3F_1 mice were treated with 320 mg/kg/day 4-vinylcyclohexene diepoxide for 5, 10, 15, 20, 25, or 30 days. Two additional groups were dosed for 30-days and allowed to recover for 30 or 60 days. Reductions in testis weight were noted after 5 days of exposure, as well as loss of Type I and B spermatogonia in Stages II to VI and of preleptotene spermatocytes in Stages VI to VIII. After 30 days there were no germ cells except spermatogonial stem cells in the testis, and reduced seminal vesicle weight. However, after a 30-day recovery period, there was 100% recovery of germ cells in the seminiferous tubules, and after 60 days, spermatozoa populated the epididymis. A dose-response study (40, 80, 160, 320 mg/kg/day ip for 30 days) showed that reduced testis weight could be detected at 80 mg/kg; at $\geq$160 mg/kg, there were greatly reduced or absent epididymal sperm and scattered empty seminiferous tubules, with some stages of spermatids and spermatocytes absent. At 320 mg/kg, seminal vesicle weight was also reduced. Treatment with 4-vinylcyclohexene (800 mg/kg ip for 30 days) or vinylcyclohexene-1,2-monoepoxide (200 mg/kg ip for 30 days) showed no effects on male reproductive organs.

Finally, differences in the susceptibility and reproductive effects in male rats and mice were seen in inhalation studies with isoprene, the 2-methyl analogue of 1,3-butadiene (Melnick et al., 1994). Male F344 rats and B6C3F_1 mice were exposed to 0, 70, 220, 700, 2,200, and 7,000 ppm isoprene, 6 h/day, 5 days/week for either 13 or 26 weeks followed by a 26-week recovery period. After 13 weeks of exposure, no effects were observed in rats at any concentration, but testicular atrophy occurred in mice at 7,000 ppm. Following 26 weeks of exposure, all treated rats in the 7,000 ppm group had hyperplasia of the interstitial cells of the testis ($p \leq 0.01$; 10/10 vs. 1/10 controls); however, following the 26-week recovery, there was only a marginal increase (not significant) in benign testicular tumors: 9/30 compared with 3/30 for controls. Mice had an increase in the incidence of testicular atrophy following 26 weeks of exposure to 7,000 ppm

($p \leq 0.05$; 5/10 vs. 0/10 controls). After 26 weeks of recovery, mice had a slight increase (not significant) in testicular atrophy at 7,000 ppm (3/29 compared with 0/29 for controls).

5.4. PLAUSIBLE MECHANISMS OF REPRODUCTIVE TOXICITY

Differences in some aspects of reproduction between test species and humans contribute to uncertainty in assigning human health reproductive risk for 1,3-butadiene. One of the limitations in using laboratory rodent models for correlating age-related changes in human reproductive physiology is the difference in the period of lifetime estrous/menstrual cycling, being much shorter in rodents (6 to 8 months duration) than in humans (30 years). This fact makes applicability of long-term exposures to presumptive toxicants between rodents and humans difficult (May and Finch, 1987-1988), but data on several agents in test animals and humans showing ovotoxicity and reproductive failure (Hoyer and Sipes, 1996) suggest that findings of estrous/menstrual irregularities, reductions in follicle numbers, and ovarian failure should be considered adverse reproductive effects and assumed to indicate the potential for reproductive toxicity in humans (Heindel, 1999). Thus, the questions raised by Christian (1996) as to the rationale for concern in the observation of ovarian atrophy in aged animals have been addressed.

In mice exposed to 1,3-butadiene for 2 years, there was dose-related atrophy in both ovary and testis (NTP, 1993), with the ovary being the more sensitive target organ. In the case of ovarian atrophy, there was a significant effect even at the lowest dose tested (6.25 ppm). Because follicle counts were not conducted in this study, there is the possibility that significant follicular loss might have been detected at even lower exposure levels and at much earlier times in the study. In support of this possibility, Hooser et al. (1994) showed that mice exposed to 4-vinylcyclohexene for 30 days (800 mg/kg/day, ip) and examined at 30, 120, 240, and 360 days from the start of exposure had a significant decrease in follicle counts (30% of control numbers) at the end of the 30-day treatment period. At 240 days after the beginning of the 30-day exposure period, follicle counts were reduced to <1% of control values with a concomitant increase in serum FSH. At 360 days, complete ovarian atrophy and increased serum FSH levels were found, at which time preneoplastic changes were detected in the ovary. Interestingly, animals continued to cycle until the 360-day time point. Thus, one of the first signs of ovarian atrophy may be the reduction in follicle counts, followed much later by ovarian atrophy and tumor formation.

Further support of this series of events comes from a study by Kao et al. (1999) in which immature mice and rats were exposed to 80 mg/kg ip of 4-vinylcyclohexene diepoxide. Increases in atretric primordial follicles were seen in the ovaries as early as 8 days of exposure in mice and 10 days in rats, with reductions in primary follicles in both species after 12 days exposure. This greater effect on primordial follicles appears to be related to the fact that 4-vinylcyclohexene

diepoxide is less efficiently detoxified in primordial and preantral follicles than in large preantral follices (Flaws et al., 1994b). In addition, 4-vinylcyclohexene diepoxide causes atresia by promoting the process of apoptosis; because the normal process of follicular atresia occurs primarily in the primordial and small growing follicles (Springer et al., 1996a and b), 4-vinylcyclohexene (and likely 1,3-butadiene) primarily affects the primordial and small growing follicles. Springer et al. (1996c) also showed that follicular viability may be affected by 4-vinylcyclohexene diepoxide via an effect on granulosa cells. Flaws et al. (1994b) showed that rat preantral follicles versus larger follicles had a reduced ability to detoxify 4-vinylcyclohexene diepoxide, another possible reason for a greater effect on primordial and primary follicles than on larger growing follicles. A recent study by Borman et al. (1999) reported a so-called protective effect of a single day of exposure to vinylcyclohexene diepoxide (80 mg/kg ip) on immature rat primary ovarian follicles. It is unclear what the mechanism underlying this effect might be, except that expression of the cell death gene *bax* was decreased, suggesting a suppression of apoptosis in favor of DNA damage repair.

There are multiple pacemakers of reproductive senescence, including the increasing de-synchronization of the temporal order of neuroendocrine signals of the central nervous system and the exhaustion of ovarian follicles (Nelson et al., 1995; Wise et al., 1997). Menopause marks the permanent end of fertility in women. If 1,3-butadiene acts on the human ovary as it does in mice, then it is possible that the exocrine function (ovulation of oocytes) could be adversely affected, leading to infertility and premature menopause. As a result the endocrine function would also be affected. There is evidence that the postmenopausal ovary retains some important endocrine functions despite follicular loss (Chakravarti et al., 1976; Judd et al., 1976; Vermeulen, 1976; Schachter et al., 1979; Aiman et al., 1986; Nagamani et al., 1986; Hughes et al., 1991). Thus, if 1,3-butadiene acts on the endocrine function of the ovary, causing a premature attenuation of steroidogenesis, then the beneficial effects of estradiol are lost, resulting in atrophy of the sensitive tissues, decreased calcium absorption, increased bone resorption, accelerated bone loss and osteoporosis, a rise in serum triglycerides, increased VLDL and LDL lipoproteins, and an increased LDL/HDL cholesterol ratio, a profile that favors atherosclerosis (Vagenakis, 1989). Clearly, there is a research need to determine human ovarian sensitivity to 1,3-butadiene and its metabolites.

Another potential concern worth discussing here is the relationship between antecedent ovarian hypoplasia and atrophy, followed by hyperplasia and subsequent ovarian neoplasia. Capen and co-workers (1995) have shown that agents that either destroy or diminish the numbers of Graafian follicles in the ovary result in decreased sex hormone secretion (especially estradiol) and lead to compensatory overproduction of pituitary gonadotropins FSH and luteinizing hormone (LH), which may place the mouse ovary at an increase risk to develop tumors via a

hormone-mediated mechanism. This hypothesized link is further discussed by Hoyer and Sipes (1996) and supported by the work of Hooser et al. (1994) showing the time course of effects on follicular atrophy, loss of feedback control of FSH secretion via the hypothalamo-pituitary axis, and subsequent observation of preneoplastic lesions in the ovary. Ovarian toxicity and carcinogenicity has been documented for at least eight chemicals recently tested in National Toxicity Program prechronic and chronic rodent studies. The chemicals that yielded treatment-related ovarian lesions, pertinent here, were 1,3-butadiene, 4-vinylcyclohexene, and vinylcyclohexene diepoxide (Maronpot, 1987). Whether these chemicals and conditions have similar effects in women is unknown at this time.

The active metabolite for inducing ovarian atrophy has been clearly shown to be the diepoxide metabolite of 1,3-butadiene (Doerr et al., 1995, 1996). This requirement for epoxidation and activation to the diepoxide form is supported by these and other studies on 1,3-butadiene and its structural analogues as well as their mono- and diepoxide metabolites (Melnick et al., 1994; Doerr et al., 1995). Flaws et al. (1994a) and Kao et al. (1999) have shown that rats, which are resistant to the ovotoxicity of 1,3-butadiene and 4-vinylcyclohexene, are affected by treatment with the diepoxide metabolite of 4-vinylcyclohexene. Thus, while there are definite species differences in effects seen between rats and mice with respect to ovarian toxicity from the parent compound, there are much less striking differences in sensitivity to the diepoxide metabolite.

The uterine atrophy observed in some of the toxicology studies (e.g., NTP, 1984, 1993) is presumed to be due to reduced estrogen stimulation of the uterus by the atrophic ovary, rather than a direct effect of the toxicant. Since the early work of Astwood (1938), it has been well known that ovarian estrogens have a trophic effect on the uterus. Recently, it has been shown that estrogen agonists promote a three- to fourfold increase in uterine wet weight and vaginal cornification in wild-type females, whereas estrogen receptor knockout mice are totally insensitive (Korach et al., 1996). Such studies clearly provide evidence for the importance of ovarian estrogens in maintaining reproductive tract tissues and organs. Although this theory is biologically plausible and the likely explanation for the uterine atrophy observed in 1,3-butadiene-exposed mice with depressed ovarian activity, additional research is needed to verify this hypothesis.

Testicular atrophy, as reflected by reduced testis weight following 1,3-butadiene exposure for 9, 15, or 24 months in male mice (NTP, 1993), indicates gonadal sensitivity in the male as well as in the female. However, the ovary is more sensitive than the testis because ovarian atrophy occurs at very low concentrations (6.25 ppm) of 1,3-butadiene compared with that seen in males after 2 years of exposure (LOAEL = 200 ppm). The sperm-head morphology study

showed that male mice are affected at concentrations ≥1,000 ppm (Hackett et al., 1988a) and the dominant lethal test showed that male mice may be affected at 200 and 1,000 ppm (Hackett et al., 1988b), again indicating that higher exposure concentrations are necessary to induce toxic effects in male mice than in female mice. Hooser et al. (1995) studied the effects of the diepoxide metabolite of vinylcyclohexene (320 mg/kg/day ip) on male mice killed after 5, 10, 15, 25, or 30 days of exposure. Decreases in testis weight began at 5 days and there was loss of Type I and B spermatogonia in Stages II to VI and of preleptotene spermatocytes in Stages VI to VIII. Following a 30-day recovery period, the seminiferous tubules were 100% repopulated, and after 60 days epididymal spermatozoa were present. Thus, the effects on the male germ cells appear to be reversible whereas those in the female are not. Chabbra et al. (1990) had earlier shown that male rats are resistant to vinylcyclohexene diepoxide, whereas females in both rats and mice are affected. The reason for these gender and species differences in sensitivity needs further investigation.

The differential intercalation of toxic chemicals with chromosomal macromolecules of oocytes and spermatocytes may be a possible reason for gender differences. The diffuse state of chromosomes of oocytes, allowing a more efficient intercalation between base pairs and potential alkylation, is different from the dense chromosomal compaction of maturing late spermatocytes. This explanation has been suggested and hypothesized as a mechanism for differential susceptibility in mice for a number of alkylating agents (Sudman et al., 1992). Because both male and female mice are adversely affected by 1,3-butadiene, albeit females more so, the exposure effect may be attributed to a dose gradient. That is, less dose is needed for intercalation of diffuse chromosomal macromolecules in oocytes than in late spermatocytes. It is of interest that a diepoxybutane-adenine adduct has been detected in Chinese hamster ovarian cells (Leuratti et al., 1994) and several guanosine adducts have been detected following butadiene monoxide exposure (Selzer and Elfarra, 1996). The reader is referred to the chapter on mutagenicity (Chapter 4) for further discussion of these issues. Whether this possible condition/mechanism holds for women exposed to 1,3-butadiene as it might for rodent species is not known. Several studies indicate that 1,3-butadiene affects spermatozoa and spermatids via a mutagenic effect, as determined by postimplantation deaths during the first 3 weeks after exposure (see Chapter 4).

The relationship between germ-cell toxicity and functional capacity has been evaluated by Grizzle et al. (1994), who exposed mice by gavage to 4-vinylcyclohexene (100, 250, or 500 mg/kg/day) in a continuous breeding protocol. A marginal reduction in testicular effects was seen at 500 mg/kg, with a highly significant reduction in ovarian follicles at the same dose level, but no effect on other measures of reproductive toxicity, fertility, or fecundity. Given the data from Hooser et al. (1994) discussed above, this is not surprising because a >90% reduction in ovarian follicle count was not associated with a change in FSH levels or estrous cycling at 120

days after the beginning of a 30-day treatment. Complete ovarian atrophy did not occur until 240 days from the beginning of treatment, with a concomitant increase in FSH levels. These data also support the gender difference in sensitivity between males and females, as testicular morphology was only marginally affected while ovarian toxicity was significantly increased. In addition, Hooser et al. (1995) showed reduced testicular weight and loss of germ cells in male mice treated with 4-vinylcyclohexene diepoxide (320 mg/kg ip) for 5 days or 80 mg/kg for 30 days, an effect that is fully reversible; in contrast, a dose of 80 mg/kg ip to female mice and rats for 12 days can lead to a permanent and irreversible loss of ovarian follicles (Kao et al., 1999).

5.5. SUMMARY AND CONCLUSIONS

Evidence has been presented showing that 1,3-butadiene induces reproductive and developmental effects in rodents. The three long-term toxicity studies conducted in Sprague-Dawley CD rats (Owen et al., 1987; Owen and Glaister, 1990) and B6C3F_1 mice (NTP, 1984, 1993) as well as the subchronic toxicity studies (NTP, 1993; Bevan et al., 1996) suggest that mice are much more sensitive than rats to the reproductive effects of 1,3-butadiene. Nonneoplastic reproductive toxicity was not observed in either male or female rats exposed by inhalation to 1,3-butadiene at concentrations up to 8,000 ppm for 2 years. Ovarian atrophy was seen in mice at all exposure levels after 2 years of exposure (NTP, 1993), a time at which ovarian atrophy is expected to appear in aged animals because of normal senescence mechanisms. However, ovarian atrophy following 1,3-butadiene exposure occurred in a dose-related fashion, and at significantly greater incidence in exposed mice versus controls. For example, ovarian atrophy was observed as early as 13 weeks in female mice exposed to 1,000 ppm (Bevan et al., 1996), after 9 months exposure to 200 and 625 ppm, after 15 months exposure to 62.5 ppm and above, and after 2 years exposure to 6.25 ppm and above (NTP, 1993). Therefore, the dose-response relationship observed for ovarian atrophy and the significant increase at the lowest dose relative to that seen in control animals of a similar age is evidence for a causal relationship between ovarian atrophy and exposure to 1,3-butadiene at 6.25 ppm.

Only a small amount of data is available regarding the reproductive or developmental effects of the metabolites of 1,3-butadiene, EB, and DEB (Doerr et al., 1995, 1996). These studies showed that mice are more sensitive than rats to the ovotoxic effects (follicular loss) of EB and DEB, and that DEB is the more potent ovotoxicant in both species. Ovarian atrophy was also observed in mice after exposure to the 1,3-butadiene dimer 4-vinylcyclohexene, administered by inhalation for 6 h/day, 5 days/week for 13 weeks at 1,000 ppm (Bevan et al., 1996), or by gavage for 5 days/week for 13 weeks at 1,200 mg/kg (NTP, 1986), or to its diepoxide, 4-vinyl-1-cyclohexene diepoxide, administered by topical application (10 mg/animal) for 13 weeks or 2 years (NTP, 1989). Ovarian atrophy induced by 1,3-butadiene, 4-vinylcyclohexene, or 4-

vinyl-1-cyclohexene diepoxide is characterized by the absence of oocytes, follicles, and corpora lutea. The functional integrity of the reproductive system in animals exposed to 1,3-butadiene has not been tested, but the severity of the ovarian lesion is indicative of reproductive dysfunction. Several studies provide a body of evidence showing that the earliest ovarian effect that can be detected is DNA degradation indicative of apoptosis in small follicles (Springer et al., 1996a), followed by atresia of follicles (Kao et al., 1999), with subsequent loss of follicles and eventual ovarian atrophy, leading to early reproductive senescence and possibly increased risk for ovarian tumor formation (Hooser et al., 1994; Hoyer and Sipes, 1996).

As for the functional consequences of the ovarian effects, Grizzle et al. (1994) showed that exposure of mice to 4-vinylcyclohexene in a continuous breeding study resulted in a significant reduction in ovarian follicle counts and a slight decrease in spermatid count, but did not show alterations of reproductive competence in F1 animals. This is not surprising because reproduction in mice is extremely robust, so that cohabitation and mating for longer periods of time to the point of complete ovarian atrophy may be necessary to see the impact of such significant follicular reductions. Rats administered 4-vinylcyclohexene orally or 4-vinyl-1-cyclohexene diepoxide dermally did not develop ovarian atrophy, although 2/10 developed ovarian atrophy when exposed by inhalation to 1,500 ppm (Bevan et al., 1996). These data demonstrate a species-specific response similar to that after exposure to 1,3-butadiene. Several other studies in which the vinylcyclohexene diepoxide metabolite was administered to rats clearly show the sensitivity of the rat ovary to the diepoxide metabolite of 4-vinylcyclohexene (Flaws et al., 1994a; Springer et al., 1996a; Kao et al., 1999) when administered intraperitoneally.

The gender differences in response to 1,3-butadiene are also supported by studies on structural analogues. For example, Melnick et al. (1994) reported testicular atrophy in male mice exposed by inhalation to 7,000 ppm isoprene for 13 or 26 weeks, and no effect was seen in rats exposed to the same concentration at 13 weeks, with some hyperplasia of the interstitial cells evident at 26 weeks. Hooser et al. (1995) explored the sensitivity of male mice to 4-vinylcyclohexene diepoxide and showed reduced testicular weight and a loss of germ cells after 5 days exposure to 320 mg/kg ip, with complete loss of germ cells except for spermatogonial stem cells after dosing for 30 days. This effect was completely reversible after a 30-60 day recovery period, whereas the ovarian toxicity reported in the female at 80 mg/kg ip for 12 days was permanent and irreversible.

Developmental effects observed after exposure to 1,3-butadiene consisted primarily of reduced fetal body weight and minor skeletal defects such as abnormal ossifications, abnormal sternebrae, and supernumerary ribs. The species-specific response for developmental effects induced by 1,3-butadiene was similar to that for reproductive effects, with mice showing greater

sensitivity than rats. This difference was also seen for maternal toxicity as manifested by decreased weight gain (whole-body and extragestational) in both rats and mice. The NOAEL for maternal effects is 200 ppm for rats (IISRP, 1982; Hackett et al., 1987a) and 40 ppm for mice (Hackett et al., 1987b). Even though the IISRP (1982) study showed increased frequencies for bipartite thoracic centers and minor skeletal defects combined at 200 ppm in rats, the response was not clearly dose-related. Several developmental effects occurred at significantly increased frequencies at 8,000 ppm and showed a dose-response relationship: major skeletal defects combined, wavy ribs, and abnormal ossification of the ribs (IISRP, 1982). The results from the IISRP (1982) study were not confirmed in the study by Hackett et al. (1987a), which showed no developmental toxicity in the same rat strain similarly exposed to 1,3-butadiene at concentrations up to 1,000 ppm. Therefore, the NOAEL for developmental effects in rats is 1,000 ppm (IISRP, 1982; Hackett et al., 1987a). A NOAEL for developmental effects could not be defined for the mouse, because male fetal body weight was decreased at 40 ppm in the Hackett et al. (1987b) study. The mechanism for the developmental effects of 1,3-butadiene or its congeners has not been explored.

Reproductive and developmental toxicity studies, therefore, show species specificity for exposure to 1,3-butadiene, with mice being much more sensitive than rats. Pharmacokinetic studies show that the uptake of 1,3-butadiene and its activation to epoxide metabolites are greater in mice than in rats (see Chapter 3). These data also indicate that the availability of 1,3-butadiene, EB, and DEB is greater in mice than in rats at comparable exposure concentrations. Although the pharmacokinetic data do not fully explain the species differences, they suggest that the basis of the difference in sensitivity may be related to the greater availability of toxic intermediates of 1,3-butadiene metabolism in mice.

A few studies provide pharmacokinetic data specifically for reproductive organs. Nose-only exposure of mice and rats to 1,3-[^{14}C]-butadiene resulted in greater or similar concentrations of radioactivity (expressed as nM/g of tissue) in tissues of rats than those of mice under conditions in which the rats were exposed to a tenfold higher concentration of 1,3-butadiene (Bond et al., 1987). If tissue uptake was expressed as 1,3-butadiene equivalents/μM inhaled 1,3-butadiene, however, radioactivity levels were 15 to 100 times higher in mice. Mammary tissue, which had 4.6-fold higher concentration in rats than in mice, was the only tissue analyzed that was relevant to evaluating reproductive effects of 1,3-butadiene. Thornton-Manning et al. (1997) exposed female Sprague-Dawley rats and female B6C3F$_1$ mice to 62.5 ppm 1,3-butadiene by inhalation for 6 hours (single exposure) or 6 hours/day for 10 days (repeated exposure) and measured EB and DEB in several tissues, including mammary (EB and DEB) and ovary (DEB only). In mammary tissue, EB levels were about 12 times greater in the mouse than in the rat following the single exposure and about 4 times greater following the repeated exposures,

whereas DEB levels were about 24 times greater following the single exposure and about 13 times greater following the repeated exposures. In addition, EB levels in mammary tissue were about 5 times greater in the rat and about 1.7 times greater in the mouse following repeated exposures than following the single exposure, whereas DEB levels in mammary tissue were about 1.4 times greater in the rat and about 1.4 times lower in the mouse following repeated exposures than following the single exposure. In the ovary, DEB levels were about 28 times greater in the mouse than in the rat following the single exposure and about 15 times greater following the repeated exposures. DEB levels in the ovary were about 1.7 times greater in the rat and about 1.1 times lower in the mouse following repeated exposures than following the single exposure. In an in vitro study, Sharer et al. (1992) showed that microsomes from the testes of rats and mice are ineffective in forming EB, but the cytosol fraction was very effective in forming glutathione conjugates. Keller et al. (1997) showed that ovarian microsomes from rats and mice were unable to form mono- or diepoxides of vinylcyclohexene. Therefore, it is unlikely that toxic effects on the testes or ovaries are due to metabolites formed within those tissues; rather, they are due to metabolites formed elsewhere.

The link among ovarian atrophy, increased FSH levels, and formation of tumors in the ovary is intriguing. Hoyer and Sipes (1996) reviewed the data related to this sequence of events and postulated that preneoplastic and neoplastic changes may result from the loss of follicles in the ovary and disruption of the feedback loop for endocrine regulation at the level of the hypothalamus and/or pituitary, resulting in increased serum FSH levels which act as a promotor of a genotoxic effect on the ovary. The data of Hooser et al. (1994) support this hypothesis, showing that the long-term sequence of events can occur after as little as 30 days of exposure of mice to the vinylcyclohexene diepoxide, which initiates accelerated follicular loss, eventual ovarian atrophy, altered hormonal balance, and preneoplastic changes.

In conclusion, the animal data suggest that there is a potential reproductive hazard for permanent and irreversible ovarian toxicity in women exposed by inhalation to 1,3-butadiene. There is also a potential risk for reproductive hazards to the testes and germ cells in men, but the effects appear to be reversible, because spermatogonial stem cells are not lost. Studies of 1,3-butadiene, vinylcyclohexene, their epoxide metabolites, and structurally related compounds provide strong evidence that the ovarian toxicity in mice is attributable specifically to the diepoxide metabolites. Thus, although there is a potential hazard for ovarian toxicity in women exposed to 1,3-butadiene, the risk would be expected to be quantitatively less than for mice because pharmacokinetic data suggest that humans form less DEB than do mice (see Chapters 3 and 10). The animal data also show that there is a potential for developmental effects in humans upon in utero exposure to 1,3-butadiene, and that these effects may occur at concentrations below those causing maternal effects.

6. TOXICITY IN ANIMALS

This chapter updates the evaluation of toxicity observed in experimental animals, reviewing subchronic and chronic studies published from 1985 through December 1998, with the exception of reproductive and developmental effects, which are reviewed in Chapter 5. The available data from acute exposure studies suggest that 1,3-butadiene has low acute toxicity. Data on acute toxicity mainly predate 1985 and are summarized by Himmelstein et al. (1997); they are not reviewed in this document. On the other hand, a review of the chronic rat bioassay conducted by Hazleton Laboratories (1981b) is included, even though the study was already reviewed by U.S. EPA (1985), because it is the major rat cancer study.

6.1. SUBCHRONIC TOXICITY

Irons et al. (1986a,b) conducted studies to assess the potential of 1,3-butadiene to induce myelotoxicity by exposing male B6C3F_1 mice and male NIH Swiss mice to 1250 ppm 1,3-butadiene, 6 h/day, 5 days/week for 6 weeks. Treatment-related hematological changes included decreases in red blood cell counts, total hemoglobin, and hematocrit, and increases in mean cell volume and circulating micronuclei in both strains of mice. The observed anemia was not accompanied by significant alterations in mean corpuscular hemoglobin concentration, increases in reticulocyte counts, or increases in the frequency of nucleated erythrocytes in peripheral blood. These hematologic changes were considered to represent a macrocytic-megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells.

Exposure of male B6C3F_1 mice to 1,250 ppm 1,3-butadiene for 6 h/day, 5 days/week, for 6 or 12 weeks did not produce any persistent effects on humoral or cell-mediated immunity (Thurmond et al., 1986). Relative thymus weights were unaffected, but relative spleen weights were decreased 20% and spleen cellularity was decreased 29% in exposed mice. Extramedullary hematopoiesis and erythroid hyperplasia in exposed mice correlated with a twofold increase in thymidine incorporation into spontaneously proliferating splenocytes. Although the number of IgM antibody plaque-forming cells (PFC) per 10^6 splenocytes was unchanged, a 30% decrease in PFC/spleen was observed. Proliferation of alloantigens was similar for 1,3-butadiene-exposed splenocytes and controls. The mitogenic response of mature T lymphocytes to phytohemagglutinin was significantly suppressed after exposure to 1,3-butadiene for 6 or 12 weeks.

6.2. CHRONIC TOXICITY

6.2.1. Rat Study

In a long-term inhalation bioassay in rats (HLE, 1981b; Owen et al., 1987; Owen and Glaister, 1990), groups of 110 male and 110 female Sprague-Dawley rats were exposed to 0, 1,000, and 8,000 ppm of 1,3-butadiene 6 h/day, 5 days/week, for 105 weeks (females) and 111 weeks (males). The low exposure level (1,000 ppm) was based on the threshold limit value (TLV) for occupationally exposed personnel, while the high exposure (8,000 ppm) was limited by safety requirements to be below the lower explosive limit of 1,3-butadiene in air. Ten males and 10 females from all groups were killed after 52 weeks for interim clinical and histopathology investigations.

Statistically significant reductions in survival occurred in both sexes at the highest exposure concentration ($p<0.05$ in males, $p<0.01$ in females). Increased mortality in female rats resulted from the sacrifice of animals with large subcutaneous masses, whereas in male rats, renal lesions were the likely major cause of the increased death rate. Following this exposure there were no effects on hematology, blood chemistry, urine analyses, or neuromuscular function that definitely could be associated with the treatment. The only treatment-associated changes were in clinical conditions, suppression of body weight, and increases in the weight of certain organs. Table 6-1 lists the relative weights of those organs that showed a treatment-related increase in either sex at 1 or 2 years. The relative liver weights of both sexes at both exposure levels were statistically significantly elevated with the exception of high-exposure females at 1 year. There were no associated changes in the liver even at the ultrastructure level. The absolute and relative kidney weights were statistically significantly increased in male rats exposed to 8,000 ppm for 2 years. This was associated with severe nephrosis compared to the controls (Table 6-2). The relative heart weights were increased in the same group, which may have been attributable to blood pressure changes resulting from the developing kidney changes. Statistically significant increases in lung and spleen weights also were noted in high-exposure males at 2 years with increased focal metaplasia in the lungs.

6.2.2. Mouse Study

A 2-year chronic inhalation toxicity and carcinogenicity study on the effects of 1,3-butadiene on B6C3F$_1$ mice was conducted by NTP (1993). In this study, groups of 70 male and 70 female mice were exposed by inhalation 6 h/day, 5 days/week to 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene for periods up to 103 weeks; groups of 90 male and 90 female mice were similarly exposed to 625 ppm 1,3-butadiene, which was the lowest exposure level in the previous NTP (1984) study. The additional animals in the 625-ppm exposure group were included because

Table 6-1. Relative organ weights (g/100 g body weight) in Sprague-Dawley rats.

	Organ weight (% Current control)							
	1 Year				2 Year			
	Male		Female		Male		Female	
Organ	1,000 ppm	8,000 ppm	1,000 ppm	8,000 ppm	1,000 ppm	8,000 ppm	1,000 ppm	8,000 ppm
Kidneys	104	102	104	102	104	124[b]	104	102
Liver	105[a]	124[b]	116[a]	110	111[b]	125[b]	118[b]	121[b]
Heart	96	100	103	103	104	116[a]	97	97
Lung	97	93	105	108	104	114[a]	106	103
Spleen	92	92	100	93	93	121[a]	107	133
Number examined	10	10	10	10	50	32	32	24

[a] Statistically significant $p<0.05$.
[b] Statistically significant $p<0.01$.

Source: Adapted from Owen et al., 1987.

Table 6-2. Incidence of nephropathy in male Sprague-Dawley rats[a,b].

	No. of animals affected		
Finding	**0 ppm**	**1,000 ppm**	**8,000 ppm**
Number examined	100 (45)	100 (50)	100 (31)
Nephropathy			
None	13 (4)	25 (7)	9 (0)
Minimal	29 (12)	32 (20)	11 (1)
Slight	38 (19)	27 (16)	42 (17)
Moderate	10 (7)	7 (4)	11 (2)
Marked	3 (2)	3 (2)	14 (9)
Severe	7 (1)	6 (1)	13 (2)

[a] The figures in parentheses are the values for animals killed at the end of the study.
[b] A statistically significant exposure-related trend ($p<0.05$) for fatal nephropathy was established. "Fatal" was defined for the statistical treatment by the Peto method of analysis.

Source: Adapted from Owen et al., 1987.

high mortality rates, observed previously at this exposure concentration, might interfere with the scheduled interim evaluations. Interim evaluations were conducted at 9 and 15 months.

Mean body weight gains of male and female mice exposed to 6.25-625 ppm 1,3-butadiene for 103 weeks were similar to those of controls. However, concentration-related decreases in survival were seen in male and female mice exposed to concentrations $\geq$20 ppm 1,3-butadiene (Table 6-3, Figures 6-1 and 6-2), primarily because of the development of malignant neoplasms. No female mice exposed to 200 or 625 ppm or male mice exposed to 625 ppm survived to the end of the study. Statistical analysis for the probability of survival was estimated using the Kaplan and Meyer (1958) procedure; Cox (1972) and Tarone's (1975) life table test was used to identify concentration-related trends.

At the 9- and 15-month interim evaluations, no clinical findings other than those associated with lesion development and moribundity were observed. Some statistically significant organ weight changes were observed at interim evaluations in male and female mice exposed to 1,3-butadiene concentrations $\geq$62.5 ppm. Effects related to toxicity to reproductive organs are discussed in Chapter 5.

Hematological indices measured at the interim evaluations showed significant ($p \leq 0.05$) decreases in erythrocyte counts, hemoglobin concentration, and packed cell volume in male mice exposed to $\geq$62.5 ppm and in female mice exposed to $\geq$200 ppm at 9 months. Mean cell volume was significantly increased in male mice exposed to 625 ppm and in females exposed to $\geq$200 ppm at 9 months. A similar profile of hematological changes was observed in male and female mice exposed to 625 ppm for 15 months. Increases in the percentage of erythrocytes with Howell-Jolly body inclusions and mean cell hemoglobin were seen at 9 and 15 months. At the 15-month interim evaluation, males exposed to 625 ppm 1,3-butadiene had a significantly increased mean platelet value, a finding that correlated with the development of neoplasms. Because these hematological changes were not associated with increases in reticulocyte counts or in frequency of polychromatic erythrocytes in peripheral blood, they were attributed to a partial or poorly regenerative bone marrow response to decreased levels of circulating erythrocytes. There were no significant changes in total serum enzyme activity of lactate dehydrogenase (LDH) or creatine kinase in mice evaluated at 9 months. LDH values at the 15-month evaluation were increased in males and females exposed to $\geq$200 ppm. At 625 ppm, LDH-1 and LDH-2 were decreased and LDH-5, the principal enzyme in skeletal muscle and liver, was increased.

Histopathological effects observed at the 9-month evaluations included bone marrow atrophy (depletion of cells) in 50% of males and in 13% of females at the highest concentration (625 ppm). The atrophy increased in severity from mild depletion of hematopoietic cells at 9 months to marked depletion in mice that died or were killed at or before 15 months. An increased incidence of bone marrow hyperplasia and an increased incidence or severity of

Table 6-3. Survival of male and female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

	Concentration (ppm)					
	0	**6.25**	**20**	**62.5**	**200**	**625**
Male						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation[a]	10	10	10	10	10	10
15-Month interim evaluation[a]	10	10	10	10	10	7
Natural deaths	6	5	11	12	23	39
Moribund kills	9	6	15	15	23	33
Accidental deaths[a]	0	0	0	0	0	1
Missing[a]	0	0	0	1	0	0
Animals surviving until study termination	35	39	24	22	4[b]	0
Percent survival at end of study[c]	70	78	49	46	8	0
Mean survival(days)[d]	597	611	575	558	502	280
Survival analysis[e]	$p<0.001$	$p=0.430$N	$p=0.044$	$p=0.021$	$p<0.001$	$p<0.001$
Female						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation[a]	10	10	10	10	10	8
15-Month interim evaluation[a]	10	10	10	10	10	2
Natural deaths	3	7	11	8	12	33
Moribund kills	10	10	14	31	37	46
Accidental deaths[a]	0	0	1	0	1	1
Animals surviving until study termination	37	33	24	11	0	0
Percent survival at end of study[c]	74	66	50	23	0	0
Mean survival (days)[d]	608	597	573	548	441	320
Survival analysis[e]	$p<0.001$	$p=0.510$	$p=0.013$	$p<0.001$	$p<0.001$	$p<0.001$

[a] Censored from survival analyses.
[b] Includes one animal that died during the last week of the study.
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations, accidental deaths, and missing animals.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice).
[e] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

Source: NTP, 1993.

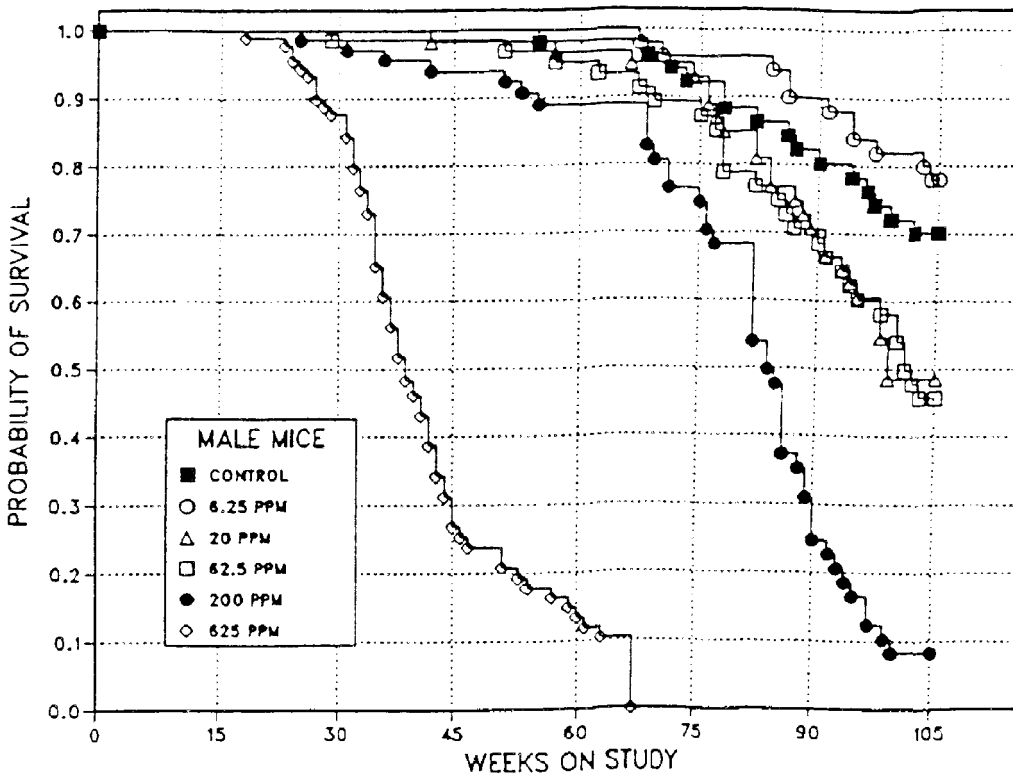

Figure 6-1. Kaplan-Meier survival curves for male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

Source: NTP, 1993.

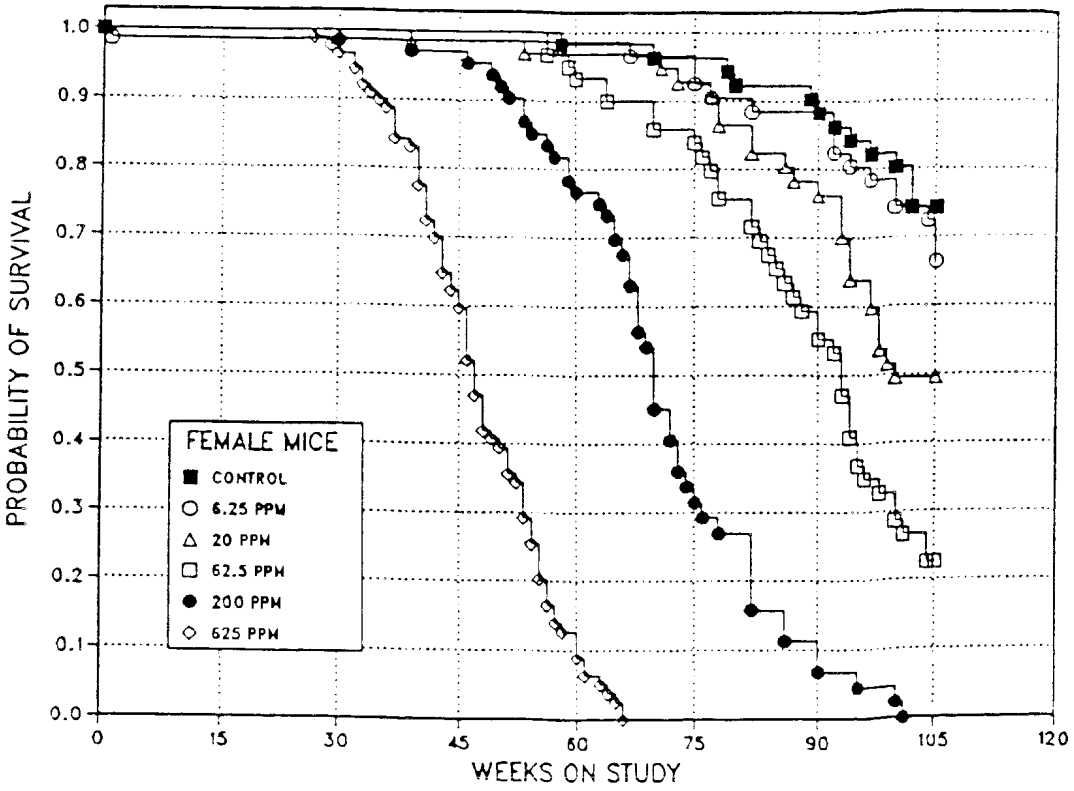

Figure 6-2. Kaplan-Meier survival curves for female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

Source: NTP, 1993.

hematopoiesis of the spleen, liver, and lung occurred in females exposed to the three highest concentrations (≥62.5 ppm). Thymic necrosis (atrophy) and decreased thymus weights were seen at the 9-month evaluation in males and females exposed to 625 ppm. Thymic necrosis also occurred in females exposed to 62.5 or 200 ppm.

In the 2-year study, bone marrow atrophy was recorded in 50% of males and 14% of females exposed to 625 ppm.

The incidence of liver necrosis was increased at the higher exposure concentrations in males and females, occurring in 8%, 10%, 16%, 27%, 29%, and 26% of males and in 4%, 4%, 14%, 10%, 38%, and 21% of females exposed to 0 (controls), 6.25, 20, 62.5, 200, and 625 ppm, respectively. Centrilobular hepatocellular necrosis of the liver was seen in 4% and 8% of males exposed to 62.5 and 625 ppm, respectively, and in 2%, 8%, and 9% of females exposed to 62.5, 200, and 625 ppm, respectively. Hepatocellular necrosis was not seen in any of the concurrent controls. Liver necrosis with no particular lobular distribution was found primarily in animals with malignant lymphoma and hemangiosarcoma; centrilobular hepatocellular necrosis was often found in animals described as anemic and in animals with atrial thrombi.

Myocardial mineralization, a lesion of unknown pathogenesis, occurred with increased frequency in both sexes at 625 ppm (males, 27%; females, 14%), but was not observed in controls. A low incidence was observed at the lower concentrations. Myocardial mineralization was also observed in a separate stop-exposure study in which male mice were exposed to 312 ppm 1,3-butadiene for 52 weeks or 625 ppm for 13 or 26 weeks, and observed for periods up to 103 weeks. The incidence of myocardial mineralization for these three exposure groups was 12%, 18%, and 28%, respectively. Details of the stop-exposure study are presented in Section 3.3.

Minimal to mild olfactory epithelial atrophy occurred in females exposed to 625 ppm and in males exposed to concentrations ≥20 ppm. However, the incidence in males exposed to 625 ppm was lower than that seen in females. The olfactory epithelial lesions were unilateral at the lower concentrations and bilateral at the higher concentrations. The lesions were similar to those seen in the NTP (1984) study, but osseous or cartilaginous metaplasia was not observed. The investigators considered olfactory nasal atrophy a possibly compound-related lesion.

Compared with controls, mice exposed to 1,3-butadiene exhibited increased incidences of proliferative lesions (hyperplasia) in several organs, including the heart, lungs, forestomach, ovaries, mammary gland, and Harderian gland (Table 6-4). Hyperplasia of the endothelium (cardiac blood vessels), alveolar epithelium, forestomach epithelium (focal), germinal epithelium and granulosa cells of the ovaries, mammary gland, and Harderian gland were all considered preneoplastic lesions. Other preneoplastic lesions identified in the 2-year study were hepatocellular foci (basophilic, clear cell, mixed cell, and eosinophilic) in female mice exposed

Table 6-4. Incidence of hyperplasia in male and female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

Organ/tissue	Sex	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Heart, endothelium	M	0/50 (0%)	1/49 (2%)	0/50 (0%)	2/48 (4%)	4/48 (8%)	5/73 (7%)
	F	0/50 (0%)	2/50 (4%)	1/50 (2%)	4/49 (8%)	5/50 (10%)	8/80 (10%)
Lung, alveolar epithelium	M	2/50 (4%)	9/50 (18%)	6/50 (12%)	13/49 (27%)	17/50 (34%)	12/73 (16%)
	F	5/50 (10%)	5/50 (10%)	3/50 (6%)	9/50 (18%)	11/50 (22%)	11/78 (14%)
Forestomach, epithelium	M	4/50 (8%)	3/50 (6%)	3/50 (6%)	5/48 (10%)	4/48 (18%)	40/72 (56%)
	F	4/50 (8%)	5/49 (10%)	4/47 (9%)	7/48 (15%)	14/50 (28%)	47/79 (59%)
Ovary, germinal epithelium	F	2/49 (4%)	3/49 (6%)	8/48 (17%)	15/50 (30%)	15/50 (30%)	19/79 (23%)
Ovary, granulosa cells	F	1/49 (2%)	0/49 (0%)	2/48 (4%)	3/50 (6%)	3/50 (6%)	2/79 (3%)
Mammary gland	F	2/50 (4%)	0/50 (0%)	2/50 (4%)	4/50 (8%)	7/50 (14%)	2/80 (3%)
Harderian gland	M	1/50 (2%)	3/49 (6%)	4/50 (8%)	6/47 (13%)	8/47 (17%)	5/40 (13%)
	F	1/50 (2%)	5/49 (10%)	9/48 (19%)	4/49 (8%)	4/49 (8%)	7/66 (11%)

Source: NTP, 1993.

to 1,3-butadiene. Hepatocellular foci were observed in 16% of controls and in 29%, 38%, 24%, 10%, and 5% of females exposed to 6.25, 20, 62.5, 200, and 625 ppm, respectively. Hyperplastic lesions were also observed in separate studies with male $B6C3F_1$ mice using variable exposure and durations (stop-exposure experiments). Hyperplasia in these studies occurred primarily in the endothelium (cardiac blood vessels), alveolar epithelium, forestomach epithelium, and Harderian gland (Table 6-5).

Table 6-5. Incidence of hyperplasia in male $B6C3F_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study.

Organ/tissue	Concentration (duration of exposure)				
	0 ppm	200 ppm (40 weeks)	312 ppm (52 weeks)	625 ppm (13 weeks)	625 ppm (26 weeks)
Heart, endothelium	0/50 (0%)	6/50 (12%)	3/50 (6%)	7/50 (14%)	7/50 (14%)
Lung, alveolar epithelium	2/50 (4%)	18/50 (36%)	14/50 (28%)	10/50 (20%)	11/50 (22%)
Forestomach, epithelium	4/50 (8%)	10/48 (21%)	20/48 (42%)	8/50 (16%)	15/50 (30%)
Harderian gland	1/50 (2%)	4/48 (8%)	6/48 (13%)	3/42 (7%)	7/36 (19%)

Source: NTP, 1993.

6.3. CARCINOGENICITY

6.3.1. Rat Study

The details of the study design and toxicity data have been described in Section 6.2.1. Under the conditions of this study, 1,3-butadiene was carcinogenic at multiple organ sites in both male and female Sprague-Dawley rats by inhalation (Table 6-6). Rats were exposed to 0, 1,000, and 8,000 ppm 1,3-butadiene, 6 h/day, 5 days/week, for 105 weeks (females) and 111 weeks (males). There were increased incidences and exposure-response trends ($p<0.05$) in several tumor types. In males, there were increased incidences of pancreatic exocrine adenomas and testicular Leydig cell tumors. Four tumor sites in females showed treatment-related effects including total mammary gland adenomas and carcinomas, thyroid follicular cell adenomas, Zymbal gland carcinomas (significant trend), and uterine sarcomas (significant trend). Furthermore, the average number of mammary gland fibroadenomas per female rat bearing a mammary gland tumor was 1.38 in controls, 3.70 in the 1,000 ppm exposure group, and 3.33 in the 8,000 ppm exposure group (Melnick and Huff, 1993). The occurrence of glial cell tumors in exposed male rats may also be related to exposure because these neurological tumors are uncommon in untreated Sprague-Dawley rats. The metabolism of 1,3-butadiene in Sprague-

Table 6-6. Incidences of tumors in Sprague-Dawley rats exposed to 1,3-butadiene by inhalation.

	Male			Female	
Tissue and tumor	**0 ppm**	**1,000 ppm**	**8,000 ppm**	**0 ppm**	**1,000 ppm**	**8,000 ppm**
Pancreas						
Exocrine adenoma	3	1	10[a,b]	2	0	0
Uterus						
Sarcoma	-	-	-	1	4	5[a]
Zymbal gland						
Adenoma	1	1	1	0	0	0
Carcinoma	0	0	1	0	0	4[a]
Mammary gland						
Benign	0	2	0	32	64[b]	55[a,b]
Malignant	1	0	0	18	15	26
Total	1	2	0	50	79[b]	81[a,b]
Thyroid						
Follicular cell adenoma	3	5	1	0	2	10[a,b]
Follicular cell carcinoma	1	0	0	0	2	1
Brain glial cell	1	4	5[a]	-	-	-
Testis						
Leydig cell tumor	0	3	8[a,b]	-	-	-
Total number of tumor-bearing rats	84	70	87	97	98	94
Subtotal tumor-bearers						
No tumors	16	30	13	3	2	6
Single tumors	26	32	30	41	24	17
Multiple tumors	58	38	57	56	74	77
Number of rats examined	100	100	100	100	100	100

[a] Increasing trend, $p<0.05$.
[b] Increased in comparison with chamber controls (0 ppm), $p<0.05$.

Source: Adapted from Owen et al., 1987, and Melnick and Huff, 1993.

Dawley rats is saturated at concentrations between 1,000 and 2,000 ppm (Laib et al., 1990), which may explain why the incidences of tumors of the brain, uterus, and mammary gland of rats exposed to 8,000 ppm were not higher than those exposed to 1,000 ppm (Melnick and Huff, 1993).

6.3.2. Mouse Studies

The first NTP mouse inhalation study of 1,3-butadiene was terminated early because of induction of fatal neoplasms (NTP, 1984); therefore, a second study (NTP, 1993) was conducted to better characterize the exposure-response relationship for neoplasms and nonneoplastic lesions induced in mice by exposure to 1,3-butadiene for 2 years. The concentrations ranged from 100-fold lower (6.25 ppm) up to the lowest concentration (625 ppm) used in the first study. In addition, stop-exposure studies were conducted to assess the relationship between concentration nd duration of exposure on the induction of neoplasms by 1,3-butadiene. Results of this study have also been published by Melnick et al. (1990a-c) and Melnick and Huff (1992). Miller and Boorman (1990) provided morphological descriptions of the neoplastic lesions induced in B6C3F_1 mice by 1,3-butadiene. The results are presented here in two parts: 2-year study and stop-exposure study.

6.3.2.1. *2-Year Study (NTP, 1993)*

The details of the study design are described in Section 6.2.2. For neoplasms that were considered to be lethal tumors, the tumor incidence was analyzed using the life table test, a survival-adjusted procedure appropriate for rapidly lethal tumors (Cox, 1972; Tarone, 1975). For incidental tumors (tumors discovered as a result of death from an unrelated cause), the primary statistical method used was the logistic regression test. Alternative statistical methods included the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart et al., 1979), analyses based on the overall proportion of tumor-bearing animals. Tests of significance included pairwise comparisons of each dose group and a test for an overall concentration-response trend.

Exposure of male and female mice to 1,3-butadiene induced a variety of common and uncommon tumors at multiple sites. The incidences of primary neoplasms associated with exposure to 1,3-butadiene (for the 2-year study) are presented in Tables 6-7 and 6-8. The percentage of animals bearing malignant tumors increased from about 30% in the controls to nearly 90% in the highest exposure group, 625 ppm. The results of interim evaluations for 9 months and 15 months are presented in Tables 6-9 and 6-10.

Table 6-7. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (histiocytic, lymphocytic, mixed, NOS, or undifferentiated)	4/50 (8%)[a] 9.8%[b] *p*<0.001[c]	2/50 (4%) 5.1% *p*=0.302N	4/50 (8%) 12.2% *p*=0.528	6/50 (12%) 17.7% *p*=0.238	2/50 (4%) 4.0% *p*=0.627	51/73 (70%) 95.4% *p*<0.001
	Histiocytic sarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0%	4/50 (8%) 10.6% *p*=0.051	5/50 (10%) 14.3% *p*=0.021	7/50 (14%) 31.9% *p*<0.001	4/73 (5%) 10.8% *p*=0.043
	Malignant lymphoma or histiocytic sarcoma	4/50 (8%) 9.8% *p*<0.001[c]	2/50 (4%) 5.1% *p*=0.302N	8/50 (16%) 21.5% *p*=0.118	11/50 (22%) 29.6% *p*=0.022	9/50 (18%) 34.7% *p*=0.005	55/73 (75%) 95.9% *p*<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/49 (0%) 0.0% NA	1/50 (2%) 3.4% *p*=0.451	5/48 (10%) 19.4% *p*=0.011	20/48 (42%) 93.3% *p*<0.001	4/73 (5%) 44.6% *p*<0.001
Lungs	Alveolar/bronchiolar adenoma	18/50 (36%) 46.9% *p*=0.200[d]	20/50 (40%) 47.3% *p*=0.517	10/50 (20%) 28.2% *p*=0.080N	25/49 (51%) 74.2% *p*=0.036	21/50 (42%) 100.0% *p*=0.061	3/73 (4%) 59.4% *p*=0.492
	Alveolar/bronchiolar carcinoma or adenocarcinoma	5/50 (10%) 14.3% *p*<0.001[c]	6/50 (12%) 15.4% *p*=0.577	11/50 (22%) 38.3% *p*=0.017	12/49 (24%) 42.9% *p*=0.006	22/50 (44%) 94.6% *p*<0.001	3/73 (4%) 59.4% *p*<0.001
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	21/50 (42%) 54.9% *p*<0.001[c]	23/50 (46%) 54.5% *p*=0.552N	19/50 (38%) 53.6% *p*=0.276	31/49 (63%) 87.9% *p*<0.001	35/50 (70%) 100.0% *p*<0.001	3/73 (4%) 59.4% *p*<0.001
Forestomach	Squamous cell papilloma	1/50 (2%) 2.5% *p*<0.001[d]	0/50 (0%) 0.0% *p*=0.535N	0/50 (0%) 0.0% *p*=0.486N	1/50 (2%) 4.5% *p*=0.739	7/50 (14%) 51.7% *p*=0.012	2/73 (3%) 40.0% *p*=0.446
	Squamous cell papilloma or carcinoma	1/50 (2%) 2.5% *p*<0.001[c]	0/50 (0%) 0.0% *p*=0.481N	0/50 (0%) 0.0% *p*=0.545N	1/50 (2%) 4.5% *p*=0.679	8/50 (16%) 54.5% *p*<0.001	4/73 (5%) 51.8% *p*<0.001
Liver	Hepatocellular adenoma	13/50 (26%) 32.1% *p*=0.042[d]	13/50 (26%) 31.3% *p*=0.552	19/50 (38%) 52.1% *p*=0.158	16/48 (33%) 57.0% *p*=0.261	23/48 (48%) 92.2% *p*=0.008	5/72 (7%) 100.0% *p*=0.253

Table 6-7. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks (continued).

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Liver (continued)	Hepatocellular carcinoma	4/49 (8%) 10.3% *p*=0.178[d]	6/49 (12%) 14.5% *p*=0.381	8/50 (16%) 25.0% *p*=0.141	9/50 (18%) 47.9% *p*=0.066	8/50 (16%) 82.7% *p*=0.006	1/80 (1%) 12.5% *p*=0.910
	Hepatocellular adenoma or carcinoma	21/50 (42%) 47.9% *p*=0.067[d]	23/50 (46%) 53.0% *p*=0.375	30/50 (60%) 70.1% *p*=0.078	25/48 (52%) 79.2% *p*=0.185	33/48 (69%) 100.0% *p*=0.030	5/72 (7%) 100.0% *p*=0.450
Harderian gland	Adenoma	6/50 (12%) 14.8% *p*<0.001[d]	7/50 (14%) 17.3% *p*=0.497	8/50 (16%) 25.8% *p*=0.395	19/50 (38%) 63.4% *p*<0.001	30/50 (60%) 95.4% *p*<0.001	6/73 (8%) 100.0% *p*=0.264
	Carcinoma	0/50 (0%) 0.0% *p*=0.720[d]	1/50 (2%) 2.6% *p*=0.522	1/50 (2%) 4.2% *p*=0.425	3/50 (6%) 11.7% *p*=0.086	2/50 (4%) 6.3% *p*=0.352	0/73 (0%) 0.0% NA
	Adenoma or carcinoma	6/50 (12%) 14.8% *p*<0.001[d]	7/50 (14%) 17.3% *p*=0.497	9/50 (18%) 29.5% *p*=0.217	20/50 (40%) 64.9% *p*<0.001	31/50 (62%) 95.5% *p*<0.001	6/73 (8%) 100.0% *p*=0.002
Preputial gland	Carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	5/50 (10%) 45.7% *p*<0.001	0/73 (0%) 0.0% NA

[a]Overall rate: number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the *p* values associated with the trend test. Beneath the dosed group incidence are the *p* values corresponding to pairwise comparison between
the control and dosed groups. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.

NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

Table 6-8. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (lymphocytic, mixed, NOS, or undifferentiated cell type)	6/50 (12%)[a] 14.6%[b] *p*<0.001[c]	12/50 (24%) 34% *p*=0.068	11/50 (22%) 38.7% *p*=0.029	7/50 (14%) 35.9% *p*=0.055	9/50 (18%) 39.7% *p*<0.001	32/80 (40%) 70.8% *p*<0.001
	Histiocytic sarcoma	3/50 (6%) 6.9% *p*<0.001[c]	2/50 (4%) 4.5% *p*=0.518N	7/50 (14%) 20.0% *p*=0.077	4/50 (8%) 17.7% *p*=0.195	7/50 (14%) 28.1% *p*=0.002	4/80 (5%) 10.3% *p*=0.038
	Malignant lymphoma or histiocytic sarcoma	9/50 (18%) 20.5% *p*<0.001[c]	14/50 (28%) 37.0% *p*=0.136	18/50 (36%) 52.1% *p*=0.005	11/50 (22%) 47.2% *p*=0.021	16/50 (32%) 56.7% *p*<0.001	36/80 (45%) 73.9% *p*<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	1/49 (2%) 4.8% *p*=0.392	21/50 (42%) 100.0% *p*<0.001	23/80 (29%) 100.0% *p*<0.001
Lungs	Alveolar/bronchiolar adenoma	4/50 (8%) 10.5% *p*=0.002[d]	11/50 (22%) 30.9% *p*=0.039	12/50 (24%) 40.7% *p*=0.013	17/50 (34%) 64.8% *p*<0.001	14/49 (29%) 100.0% *p*=0.002	17/78 (22%) 100.0% *p*=0.010
	Alveolar/bronchiolar adenocarcinoma or carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	5/50 (10%) 13.3% *p*=0.029	11/50 (22%) 42.9% *p*<0.001	9/50 (18%) 40.8% *p*<0.001	19/49 (39%) 100.0% *p*<0.001	8/78 (10%) 100.0% *p*<0.001
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	4/50 (8%) 10.5% *p*<0.001[c]	15/50 (30%) 39.5% *p*=0.004	19/50 (38%) 63.7% *p*<0.001	24/50 (48%) 78.5% *p*<0.001	25/49 (51%) 100.0% *p*<0.001	22/78 (28%) 100.0% *p*<0.001
Forestomach	Squamous cell papilloma	0/50 (0%) 0.0% *p*<0.001[d]	0/50 (0%) 0.0% NA	2/50 (4%) 8.3% *p*=0.149	1/50 (2%) 9.1% *p*=0.260	3/50 (6%) 100.0% *p*=0.078	16/80 (20%) 100.0% *p*=0.002
	Squamous cell carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	1/50 (2%) 4.2% *p*=0.414	1/50 (2%) 8.3% *p*=0.277	1/50 (2%) 3.8% *p*=0.374	6/80 (8%) 70.5% *p*<0.001
	Squamous cell papilloma or carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	3/50 (6%) 12.5% *p*=0.056	2/50 (4%) 16.7% *p*=0.044	4/50 (8%) 100.0% *p*=0.001	22/80 (28%) 100.0% *p*<0.001
Liver	Hepatocellular adenoma	11/49 (22%) 29.7% *p*=0.599N	10/49 (20%) 27.8% *p*=0.531N	9/50 (18%) 30.3% *p*=0.519N	14/48 (28%) 65.8% *p*=0.025	15/50 (29%) 89.0% *p*=0.009	1/80 (1%) 100.0% *p*=0.505
	Hepatocellular carcinoma	4/49 (8%) 10.3% *p*=0.178[d]	6/49 (12%) 14.5% *p*=0.381	8/50 (16%) 25.0% *p*=0.141	9/50 (18%) 39.9% *p*=0.066	8/50 (16%) 82.7% *p*=0.006	1/80 (1%) 12.5% *p*=0.910

Table 6-8. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks (continued).

Target organ	Neoplastic lesion	Concentration (ppm)					
		0	6.25	20	62.5	200	625
Liver (continued)	Hepatocellular adenoma or carcinoma	15/49 (31%) 39.3% *p*=0.497[d]	14/49 (29%) 34.3% *p*=0.504N	15/50 (30%) 45.5% *p*=0.441	19/50 (38%) 74.8% *p*=0.027	16/50 (32%) 91.7% *p*=0.008	2/80 (3%) 100.0% *p*=0.302
Ovary	Benign granulosa cell tumor	1/49 (2%) 2.8% *p*=0.030[d]	0/49 (0%) 0.0% *p*=0.517N	1/48 (2%) 3.2% *p*=0.735	6/50 (12%) 28.5% *p*=0.026	6/50 (12%) 100.0% *p*=0.020	6/79 (8%) 27.1% *p*=0.303
	Malignant granulosa cell tumor	0/49 (0%) 0.0% *p*=0.068[d]	0/49 (0%) 0.0% NA	0/48 (0%) 0.0% NA	3/50 (6%) 19.3% *p*=0.046	2/50 (4%) 54.2% *p*=0.037	0/79 (0%) 0.0% NA
	Benign or malignant granulosa cell tumor	1/49 (2%) 2.8% *p*=0.006[d]	0/49 (0%) 0.0% *p*=0.517N	1/48 (2%) 3.2% *p*=0.735	9/50 (18%) 42.9% *p*=0.001	8/50 (16%) 100.0% *p*=0.001	6/79 (8%) 27.1% *p*=0.303
Mammary gland	Adenoacanthoma	0/50 (0%) 0.0% *p*=0.025[c]	1/50 (2%) 2.9% *p*=0.489	2/50 (4%) 7.7% *p*=0.152	6/50 (12%) 32.5% *p*<0.001	4/50 (8%) 13.6% *p*=.021	0/80 (0%) 0.0% NA
	Carcinoma	0/50 (0%) 0.0% *p*<0.001[c]	2/50 (4%) 5.8% *p*=0.221	2/50 (4%) 5.7% *p*=0.192	6/50 (12%) 16.2% *p*=0.008	11/50 (22%) 39.1% *p*<0.001	12/80 (15%) 100.0% *p*<0.001
	Malignant mixed tumor	0/50 (0%) 0.0% *p*<0.001[c]	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	0/50 (0%) 0.0% NA	4/80 (5%) 29.4% *p*=0.003
Harderian gland	Adenoma	8/50 (16%) 20.8% *p*=0.046[d]	10/50 (20%) 29.2% *p*=0.356	6/50 (12%) 20.7% *p*=0.511N	15/50 (30%) 61.0% *p*=0.016	20/50 (40%) 89.3% *p*=0.001	9/80 (11%) 45.2% *p*=0.176
	Carcinoma	0/50 (0%) 0.0% *p*=0.873N[d]	1/50 (2%) 2.7% *p*=0.493	1/50 (2%) 2.3% *p*=0.631	0/50 (0%) 0.0% NA	1/50 (2%) 50.0% *p*=0.085	0/80 (0%) 0.0% NA
	Adenoma or carcinoma	8/50 (16%) 20.8% *p*=0.061[d]	10/50 (20%) 29.2% *p*=0.356	7/50 (14%) 22.5% *p*=0.575N	15/50 (30%) 61.0% *p*=0.016	20/50 (40%) 89.3% *p*=0.001	9/80 (11%) 45.2% *p*=0.176

[a]Overall rate; number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. Beneath the control incidence are the *p* values associated with the trend test. Beneath the dosed group incidence are the *p* values corresponding to pairwise comparison between
the control and dosed groups. The life table analysis regards neoplasm in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.
NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.
Source: NTP, 1993.

Table 6-9. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months[a].

Target organ	Neoplastic lesion		Concentration (ppm)					
			0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (histiocytic, lymphocytic, mixed, NOS, or undifferentiated)	9 months						1/10
		15 months						2/7
Heart	Hemangiosarcoma	9 months						
		15 months					1/10	3/7
Lungs	Alveolar/ bronchiolar adenoma, adenocarcinoma, or carcinoma	9 months	1/10	1/1	1/2	0/10	2/10	3/10
		15 months				2/10	4/10	5/7
Forestomach	Squamous cell papilloma or carcinoma	9 months						1/10
		15 months					1/10	3/7
Liver	Hepatocellular adenoma or carcinoma	9 months	4/10	0/10	1/10	0/10	1/10	1/10
		15 months	2/10	1/10	4/10	3/10	4/10	5/7
Harderian gland	Adenoma or carcinoma	9 months						
		15 months			2/10	4/10	3/10	3/7

[a]Overall rate: number of tumor-bearing animals/number of animals examined.

NA = not applicable; no tumors in these groups.

NOS = not otherwise specified.

N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

Table 6-10. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months[a].

Target organ	Neoplastic lesion		Concentration (ppm)					
			0	6.25	20	62.5	200	625
All organs	Malignant lymphoma (lymphocytic, mixed, NOS, or undifferentiated cell type)	9 months						1/8
		15 months	1/10				1/10	0/2
Heart	Hemangiosarcoma	9 months						
		15 months					1/10	2/2
Lungs	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	9 months					2/10	1/8
		15 months				3/10	3/10	1/2
Forestomach	Squamous cell papilloma or carcinoma	9 months						
		15 months				1/10	2/10	1/2
Liver	Hepatocellular adenoma or carcinoma	9 months						
		15 months	1/10	1/10	0/10	1/10	3/10	1/2
Ovary	Benign or malignant granulosa cell tumor	9 months					1/10	
		15 months				1/10	4/10	1/2
Mammary gland	Adenoacanthoma, adenocarcinoma, carcinoma, or malignant mixed tumor	9 months						
		15 months					2/10	1/2
Harderian gland	Adenoma or carcinoma	9 months						1/5
		15 months	2/9	1/1	1/1	1/10	3/10	0/2

[a]Overall rate; number of tumor-bearing animals/number of animals examined.
NA = not applicable; no tumors in these groups.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

As in the previous study (NTP, 1984), exposure of mice to 1,3-butadiene was associated with the development of malignant lymphocytic lymphomas and to a lesser extent with histiocytic sarcomas. The incidence of malignant lymphomas, particularly lymphocytic lymphomas, was significantly increased in males and females exposed to 625 ppm and in females exposed to 20 and 200 ppm (survival-adjusted) compared with controls. In addition, there were significant exposure-response trends ($p<0.001$) in both sexes. The lymphocytic lymphomas were well differentiated and occurred as early as week 23, peaking before the 15-month interim evaluation. Many organs, particularly the spleen, lymph nodes, liver, lung, and kidney, were affected in mice with lymphocytic lymphoma; however, the thymus was involved in most mice and was the primary organ affected in some. The lymphocytic lymphomas consisted of uniform populations of small- to medium-sized lymphocytes, whereas the mixed and undifferentiated lymphomas generally consisted of more heterogeneous populations of lymphocytes with pleomorphism and atypia. Other histological types of malignant lymphomas (mixed and undifferentiated), commonly associated with the spontaneous lymphomas in aging $B6C3F_1$ mice, were seen at low incidence in some groups. The incidences of histiocytic sarcoma were significantly increased in males and females exposed to 200 and 625 ppm and in males exposed to 62.5 ppm. The histiocytic sarcomas (previously referred to as reticulum cell sarcomas or type A sarcomas) were large and monomorphic, with dark basophilic nuclei and relatively abundant eosinophilic cytoplasm.

Hemangiosarcomas of the heart were observed in male (at $\geq$20 ppm) and female (at $\geq$62.5 ppm) mice exposed to 1,3-butadiene for 2 years. The incidences of hemangiosarcomas of the heart were significantly increased in male mice exposed to $\geq$62.5 ppm and in female mice exposed to $\geq$200 ppm. There was a significant exposure-response trend in both sexes. The cardiac hemangiosarcomas were observed in all ventricular locations, but were more frequent in the left ventricular wall. Typical hemangiosarcomas had solid foci of anaplastic, pleomorphic spindle cells at the center with a loose arrangement at the periphery. They were occasionally multifocal and frequently coexisted with foci of endothelial hyperplasia distant and separate from the main neoplasm. Hemangiosarcomas of the heart are considered uncommon in untreated $B6C3F_1$ mice (none were observed in 573 male and 558 female historical controls in NTP inhalation studies). In male mice, the lower incidence of cardiac hemangiosarcoma at 625 ppm compared with that at 200 ppm was attributed to the early mortality from induction of lethal lymphocytic lymphoma at 625 ppm. The time-to-tumor detection for all hemangiosarcomas of the heart ranged from 682 days at 20 ppm to 289 days at 625 ppm for males and from 649 days at 20 ppm to 307 days at 625 ppm for females. When hemangiosarcomas occurred in multiple organs, the cardiac neoplasms were usually designated as primary, because the incidence of hemangiosarcomas was highest in the heart and the earliest lesions occurred in the heart.

However, it could not be determined with certainty if the hemangiosarcomas observed in other organs were metastases or primary neoplasms. Subcutaneous, splenic, and hepatic hemangiosarcomas that were found in the absence of cardiac hemangiosarcomas may reflect the development of spontaneous vascular neoplasms known to occur in $B6C3F_1$ mice.

Exposure of mice to 1,3-butadiene was also associated with an increased incidence of pulmonary neoplasms in male and female mice. Although the incidence of alveolar/bronchiolar adenomas was not significantly increased in male mice in the 2-year study, the combined incidences of alveolar/bronchiolar adenocarcinomas and carcinomas and the combined incidences of the benign and malignant pulmonary neoplasms were significantly increased at 62.5, 200, and 625 ppm. In female mice, the incidences of the benign and malignant neoplasms analyzed separately or together were significantly increased in all exposure groups compared with controls. Thus, even at 6.25 ppm, 1,3-butadiene was carcinogenic to female $B6C3F_1$ mice. The lower incidence of lung neoplasms at 625 ppm compared with the incidence at 200 ppm was attributed to the high rate of early deaths due to the competing risks of lymphocytic lymphoma in female mice exposed to 625 ppm. There was a significant exposure-response trend for combined adenomas and carcinomas in both sexes. The time to tumor detection for lung tumors combined ranged from 587 days at 6.25 ppm to 251 days at 625 ppm for males, and from 519 days at 6.25 ppm to 275 days at 625 ppm for females. The spectrum of lung lesions ranged from alveolar epithelial hyperplasia to adenomas, carcinomas, and adenocarcinomas. Histologically, the alveolar/bronchiolar adenomas exhibited distortion of the alveolar structure through the formation of complex, irregular papillary patterns; the alveolar/bronchiolar carcinomas were similar, but consisted of heterogeneous cell populations with various degrees of cellular pleomorphism and atypia. The adenocarcinomas were larger, highly anaplastic neoplasms, often accompanied by hemorrhage or necrosis.

In the forestomach, significant increases in squamous cell papillomas and carcinomas combined were observed in male mice exposed to $\geq$200 ppm and in female mice exposed to $\geq$62.5 ppm compared with controls. There was a significant exposure-response trend for papillomas and carcinomas combined in both sexes. The combined incidence of squamous cell papillomas and carcinomas of the forestomach (males, 4/575 [0.7%]; females, 9/561 [1.6%]) for historical controls suggests that these lesions are relatively uncommon in $B6C3F_1$ mice.

Increased incidences of hepatocellular adenomas and carcinomas were also seen in 1,3-butadiene-exposed mice (Tables 6-7 and 6-8). The hepatocellular adenomas were discrete, expansile masses; the carcinomas were larger than the adenomas and consisted of markedly disorganized hepatocytes. The low incidence of liver neoplasms observed in males and females at 625 ppm probably reflects increased early deaths from malignant lymphoma. Hepatocellular adenomas and carcinomas are common neoplasms in $B6C3F_1$ mice, occurring in 196/572 (34%)

of male and 87/558 (15.6%) of female historical controls in NTP inhalation studies. The data suggest that 1,3-butadiene has only a weak tumorigenic effect in the livers of male and female mice. However, a chemical-related effect is supported by the detection of an activated K-ras oncogene in liver neoplasms obtained from mice exposed to 1,3-butadiene (Goodrow et al., 1990). According to Reynolds et al. (1987), activated K-ras oncogene had never been detected in liver neoplasms from untreated $B6C3F_1$ mice.

Although a variety of neoplasms were seen in the ovaries of female mice, only benign and malignant granulosa cell tumors were definitely attributed to exposure to 1,3-butadiene (Table 6-5). The ovarian granulosa cell tumors varied from small benign tumors to large cystic tumors with thick trabeculae and spaces filled with blood or clear fluid. The overall historical control incidence at NTP for benign and malignant granulosa cell tumors each was 1/548 (0.2%).

Increased incidences of mammary gland neoplasms were seen in female mice exposed to $\geq$62.5 ppm 1,3-butadiene. Mammary tumors included adenoacanthomas, adenocarcinomas, and malignant mixed tumors, the latter occurring only at 625 ppm. The mammary gland tumors combined exhibited a significant exposure-response relationship. The adenoacanthomas were considered variants of adenocarcinomas that have prominent squamous differentiation. The malignant mixed tumors consisted of epithelial components arranged in glandlike structures and anaplastic spindle-cell components. Mammary gland adenocarcinomas and adenoacanthomas were considered uncommon in female $B6C3F_1$ mice; the overall historical incidence at NTP was 21/561 (3.7%) for carcinomas and 1/561 (0.2%) for adenoacanthomas in female control mice.

The Harderian gland was identified as another site of 1,3-butadiene-induced neoplasms in male and female mice (Tables 6-7 and 6-8), with significant exposure-related increases in adenomas at 62.5 and 200 ppm and a low incidence of carcinomas in males exposed to 6.25, 20, 62.5, or 200 ppm. The low incidence of Harderian gland tumors at 625 ppm was attributed to early deaths due to lymphocytic lymphoma, which precluded the development of Harderian gland tumors. The investigators noted that the occurrence of Harderian gland carcinomas in mice, particularly males, is unusual. The overall incidence of Harderian gland carcinomas was 2/575 (0.3%) in male and 3/561 (0.5%) in female historical controls at NTP. The 2-year historical incidence of adenomas and carcinomas (combined) of the Harderian gland for control groups in NTP inhalation studies was 25/575 (4.3%) for males and 13/561 (2.3%) for females.

Preputial gland carcinomas, also considered to be rare neoplasms in $B6C3F_1$ mice, were seen in five males ($p<0.05$) exposed to 200 ppm (none were reported in one survey of NTP historic control data). These tumors were also thought to be exposure-related lesions. Some preputial carcinomas were composed of large eosinophilic epithelial cells that were well differentiated; more frequently, the carcinomas had necrotic cores and a thin layer of very

anaplastic basophilic epithelial cells that aggressively invaded surrounding tissue and blood vessels.

Renal tubule adenomas were seen in 2/50 females exposed to 200 ppm 1,3-butadiene and in 1/50, 3/48, and 1/49 of males exposed to 6.25, 62.5, and 200 ppm, respectively. At the 15-month evaluation, renal tubular adenoma occurred in 1/7 males exposed to 625 ppm. The historical incidence of spontaneous renal tubule adenomas in untreated control groups in NTP inhalation studies was 1/571 (0.2%) for males and 0/559 (0.0%) for females. Histologically, the renal tubule adenomas contained multiple dilated tubules separated by thin connective tissue septa. These renal lesions were probably related to exposure to 1,3-butadiene in males and possibly related to exposure in females.

One neurofibrosarcoma of the subcutaneous tissue was observed in two females exposed to 625 ppm at the 15-month evaluation. In the 2-year study, the combined incidences of neurofibrosarcomas and sarcomas of the subcutaneous tissue were significantly increased in female mice exposed to 62.5 ppm (p=0.017), 200 ppm (p=0.002), and 625 ppm (p=0.013) by the life table test. Subcutaneous tissue sarcomas (all types) were considered uncommon spontaneous neoplasms; the historical incidence was 2/561 (0.4%) for female controls at NTP, suggesting that these subcutaneous tissue neoplasms may have been exposure-related. The historical incidence for NTP inhalation studies was not reported.

One adenoma and one carcinoma of the Zymbal's gland were seen in females exposed to 625 ppm; one adenoma also occurred in a concurrent control male mouse, but none were reported in historical controls. The report indicated that these Zymbal's gland neoplasms may be related to 1,3-butadiene exposure.

Carcinomas of the small intestine, another uncommon tumor in the $B6C3F_1$ mouse, were seen in two females exposed to 6.25 ppm and in one female exposed to 62.5 ppm. One carcinoma each was seen in one male each exposed to 6.25, 20, or 62.5 ppm, and in two males exposed to 200 ppm. The relationship of these neoplasms to exposure to 1,3-butadiene could not be determined; however, controls did not exhibit proliferative lesions of the intestine.

In supplemental analyses, the authors performed a "Poly-3" quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989) as an alternative to the logistic regression analyses, whose sensitivity was reduced by the decreased survival in the higher exposure groups. For tumor sites related to butadiene exposure, the "Poly-3" test detected significant responses in some of the exposure groups that had not been detected by the logistic regression analyses. The overall results were consistent with those already presented.

The authors also fitted a modified Weibull model (Portier et al., 1986) to the "Poly-3" survival-adjusted tumor rates to determine the shape parameters for the exposure-response relationships. About half of the tumor sites associated with butadiene exposure had exposure-

response relationships consistent with a linear model (i.e., shape parameter of 1). Most of the other tumor sites exhibited supralinear exposure-response relationships (i.e., steep slope in low-exposure region; shape parameter significantly <1). These sites were the liver in male mice, the mammary gland in females, and the Harderian gland and lung in both sexes. Only the malignant lymphoma in males and heart hemangiosarcoma in females had a shape parameter significantly greater than 1, suggestive of a sublinear exposure-response relationship.

6.3.2.2. *2-Year Stop-Exposure Study (NTP, 1993)*

An additional study with $B6C3F_1$ mice, referred to as "stop-exposure study," was conducted to assess the relationship between exposure level and duration of exposure to outcome of 1,3-butadiene carcinogenicity. Groups of 50 male mice were exposed 6 h/day, 5 days/week at concentrations of (a) 200 ppm for 40 weeks, (b) 625 ppm for 13 weeks, (c) 312 ppm for 52 weeks, or (d) 625 ppm for 26 weeks. After the exposures were stopped, the animals were placed in control chambers for the remainder of the 103-week studies. The total exposures to 1,3-butadiene (concentration × duration of exposure) were approximately 8,000 ppm·weeks for groups exposed to 200 ppm for 40 weeks or 625 ppm for 13 weeks; the total exposures were approximately 16,000 ppm·week for groups exposed to 312 ppm for 52 weeks or 625 ppm for 26 weeks. No additional controls were included for these studies, because they were run concurrently with the 2-year studies.

Using the stop-exposure protocol, inhalation exposure to 1,3-butadiene had no effect on mean body weights. However, exposure to 1,3-butadiene markedly reduced survival in all stop-exposure groups as a result of the development of neoplasms, particularly malignant lymphomas and hemangiosarcomas of the heart (Figure 6-3). A comparison of the two groups receiving total exposures of 8,000 ppm·weeks showed that the survival of mice exposed to 625 ppm (13 weeks) was similar to that of mice exposed to 200 ppm (40 weeks). By contrast, in the groups exposed to 16,000 ppm·weeks, survival of mice exposed to 625 ppm (26 weeks) was significantly lower than that of mice exposed to 312 ppm (52 weeks).

Neoplasms induced in the stop-exposure studies are summarized in Table 6-8. Overall, the data show that exposure of male mice to 1,3-butadiene using the stop-exposure protocol induced neoplasms at the same sites as those observed in the 2-year study.

Lymphocytic lymphomas of thymic origin occurred at a markedly increased incidence in mice exposed to 625 ppm for 13 or 26 weeks. According to the life table test, the incidence of lymphocytic lymphoma was also significantly increased in mice exposed to 200 ppm for 40 weeks or 312 ppm for 52 weeks. The incidence of histiocytic sarcomas was significantly increased (life table test) in mice in all stop-exposure groups as well.

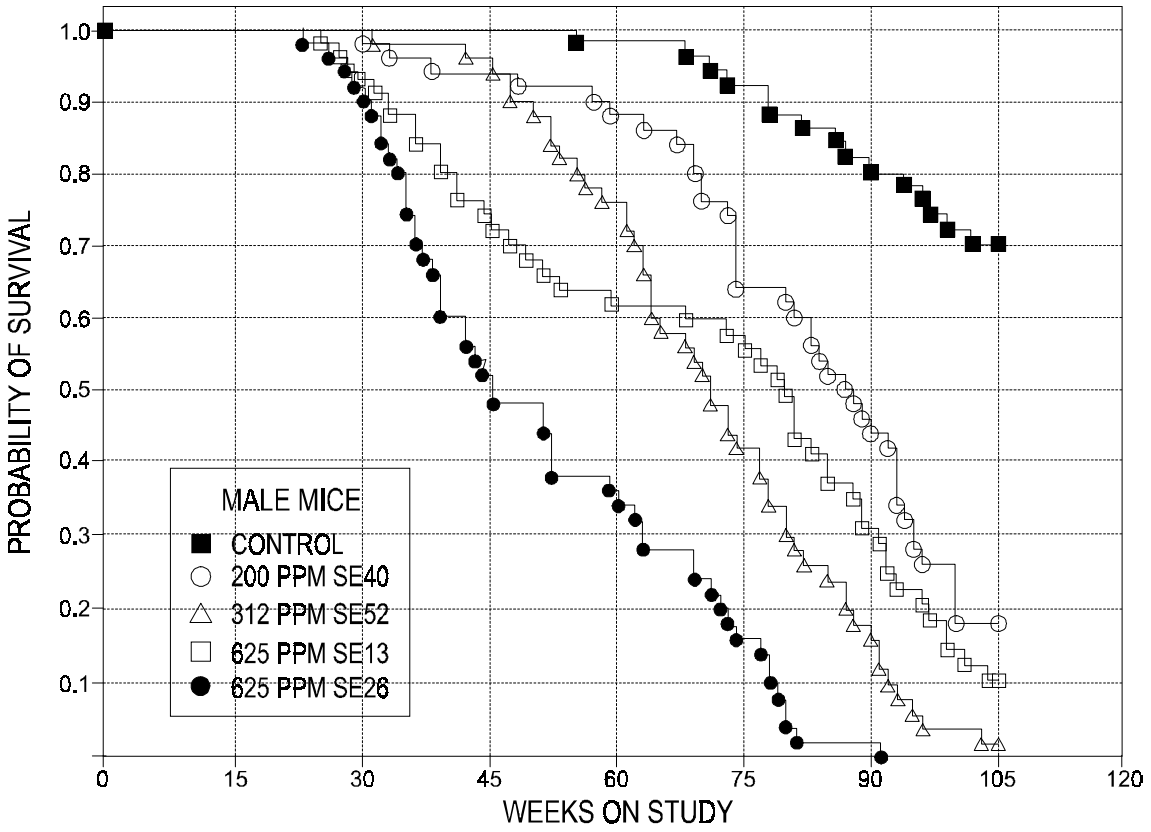

Figure 6-3. Kaplan-Meier survival curves for male mice in the stop-exposure inhalation of 1,3-butadiene.

Source: NTP, 1993.

The lower incidences of lymphocytic lymphomas at 200 ppm (40 weeks) and 312 ppm (52 weeks) compared to 625 ppm for 13 and 26 weeks, respectively, demonstrate that the concentration of 1,3-butadiene is a greater contributing factor in the development of this lesion than the duration of exposure, i.e., a high concentration for a short duration is more effective than a lower concentration of longer duration. A comparison of the 200 ppm (40 weeks) versus the 625 ppm (13 weeks) and of the 312 ppm (52 weeks) versus the 625 ppm (26 weeks) lymphocytic lymphoma results using a life table test confirms that the higher concentration/shorter duration regimen is significantly more effective than the lower concentration/longer duration regimen within each cumulative exposure grouping (p=0.005 for 8,000 ppm·weeks and p<0.001 for 16,000 ppm·weeks) after survival differences are taken into account.

As observed in the 2-year study, lymphocytic lymphomas occurred very early after exposure started: as early as 23 weeks in the group exposed to 625 ppm for 26 weeks and as early as 24 weeks in the group exposed to 625 ppm for 13 weeks. This lesion accounted for 24 and 17, respectively, of the first 25 deaths occurring in these groups by weeks 45 and 79, respectively. Therefore, early deaths due to lymphocytic lymphoma would have a tremendous negative effect on the incidence of late-developing lesions.

Hemangiosarcomas of the heart, which also accounted for some of the early deaths, were significantly increased in most stop-exposure groups compared with the controls. The highest incidence, which was about twice as high as that of other groups, occurred in the group exposed to 312 ppm, followed by the groups exposed to 200 ppm and 625 ppm (26 weeks). The lowest incidence occurred in the group exposed to 625 ppm for 13 weeks. Hemangiosarcomas appeared at about 9 months in the 200, 312, and 625 ppm (26-week) stop-exposure groups. Comparison (life table test) of groups having the same total exposures showed that the incidences of hemangiosarcomas in mice exposed to 625 ppm were significantly lower than that of the corresponding group exposed to 312 ppm (p=0.032) but not 200 ppm. The incidences of hemangiosarcomas in both 625-ppm stop-exposure groups were higher than that in the 625-ppm 2-year exposure group, probably because of longer survival of the stop-exposure groups.

The incidences of neoplastic lesions of the lung (alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma) were significantly elevated in each exposure group. The highest incidence occurred in the 200-ppm stop-exposure group, followed by the 312-, 625- (13 weeks), and 625-ppm (26 weeks) groups. The adenomas developed after week 47 and the adenocarcinomas and carcinomas developed after week 53; the late appearance of these lesions relative to lymphocytic lymphomas probably accounted for the lowest incidence of lung neoplasms occurring in 625 ppm (26 weeks) group. A life table analysis suggested the incidence of lung lesions in the 625 ppm (26 weeks) group was significantly *greater* than in the 312 ppm

(52 weeks) group (p=0.013), but no difference was detected between the 200 ppm (40 weeks) and 625 ppm (13 weeks) groups.

Mice exposed to 200 ppm 1,3-butadiene for 40 weeks had significantly increased incidences of hepatocellular adenomas and adenomas/carcinomas combined; the incidences of hepatocellular carcinomas analyzed alone were not significantly increased. Exposure to 1,3-butadiene at 312 ppm or 625 ppm (13 or 26 weeks) did not increase the incidence of hepatocellular neoplasms of any type. The earliest detection of these neoplasms was 67 weeks for the 625 ppm (13 weeks), 57 weeks for the 200 ppm, 47 weeks for the 312 ppm, and 45 weeks for the 625 ppm (26 weeks) stop-exposure groups. A logistic regression analysis found no differences between the 200 ppm and 625 ppm (13 weeks) or the 312 ppm and 625 ppm (26 weeks) groups.

A low incidence of squamous cell papillomas of the forestomach occurred in each of the groups, and squamous cell carcinomas were seen in mice exposed to 312 ppm or 625 ppm for 13 and 26 weeks. The incidences of squamous cell papillomas were not significantly greater than those of controls for any group, but the incidences of squamous cell carcinomas were significantly greater by the life table test, which is considered to be the appropriate test (NTP, 1993) for these fatal neoplasms. A life table analysis also revealed a statistically significant exposure-rate effect for the squamous cell carcinomas in both of the total exposure groupings (p=0.019 for 8,000 ppm·weeks and p=0.015 for 16,000 ppm·weeks), suggesting that the higher concentration/shorter duration exposures were more potent.

The incidence of adenomas of the Harderian gland was significantly greater in each exposure group than in the controls by a logistic regression test. A low incidence of Harderian gland carcinomas occurred in mice exposed to 200 ppm for 40 weeks (not significant), 312 ppm for 52 weeks (p=0.006), and 625 ppm for 13 weeks (not significant). No Harderian gland carcinomas were observed in the controls or in mice exposed to 625 ppm for 26 weeks. A logistic regression analysis did not detect any exposure-rate effects.

Other neoplasms occurred at low incidence in the stop-exposure studies; they were considered to be related to exposure because of their low spontaneous incidences in NTP historical control male mice. These neoplasms occurred in the kidney, brain, Zymbal's gland, and preputial gland. The incidences of these neoplasms are also summarized in Table 6-11.

Renal tubule neoplasms occurred in historical male control mice; the range was 0 to 1%. The small number of these neoplasms in each of the exposure groups are considered to be related to administration of 1,3-butadiene because the incidences were greater than the upper range for historical controls.

Table 6-11. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study.

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		103	40	13	52	26
Total exposure (ppm·weeks)		0	8,000	8,000	16,000	16,000
Target organ	Neoplastic lesion					
Hematopoietic	Lymphocytic malignant lymphoma	2/50[a] (4%) 4.7%[b] --	6/50 (12%) 26.7% p=0.033[c]	17/50 (34%) 35.8% p<0.001	4/50 (8%) 100.0% p=0.034	30/50 (60%) 81.5% p<0.001
	Lymphoma (mixed or NOS)	2/50 (4%) 5.3% --	2/50 (4%) 7.8% p=0.382[c]	5/50 (10%) 34.8% p=0.010	4/50 (8%) 58.0% p=0.005	3/50 (6%) 43.3% p=0.002
	Histiocytic sarcoma	0/50 (0%) 0.0% --	5/50 (10%) 21.3% p=0.006[c]	2/50 (4%) 28.9% p=0.011	7/50 (14%) 43.0% p<0.001	2/50 (4%) 15.6% p=0.036
	Malignant lymphoma or histiocytic sarcoma	4/50 (8%) 9.8% --	13/50 (26%) 46.8% p<0.001[c]	24/50 (48%) 72.1% p<0.001	15/50 (30%) 100.0% p<0.001	35/50 (70%) 91.2% p<0.001
	Malignant lymphoma (lymphocytic, mixed, or NOS)	4/50 (8%) 9.8% --	8/50 (16%) 32.4% p=0.023[c]	22/50 (44%) 58.2% p<0.001	8/50 (16%) 100.0% p<0.001	33/50 (66%) 89.5% p<0.001
Heart	Hemangiosarcoma	0/50 (0%) 0.0% --	15/50 (30%) 76.2% p<0.001[c]	7/50 (14%) 61.8% p<0.001	33/50 (66%) 100.0% p<0.001	13/50 (26%) 100.0% p<0.001
Lungs	Alveolar/bronchiolar adenoma	18/50 (36%) 46.9% --	24/50 (48%) 94.3% p=0.015[d]	17/50 (34%) 85.3% p=0.044	26/50 (52%) 100.0% p=0.001	12/50 (24%) 100.0% p<0.001
	Alveolar/bronchiolar adenocarcinoma or carcinoma	5/50 (10%) 14.3% --	22/50 (44%) 89.5% p<0.001[c]	18/50 (36%) 87.7% p<0.001	16/50 (32%) 100.0% p<0.001	11/50 (22%) 100.0% p<0.001

Table 6-11. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued).

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		103	40	13	52	26
Total exposure (ppm·weeks)		0	8,000	8,000	16,000	16,000
Target organ	Neoplastic lesion					
Lungs (continued)	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	21/50 (42%) 54.9% --Sum.	36/50 (72%) 100.0% $p<0.001^{c}$Sum.	28/50 (56%) 100.0% $p<0.001$Sum.	32/50 (64%) 100.0% $p<0.001$Sum.	17/50 (34%) 100.0% $p<0.001$
Liver	Hepatocellular adenoma	13/50 (26%) 32.1% --	27/49 (55%) 91.1% $p<0.001^{d}$Sum.	19/49 (39%) 91.1% *pSum.*=0.042Sum.	19/50 (38%) 100.0% *pSum.*=0.045Sum.	11/50 (22%) 100.0% $p=0.284$Sum.
	Hepatocellular carcinomaSum.	11/50 (22%) 26.0% --Sum.	14/49 (29%) 50.3% $p=0.530^{d}$Sum.	14/49 (29%) 90.9% $p=0.142$Sum.	10/50 (20%) 74.6% $p=0.453$Sum.	4/50 (8%) 50.5% $p=0.393$Sum.
	Hepatocellular adenoma or carcinoma	21/50 (42%) 47.9% --	33/49 (67%) 93.4% $p=0.004^{d}$	24/49 (49%) 94.4% $p=0.063$	24/50 (48%) 100.0% $p=0.169$	13/50 (26%) 100.0% $p=0.561$
Forestomach	Squamous cell papilloma	1/50 (2%) 2.5% --	3/50 (6%) 21.4% $p=0.195^{d}$	4/50 (8%) 28.3% $p=0.260$	4/50 (8%) 100.0% $p=0.181$	4/50 (8%) 20.1% $p=0.301$
	Squamous cell carcinoma	0/50 (0%) 0.0% --	0/50 (0%) 0.0% NA	4/50 (8%) 51.6% $p<0.001^{d}$	5/50 (10%) 33.1% $p<0.001$	6/50 (12%) 40.9% $p<0.001$
	Squamous cell papilloma or carcinoma	1/50 (2%) 2.5% --	3/50 (6%) 21.4% $p=0.065^{c}$	7/50 (14%) 56.6% $p<0.001$	9/50 (18%) 100.0% $p<0.001$	10/50 (20%) 52.8% $p<0.001$
Harderian gland	Adenoma	6/50 (12%) 14.8% --	26/50 (52%) 87.9% $p<0.001^{d}$	20/50 (40%) 94.3% $p=0.001$	28/50 (56%) 100.0% $p<0.001$	13/50 (26%) 100.0% $p=0.046$

Table 6-11. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued).

Parameters		Concentration (duration of exposure)				
		0 ppm	200 ppm	625 ppm	312 ppm	625 ppm
Duration of exposures (weeks)		103	40	13	52	26
Total exposure (ppm·weeks)		0	8,000	8,000	16,000	16,000
Target organ	Neoplastic lesion					
Harderian gland (continued)	Carcinoma	0/50 (0%) 0.0% --	2/50 (4%) 5.6% *p*=0.397[d]	4/50 (8%) 38.8% *p*=0.190	2/50 (4%) 51.5% *p*=0.006	0/50 (0%) 0.0% NA
	Adenoma or carcinoma	6/50 (12%) 14.8% --	27/50 (54%) 88.3% *p*<0.001[d]	23/50 (46%) 100.0% *p*<0.001	30/50 (60%) 100.0% *p*<0.001	13/50 (26%) 100.0% *p*=0.046
Kidney	Renal tubule adenoma	0/50 (0%) 0.0% --	4/48 (8%) 17.4% *p*=0.073[d]	1/50 (2%) 14.3% *p*=0.273	3/49 (6%) 27.8% *p*=0.075	1/50 (2%) 6.3% *p*=0.731
Brain[e]	Malignant glioma	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
	Malignant neuroblastomas	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Preputial gland	Carcinoma	0/50 (0%) 0.0% --	1/50 (2%) 10.0% *p*=0.368[c]	4/50 (8%) 16.9% *p*=0.039	4/50 (8%) 100.0% *p*<0.001	3/50 (6%) 100.0% *p*=0.002
	Adenoma or carcinoma	0/50 (0%) 0.0% --	1/50 (2%) 10.0% *p*=0.368[c]	5/50 (10%) 22.9% *p*=0.013	4/50 (8%) 100.0% *p*<0.001	3/50 (6%) 100.0% *p*=0.002
Zymbal's gland	Adenoma or carcinoma	1/50 (2%) 2.9% --	1/50 (2%) 4.8% *p*=0.531[c]	2/50 (4%) 8.8% *p*=0.178	0/50 (0%) 0.0% *p*=0.998N	2/50 (4%) 37.3% *p*=0.009

[a]Overall rate, number of tumor-bearing animals/number of animals examined.
[b]Survival-adjusted rate. Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[c]Life table test. The *p* values for pairwise comparison of exposed groups with controls are beneath the exposed group incidence. N refers to negative association with control group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.
[d]Logistic regression test. This test regards the neoplasms as nonfatal.
[e]No statistical analysis.
NA = not applicable.
NOS = not otherwise specified.
N = incidence in dose group is lower than in control group.

Source: NTP, 1993.

Brain neoplasms, including two neuroblastomas and two malignant gliomas observed in male mice exposed to 625 ppm for 13 weeks and one malignant glioma observed in male mice exposed to 625 ppm for 26 weeks, may have been related to 1,3-butadiene exposure. Brain neoplasms are rare in untreated B6C3F_1 mice; none have been reported in 574 NTP historical control male mice. Furthermore, a low incidence of gliomas was also reported in the previous NTP (1984) study. For these reasons, the brain neoplasms are considered exposure-related lesions.

A low incidence of preputial gland carcinomas occurred in the exposed groups in the stop-exposure studies, and none were seen in controls. Compared with the incidence in concurrent controls, the combined incidences of preputial gland tumors (adenoma and carcinoma) were significant in male mice exposed to 312 ppm (52 weeks) and to 625 ppm (13 and 26 weeks) by the life table test. Preputial gland carcinomas were not reported in a survey of NTP historical control mice, further indicating that these neoplasms are probably related to exposure to 1,3-butadiene.

One male exposed to 200 ppm for 40 weeks, two males exposed to 625 ppm for 13 weeks, and two males exposed to 625 ppm for 26 weeks developed Zymbal's gland carcinomas. This lesion did not occur in male mice exposed to 312 ppm for 52 weeks; one control male, however, developed an adenoma. The combined incidence of Zymbal's gland adenomas and carcinomas in animals exposed to 625 ppm for 26 weeks was significantly increased compared with controls by the life table test. Zymbal's gland neoplasms are rare spontaneous neoplasms that had not been observed in any NTP historical controls before the only occurrence of this adenoma in the control male mice for these studies.

To summarize the results of the stop-exposure study pertaining to the relationship between exposure level and duration of exposure: For lymphocytic lymphomas, there is strong evidence that higher concentration/shorter duration exposures are more potent than lower concentration/longer duration exposures for both the 8,000 ppm·weeks and 16,000 ppm·weeks total exposure groupings. There is also some evidence for a similar exposure-rate effect for forestomach squamous cell carcinomas in both total exposure groupings. Any exposure-rate effects at other sites are less clear, especially because it is difficult to distinguish a small apparent increased potency effect of higher concentration/shorter duration exposures from an effect of longer potential postexposure follow-up times following the shorter duration exposures.

6.3.2.3. *Summary of NTP (1993) Study*

The 2-year inhalation study showed that 1,3-butadiene is a potent carcinogen in mice at all concentrations evaluated. It also demonstrated that exposure to lower concentrations of 1,3-

butadiene than those used in the previous NTP (1984) study allowed expression of neoplasms at other sites and provided clearer exposure-response relationships because of increased survival. Statistically significant increases in the incidences of malignant tumors at one or more sites occurred in male mice exposed to ≥20 ppm and in females exposed to ≥6.25 ppm 1,3-butadiene (the lowest exposure concentration used) for periods up to 103 weeks. The possibility therefore exists that lower exposure concentrations would also cause cancer in $B6C3F_1$ mice. The percentage of animals bearing malignant tumors increased from about 30% in the controls to nearly 90% in the highest exposure group, 625 ppm. Lymphocytic lymphomas, hemangiosarcomas of the heart, lung neoplasms, and neoplastic lesions of the forestomach, mammary gland, ovary, and liver, lesions identified in the NTP (1984) study, were again increased in this study. In addition, the Harderian gland and preputial gland were identified as sites of 1,3-butadiene-induced neoplasms. Tumors observed in the kidneys, skin, Zymbal's gland, and intestine may also have been related to 1,3-butadiene exposure.

The stop-exposure study demonstrated that limited exposure to 1,3-butadiene also induces neoplasms at multiple organ sites in male $B6C3F_1$ mice. Incidences of lymphocytic lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, forestomach squamous cell neoplasms, Harderian gland neoplasms, and preputial gland neoplasms were increased compared with controls after exposure to 625 ppm 1,3-butadiene for only 13 weeks. The stop-exposure study also demonstrated an apparent exposure-rate effect for the induction of lymphocytic lymphomas by 1,3-butadiene. At equivalent total exposures, the induction of lymphocytic lymphomas was greater with exposure to a higher concentration of 1,3-butadiene for a shorter time than for exposure to a lower concentration for a longer duration.

Overall, the NTP (1993) was a very well conducted study with a precise and comprehensive presentation of the data. Adequate numbers of animals of both sexes were exposed to multiple concentration levels of 1,3-butadiene for a major portion of their life span. Comprehensive histopathologic evaluations were performed and mortality and tumor incidences were analyzed statistically using multiple methods.

6.3.2.4. *1-Year Study (Irons et al., 1989; Irons, 1990)*

To elucidate the mechanism of murine leukemogenesis, Irons and co-workers compared the induction of thymic lymphomas and expression of murine leukemia virus in NIH Swiss male mice and $B6C3F_1$ male mice by exposing them to 1,250 ppm 1,3-butadiene, 6 h/day, 5 days/week for 52 weeks. Activation of an endogenous esotropic retrovirus has been associated with spontaneous lymphomas in the $B6C3F_1$ mouse. The NIH mouse strain was used because it does not express the esotropic murine leukemia viruses expressed in $B6C3F_1$ mice. The background rate for thymic lymphoma in NIH mice is nearly zero. Although there was a marked

difference between the incidence of thymic lymphoma/leukemia in $B6C3F_1$ mice (57%) and the incidence in similarly exposed NIH mice (14%), the study showed that 1,3-butadiene can induce thymic lymphomas independently of an activated retrovirus. In addition, because these studies were for only 52 weeks, they did not necessarily allow for a full response for induction of lymphomas by 1,3-butadiene.

6.4. STUDIES OF 1,3-BUTADIENE METABOLITES

The 1,3-butadiene metabolites 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane have been shown to be carcinogenic in rats when administered by skin application or subcutaneous injection (Van Duuren et al., 1963, 1966).

6.5. RELATED COMPOUNDS

1,2-Epoxybutane, a related compound that is used as a stabilizer for chlorinated hydrocarbon solvents, was administered by inhalation 6 h/day, 5 days/week for 24 months at exposure concentrations of 0, 200, or 400 ppm to F344/N rats and 0, 50, or 200 ppm to $B6C3F_1$ mice (Dunnick et al., 1988). The treatment and control groups consisted of 50 male and 50 female animals of each species. Exposure-related inflammatory, degenerative, and proliferative lesions occurred in the nasal cavity of both rats and mice. Neoplastic lesions were restricted to the respiratory tract in rats. At 400 ppm, nasal papillary adenomas were observed in seven male rats and two female rats; none were observed in controls. In male rats exposed to 400 ppm, there was also an increased incidence of alveolar/bronchiolar adenomas or carcinomas (combined) (5/50) compared with controls (0/50). No exposure-related neoplastic lesions were seen in male or female mice.

The toxicology and carcinogenicity of 4-vinyl-1-cyclohexene, a dimer of 1,3-butadiene, were reviewed in the 1985 U.S. EPA 1,3-butadiene report, based on a draft NTP report. The final NTP report (NTP, 1986) contains the same information; therefore, the data are not summarized in this update. The basic conclusion was that there was clear evidence of carcinogenicity of 4-vinyl-1-cyclohexene (by gavage) in female mice on the basis of increased ovarian neoplasms and equivocal evidence in male mice based on marginal increases of malignant lymphomas and alveolar/bronchiolar adenomas. In male mice, the sensitivity for detecting a carcinogenic response was limited by poor survival at the high exposure. In rats, there was inadequate evidence in males, at least in part because of excessive mortality, and equivocal evidence in females based on increased neoplasms of the clitoral gland.

Chloroprene (2-chloro-1,3-butadiene) and isoprene (2-methyl-1,3-butadiene) are structurally related to 1,3-butadiene. In an NTP (1998) inhalation bioassay, chloroprene produced multiple organ carcinogenic responses in rats and mice, with several sites being the same as those

targeted by 1,3-butadiene. Groups of 50 male and 50 female F344/N rats and 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 12.8, 32, or 80 ppm chloroprene 6 h/day, 5 days/week, for 2 years. Chloroprene was carcinogenic to the oral cavity, thyroid gland, and kidney of male and female rats, as well as the lung of male rats and the mammary gland (fibroadenomas) of female rats. It also produced tumors of the lung, circulatory system (hemangiomas and hemangiosarcomas), Harderian gland, and forestomach (papillomas) of male and female mice, as well as kidney tubular cell adenomas in male mice and tumors of the liver, mammary gland, skin, mesentery, and Zymbal's gland in female mice. Melnick et al. (1999) calculated EC_{10}s for both 1,3-butadiene and chloroprene for the various treatment-related tumors and concluded that the overall carcinogenic potency of chloroprene and 1,3-butadiene appeared to be similar in mice. In particular, female lung tumors, which appear to be the most sensitive tumor response in mice from both 1,3-butadiene and chloroprene exposures, yielded the same EC_{10}s for both 1,3-butadiene and chloroprene. With respect to rats, chloroprene has greater carcinogenic potency in the F344/N rat than 1,3-butadiene has in the Sprague-Dawley rat.

In a different inhalation bioassay (Trochimowicz et al., 1998), male and female Wistar rats and Syrian golden hamsters were exposed to up to 50 ppm chloroprene for 6 h/day, 5 days/week, for up to 24 months (rats) or 18 months (hamsters). These investigators reported that "there was no evidence of carcinogenicity related to (chloroprene) exposure" in either species under these exposure conditions. However, there was a statistically significant increased incidence of mammary gland fibroadenomas in the female rat, as was seen in the NTP study.

In a 2-year rat inhalation bioassay of isoprene (NTP, 1997), groups of 50 male and 50 female rats were exposed to 0, 220, 700, or 7,000 ppm isoprene, 6 h/day, 5 days/week, for 104 weeks. In males, there were increased incidences of mammary gland neoplasms (fibroadenomas plus carcinomas), renal tubule adenomas, and testicular adenomas. In female rats, there were increased incidences and multiplicities of mammary gland fibroadenomas. A few rare brain neoplasms in exposed females may also have been treatment-related.

There are no 2-year bioassays of isoprene exposure in mice; however, there are two inhalation studies that used shorter exposure periods (Placke et al., 1996; Melnick et al., 1996b). Placke et al. (1996) exposed 12 groups of 50 male B6C3F$_1$ mice to various concentration × time scenarios, with exposure concentrations up to 2,200 ppm and exposure durations up to 80 weeks. The mice were then held until week 104, with the exception of 5 exposure groups that had excessive mortality and were slaughtered after 96 weeks. Groups of 50 female mice were exposed to 0, 10, and 70 ppm isoprene, 8 h/day for 80 weeks, and similarly held until week 104. The male mice exhibited most of the same treatment-related tumors as mice exposed to 1,3-butadiene—tumors of the lung, liver, Harderian gland, and forestomach, as well as heart (and spleen) hemangiosarcomas and histiocytic sarcomas. As with chloroprene, a major difference

from the results of 1,3-butadiene exposure was the absence of the early-onset T-cell lymphomas. The female mice were exposed to lower concentrations and only showed treatment-related increased incidences of Harderian gland adenomas, pituitary gland adenomas, and possibly hemangiosarcomas of the spleen. Placke et al. concluded that isoprene appears to be about one order of magnitude less potent than 1,3-butadiene in mice. Melnick et al. (1996b) exposed male $B6C3F_1$ mice to isoprene at concentrations up to 7,000 ppm for 26 weeks followed by a 26-week "recovery" period. Treatment-related tumors were observed in the lung, liver, Harderian gland, and forestomach, but no hemangiosarcomas or lymphomas were induced under these conditions.

6.6. DISCUSSION AND CONCLUSIONS

The long-term inhalation studies have shown that 1,3-butadiene is carcinogenic in rats and mice, inducing tumors at multiple organ sites (NTP, 1984, 1993; Owen et al., 1987). Results of the 1993 NTP mouse study and the Owen et al. rat study are summarized in Table 6-12.

Results of the 1993 NTP mouse bioassay detailed in this report confirmed the carcinogenicity of 1,3-butadiene in male and female $B6C3F_1$ mice demonstrated in an earlier 1984 NTP study. Of particular interest in the 1993 study were the large number of primary organ sites of tumor induction by 1,3-butadiene; the early and extensive development of lymphomas; the induction of uncommon tumors, such as hemangiosarcomas of the heart and squamous cell neoplasms of the forestomach; and the development of malignant lung tumors at exposure concentrations as low as 6.25 ppm. Because there were no exposure levels of 1,3-butadiene at which a carcinogenic response was not induced, it is likely that exposure to concentrations below 6.25 ppm would also cause cancer in mice.

Exposure to 1,3-butadiene at concentrations ranging from 6.25 to 625 ppm for 2 years caused increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, mammary gland, ovary, and liver, all lesions identified in the NTP (1984) study. The Harderian gland and preputial glands were identified as additional sites, and tumors in the kidneys, skin, Zymbal's gland, and intestine were marginally associated with 1,3-butadiene. Because of increased survival, the study also established clearer concentration-response relationships than the 1984 study. Competing risks of early-developing lethal lymphocytic lymphomas at high concentrations preempted the appearance of late-developing neoplasms at some organ sites.

Separate experiments with reduced exposure durations (stop-exposure study) showed that continued exposure is not necessary for development of neoplasms. The incidences of lymphocytic lymphomas, hemangiosarcomas of the heart, and tumors of the lung, forestomach, Harderian gland, and preputial gland were increased in mice exposed for only 13 weeks to 625 ppm 1,3-butadiene, and it is likely that even shorter exposure durations would have produced a carcinogenic response. The stop-exposure study also suggests that concentration is a greater

Table 6-12. Significant positive primary tumor incidences in mice and rats exposed to 1,3-butadiene for 2 years[a].

Target tissue	Neoplasm	Exposure concentration (ppm)						
		6.25	20	62.5	200	625	1,000	8,000
Systemic	Malignant lymphoma		F		F	MF		
	Histiocytic sarcoma			M	MF	MF		
Heart	Haemangiosarcoma			M	MF	MF		
Lung	Alveolar bronchiolar adenoma or carcinoma or adenocarcinoma	F	F	MF	MF	MF		
Forestomach	Squamous-cell papilloma or carcinoma			F	MF	MF		
Harderian gland	Adenoma or carcinoma			MF	MF	M		
Liver	Hepatocellular adenoma or carcinoma			F	MF			
Preputial Gland	Carcinoma				M			
Ovary	Granulosa cell tumors, benign or malignant			F	F	F		
Mammary gland	Adenocanthoma, carcinoma, malignant mixed tumors carcinoma, fibroadenoma			F	F	F	F	F
Brain	Glioma[b]							
Testis	Leydig cell tumor							M
Pancreas	Exocrine adenoma							M
Zymbal gland	Carcinoma[c]							
Thyroid	Follicular cell adenoma or carcinoma							F
Uterus	Stromal sarcoma[c]							

Unshaded column - mouse.
Shaded column - rat.
M - male, F- female.
[a]Results from the 1993 NTP mouse study and the 1987 Owen et al. rat study. The 1984 NTP mouse study is not included because it was terminated at 61 weeks because of excessive mortality.
[b]No significant increases by pairwise comparison, but increasing trend in male SD rats ($p<0.05$).
[c]No significant increases by pairwise comparison, but increasing trend in female SD rats ($p<0.05$).

contributing factor in the development of lymphocytic lymphomas than duration of exposure. At comparable total exposures, the incidence of lymphocytic lymphomas was greater with exposure to a high concentration of 1,3-butadiene for a short time compared with a lower concentration for a longer duration.

A morphological continuum of 1,3-butadiene-induced proliferative lesions to neoplasia or the progression of benign to malignant neoplasms was evident for a number of sites in both the 2-year and the stop-exposure study (NTP, 1993). Increased incidences of proliferative, nonneoplastic lesions (hyperplasia) of the cardiac endothelium, alveolar epithelium, forestomach epithelium, germinal epithelium and granulosa cells of the ovaries, mammary gland, and Harderian gland probably represent treatment-related preneoplastic changes at these target sites. The distinction between adenoma and carcinoma further reveals the biological progression of the benign lesions to malignant neoplasia. For example, in the lungs of male mice, progression from alveolar-bronchiolar adenoma to carcinoma was evident in the 200-ppm exposure group and in all of the stop-exposure groups.

The mechanism of 1,3-butadiene-induced carcinogenicity is not known; however, metabolism likely involving two reactive metabolites, 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane, is thought to be an important factor (Chapters 3 and 4).

The carcinogenic response of 1,3-butadiene in rats and mice is well documented. Also, studies reviewed have shown that mice are more sensitive to the induction of carcinogenic effects than are rats (see Table 6-12). The carcinogenic activity in Sprague-Dawley rats exposed to 1,000 or 8,000 ppm 1,3-butadiene was largely limited to endocrine tissues or hormonal responsive tissues, such as pancreas, Leydig cells of the testis, uterus, Zymbal's gland, mammary gland, and thyroid (Owen et al., 1987), whereas exposure of $B6C3F_1$ mice to much lower concentrations of 1,3-butadiene caused significantly increased incidences of mammary gland neoplasms and granulosa cell neoplasms of the ovary as well as malignant lymphomas, histiocytic sarcomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, squamous cell neoplasms of the forestomach, Harderian gland neoplasms, and hepatocellular neoplasms. The reason for the species difference is not known, but may in part be due to differences in toxicokinetics. For example, metabolism studies have shown that blood concentrations of 1,3-butadiene are higher in mice than in rats, and are lower in monkeys than in either rodent species (Chapter 3). In vitro studies using liver microsomes have shown that the metabolism of the reactive intermediate, 1,2-epoxy-3-butene, to the non-DNA-reactive 1,2-dihydroxybut-3-ene is the prevalent pathway in human and rat preparations, whereas mouse liver microsomes convert 1,2-epoxy-3-butene to DNA-reactive 1,2:3,4-diepoxybutane in addition to the nonreactive 1,2-dihydroxybut-3-ene (Csanády and Bond, 1991).

Investigations by Irons and co-workers (Irons et al., 1989; Irons, 1990) to explain the species differences of 1,3-butadiene-induced carcinogenicity have focused on the possibility that activation of an endogenous leukemia retrovirus may play a critical role in 1,3-butadiene-induced lymphoma in $B6C3F_1$ mice. The incidence of thymic lymphomas was greater in $B6C3F_1$ mice (57%) than in NIH Swiss mice (14%) exposed to 1,250 ppm 1,3-butadiene for 1 year. However, the NIH Swiss mouse does not express the endogenous leukemia retrovirus and has a very low background rate for thymic lymphomas. Thus, the finding that exposure to 1,3-butadiene caused a 14% incidence of thymic lymphomas in NIH Swiss mice suggests that 1,3-butadiene can induce thymic lymphomas independently of an activated retrovirus.

Identification of activated oncogenes in chemically induced tumors also may provide information regarding the mechanism of tumor induction by butadiene. For example, because K-ras is the most commonly detected oncogene in human cancers, tumors from the NTP (1993) study were evaluated for the presence of K-ras oncogenes (Goodrow et al., 1990). Activated K-ras oncogenes were detected in 6/9 lung tumors, 3/12 hepatocellular carcinomas, and 2/11 lymphomas obtained from $B6C3F_1$ mice exposed to 1,3-butadiene at concentrations ranging from 62.5 to 625 ppm. A specific codon 13 mutation was found in most of the activated K-ras oncogenes, suggesting a chemical-specific effect. Activated K-ras genes have not been found in spontaneously occurring liver tumors or lymphomas (Goodrow et al., 1990) and were observed in only 1/10 of spontaneous lung tumors in $B6C3F_1$ mice (Goodrow et al., 1990; Reynolds et al., 1987). Furthermore, it was shown that tumor suppressor genes are inactivated during 1,3-butadiene carcinogenesis. Soderkvist et al. (1992) identified allelic losses in the p53 tumor suppressor gene in lung and mammary carcinomas and lymphomas of $B6C3F_1$ mice exposed to 1,3-butadiene, that were analogous to those observed in a variety of human cancers.

Immune-function assays conducted by Thurmond et al. (1986) in which $B6C3F_1$ mice were exposed by inhalation to 1,250 ppm 1,3-butadiene for 6 or 12 weeks showed that 1,3-butadiene exerts no significant immunosuppressive effects, suggesting that 1,3-butadiene causes neoplasia by mechanisms other than compromise of immune function.

In addition to the carcinogenic effects noted in the NTP (1993) study, exposure to 1,3-butadiene caused hematological changes indicative of a partially regenerative anemia in mice exposed to $\geq$62.5 ppm 1,3-butadiene. Mice exposed to 625 ppm exhibited bone marrow atrophy and splenic and hepatic extramedullary hematopoiesis. Increases in mean cell volume and mean cell hemoglobin at 625 ppm 1,3-butadiene suggested that although 1,3-butadiene caused suppression o-f hematopoiesis in the bone marrow, younger larger cells may have been released into the blood from extramedullary sites. A macrocytic-megaloblastic anemia was reported in $B6C3F_1$ mice exposed to 1,250 ppm 1,3-butadiene for 6 weeks (Irons et al., 1986a,b).

7. EPIDEMIOLOGIC STUDIES OF CARCINOGENICITY

This updated review presents the evaluation of studies published from 1985 through December 1998. The follow-up proposed by Lemen et al. (1990) of the cohort studied by Meinhardt et al. (1982) and Downs et al. (1992), an abstract submitted for the International Symposium, is not reviewed in this evaluation. Lemen et al. (1990) did not present any results, and no details of study design and analysis were available for Downs et al. (1992). Since 1985, investigators have conducted studies of workers who produce 1,3-butadiene as a raw material (monomer production) or who use 1,3-butadiene in styrene-butadiene rubber (SBR) production (polymer production).

7.1. MONOMER PRODUCTION

7.1.1. Texaco Cohort

7.1.1.1. *Downs et al. (1987): Mortality Among Workers at a Butadiene Facility*

Investigators examined a cohort of 2,586 permanent male employees who worked a minimum of 6 months in a Texaco butadiene manufacturing plant (monomer production) that supplied the raw material to two adjacent SBR plants studied by Meinhardt et al. (1982) and for which an update has been proposed by Lemen et al. (1990). Data were available for the 37-year period from January 1, 1943, through December 31, 1979. Vital status of the cohort was determined through the Social Security Administration (SSA). Individuals whose vital status was unverifiable through SSA were traced through the Texas Department of Public Safety. Death certificates were obtained from the health departments of the States where the individual resided at the time of death. When this effort was unsuccessful, the individual's name was placed on a list, which was submitted to the health departments of Texas and Louisiana, to obtain the death certificates. A trained nosologist coded the death certificates using the eighth revision of the International Classification of Diseases (ICD).

Because quantitative exposure data had not been accumulated for individual workers, the investigators used department codes to construct a qualitative exposure scale composed of four groups: Group I, low exposure (included utility, office, and management workers, N = 432); Group II, routine exposure (included process, laboratory, storage, and transport workers, N = 710); Group III, nonroutine exposure (included skilled maintenance workers, N = 993); and Group IV, unknown exposures (N = 451). The investigators postulated that Group III workers may have had exposure to higher concentrations with a lesser frequency than Group II workers.

Of 2,586 employees in the cohort, 175 (6.8%) were black. Scrutiny of death certificates revealed that 45 blacks (7.5% of total deaths) were improperly coded as whites. At this point, investigators conducted a preliminary analysis on the total cohort, using both black and white

national death rates. The standard mortality ratios (SMRs) were higher based on black rates as compared with white rates for four cause-specific deaths only: all lymphohematopoietic cancers (SMR = 169 vs. 138), lymphosarcoma (SMR = 336 vs. 220), Hodgkin's disease (SMR = 135 vs. 102), and leukemia (SMR = 155 vs. 119). Most of the other SMRs for both cancers and noncancers were decreased based on black rates. Therefore, using black rates would have underestimated the risks. Thus, the entire cohort was treated as white, and all further analyses were conducted using white death rates.

Expected deaths were calculated using two referent populations: U.S. white males (national comparison) and white males in a seven-county area surrounding the plants (local comparison). The rates were standardized for age, race, sex, and calendar year. SMRs (labeled NSMR for national comparisons and LSMR for local comparisons) were calculated in the customary manner by dividing the observed deaths by the expected deaths and multiplying the ratio by 100. Under the null hypothesis, the significance of the ratios of observed to expected deaths was tested assuming that the observed (O) deaths followed a Poisson distribution using a two-sided test and assuming a p value of <0.05 to be significant. Comparisons between Groups I, II, and III were done by using the Mantel-Haenzel procedure for computation of relative risks in follow-up studies with stratified data (Rothman and Boice, 1982), and power calculations were performed using the normal approximation to the Poisson distribution (Beaumont and Breslow, 1981). The person-years at risk were not accrued until after the sixth month of employment.

A total of 64,800 person-years was accrued for the follow-up period. There were 603 deaths from 1943 through 1979; death certificates were obtained for 579 individuals (96%). The vital status was unknown for 73 individuals (2.8% of the total cohort).

Results of this investigation indicated lower than expected mortality for these workers from all causes (NSMR = 80, $p<0.05$ and LSMR = 96, $p>0.05$, O = 603) and from all cancers (NSMR = 84, $p>0.05$ and LSMR = 76, $p<0.05$, O = 122). However, a site-specific comparison indicated a statistically significant increase in mortality from lymphosarcoma and reticulosarcoma (ICD code 200, NSMR = 235, 95% confidence intervals [CI] = 101-463, O = 8) compared with national rates and a nonsignificant excess (LSMR = 182, $p>0.05$) compared with local rates.

Since the Meinhardt et al. (1982) study had indicated lymphohematopoietic cancers may be increased among the individuals hired during wartime,[1] the Texaco cohort was subdivided into wartime workers and postwar workers, although no process change took place in monomer production after the war. A comparison of wartime workers (N = 1,061; 452 deaths) who had worked for at least 6 months prior to 1945 and postwar workers (N = 1,525; 151 deaths) found an

[1]Meinhardt et al. (1982); the war/postwar comparison was related to process change.

increase for all lymphohematopoietic cancers among wartime workers (NSMR = 150, 95% CI = 84-247, O = 15) and among postwar workers (NSMR = 134, $p>0.05$, O = 6). However, stratification reduced sample sizes considerably. Of a total of 7 deaths from leukemia, 6 were in wartime workers while 4.2 were expected.

The analyses by duration of employment on mortality showed an increase among those who worked <5 years for all lymphohematopoietic cancers (NSMR = 167, $p>0.05$, O = 11), with most of the increase attributed to leukemia (NSMR = 187, $p>0.05$, O = 5) and residual lymphohematopoietic cancers[2] (i.e., non-Hodgkin's lymphoma [NHL], multiple myeloma, and other lymphohematopoietic cancers) (NSMR = 172, $p>0.05$, O = 5). Among those who worked >5 years, a nonsignificant increase was found for all lymphohematopoietic cancers (NSMR = 127, O = 10), mainly due to an increase in residual lymphohematopoietic cancers (NSMR = 200, O = 7).

Further analyses were conducted for the four groups identified on the qualitative exposure scale. For those with routine exposure (Group II), increases were noted for all lymphohematopoietic cancers, Hodgkin's disease, and residual lymphohematopoietic cancers. An excess of kidney cancer was also observed in this group based on one case. Similarly, in those with nonroutine exposure (Group III), excesses were observed for all lymphohematopoietic cancers, Hodgkin's disease, leukemia (of seven leukemia cases five were in this group), and residual lymphohematopoietic cancers. For those in the low-exposure group (Group I), excess mortality was seen for the same cancers (excluding Hodgkin's disease): all lymphohematopoietic cancers, leukemia, and residual lymphohematopoietic cancers. In general, use of local southeast Texas coastal rates resulted in lower SMRs for the above three groups except for Hodgkin's disease in routine and nonroutine exposure groups, which showed slight increases over national rates. Both of these SMRs were based on one observed case in each group. None of the excess found in these three groups was statistically significant.

The comparison of Groups II, III, and IV with the low-exposure group (Group I) resulted in inconsistent findings due to a small number of cause-specific deaths and could not be reliably interpreted.

Analyses were also done by latency and number of years worked using national rates. Although the results for number of years worked were inconsistent for total cancers, the SMRs increased from 80 to 93, with increasing latency for this category. Similarly, excess SMRs for all lymphohematopoietic deaths were observed in all latency periods (0 to 9, 20 to 29, 30 to 39) except for 10 to 19 years. The number of years of employment results showed an inverse relationship for these cause-specific deaths. For cause-specific deaths due to lymphosarcoma and

[2]Residual lymphohematopoietic cancers include ICD codes 200, 202, 203, 208, and 209.

reticulosarcoma (ICD code 200), both the latency as well as number of years employed showed an inverse relationship. The notable finding in this analysis was for workers who had a latency of 0 to 9 years and had worked for less than 10 years (NSMR = 1,198, $p<0.01$, O = 4). This increase was statistically highly significant (tested by the author of this document using the Poisson distribution).

This is an extensively analyzed cohort mortality study. As correctly acknowledged by the investigators, there are a few methodological limitations to this study, the major ones being a lack of industrial hygiene (IH) data and a lack of personal work histories. In addition, half of the total cohort worked less than 5 years in the plant. Some of the workers from this cohort had also worked in two neighboring SBR plants. The exposures to other chemicals in the SBR plants and in prior jobs are the confounders that were not adjusted for in this study. The cohort is relatively small to start with, but stratification in several subgroups further reduced the power.

The major strength of the study is that it was conducted in a butadiene (monomer) production facility in a cohort where confounding exposure from styrene was absent. The excesses observed are in cancers of the lymphohematopoietic system, which are consistent with cancer findings of the SBR plant workers. Most of the cases of lymphohematopoietic cancers are concentrated in workers employed for less than 10 years and in wartime workers, which may be due to the occurrence of higher exposures during wartime years. The exposures during subsequent periods were lower. Thus, the finding of excess cancer mortality in short-term employees is not evidence against dose-response relationship.

7.1.1.2. *Divine (1990): An Update on Mortality Among Workers at a 1,3-Butadiene Facility—Preliminary Results*

In 1990, Divine reported an updated analysis of the same Texaco plant (monomer production) cohort. The follow-up on the original cohort was extended through 1985 (adding 6 more years) by updating the information on workers from company data and the SSA. Death certificates were obtained from the health departments of Texas, Louisiana, Ohio, and Mississippi and were coded by a trained nosologist according to the eighth revision of the ICD. The National Death Index (NDI) records were searched for workers for whom the SSA failed to provide the vital status.

Mortality analyses were performed using Monson's computer program (Monson, 1974). Again, the white male death rates of the U.S. population were used due to uncertainties about race information in the company files and because there were few blacks in the cohort. Person-years were accrued similarly to the Downs et al. (1987) study.

The qualitative exposure categories remained the same. IH sampling data at the time of this study supported the exposure categories developed earlier. For this study, lymphosarcoma

(ICD code 200) was reported separately from the cancers of other lymphatic tissues (ICD codes 202, 203, and 208).

A total of 74,219 person-years had accrued through 1985. The number of deaths had increased to 826, and death certificates were not available for 49 individuals (6%). Of 2,582 employees in the cohort,[3] 1,708 individuals were still alive and 48 (1.9%) were lost to follow-up. Overall, the pattern of results was unchanged from the report by Downs et al. (1987) for this cohort. For the total cohort, the SMRs for all lymphohematopoietic cancers and Hodgkin's disease were increased but not significantly; however, for lymphosarcoma and reticulosarcoma, the excess was significantly larger (SMR = 229, 95% CI = 104-435, O = 9) and accounted almost entirely for the increase in overall lymphohematopoietic cancers. Analyses by various subcohorts also yielded results similar to those observed in the earlier study (Downs et al., 1987). The highest increase was observed in lymphosarcoma and reticulosarcoma among workers who had worked more than 5 years but less than 10 years (SMR = 245, 95% CI = 79-572, O = 5). Analyses by wartime and postwar subcohorts demonstrated a statistically significant increase among the wartime subcohort for the same cause-specific deaths (SMR = 269, 95% CI = 108-555, O = 7), while an excess in the postwar subcohort had two deaths from lymphosarcoma and reticulosarcoma and was not statistically significant. There was one additional death from leukemia, bringing the total to eight deaths in the total cohort; this death occurred in the postwar subcohort.

Among the subcohorts based on exposure levels, the only statistically significant excess was observed for lymphosarcoma and reticulosarcoma among workers who were ever employed in the routine exposure category, exposure Group II (SMR = 561, 95% CI = 181-1,310, O = 5). Among workers who were ever employed in the nonroutine exposure category (Group III), an excess was observed for all lymphohematopoietic cancers based on 11 deaths, mainly due to an increase in leukemia (SMR = 185, 95% CI = 68-403, O = 6). Lymphosarcoma in this group was slightly increased based on two cases.

For the total cohort, no pattern with latency or duration of years worked was observed for either all deaths or total cancer deaths. For all lymphohematopoietic cancers, excesses were observed in the latency groups of 30+ years (SMR = 205, O = 8) and 0 to 9 years (SMR = 200, O = 4). Both of these groups had worked less than 10 years. Deaths from lymphosarcoma were also increased in the same duration and latency groups. For duration of less than 10 years and latency of 30+ year and 0 to 9 year groups, the SMRs were 3,333 (O = 2) and 1,333 (O = 4), respectively. For leukemia the SMRs increased with increasing latency among the workers who had worked less than 10 years. The SMRs were 125 (O = 1), 182 (O = 2), and 250 (O = 4)

[3]The cohort was reduced to 2,582 from 2,586 because of some double counting and some coding error where a female was coded as male.

respectively for latency of 10-19 year, 20-29 year, and 30+ year groups, respectively. No statistical test results were presented for this analysis. Similar analyses by different exposure groups failed to show any pattern for all lymphohematopoietic deaths and lymphosarcoma deaths among low-exposure and unknown-exposure groups. Among routinely exposed groups, the excesses were observed for the same two latency and duration groups as for the total cohort, whereas for nonroutine exposure the excesses were observed only for 20 to 29 and 30+ years' latency groups who had worked for less than 10 years. All of these excesses were based on ≤ 3 deaths in each group, making interpretation of these findings by exposure levels very difficult.

The investigator stipulated that the increased lymphosarcoma and reticulosarcoma among wartime workers may be due to higher exposures during wartime, or that this group was somehow different from those hired at other times; perhaps they were not eligible for the draft because of age or health reasons. It should be noted that although the excesses in leukemia deaths were not statistically significant, they occurred in workers who had worked less than 10 years and increased with increasing latency.

This also is a well-conducted study; unfortunately, the same methodological limitations that were present in the Downs et al. (1987) study are applicable to this study. However, the findings of this study are consistent with the earlier study as well as with other SBR plant studies.

7.1.1.3. *Divine et al. (1993): Cancer Mortality Among Workers at a Butadiene Production Facility*

This update added another 5 years of follow-up to the earlier cohort of monomer workers (Divine, 1990). Cohort inclusion criteria remained the same but were extended from December 31, 1979, to December 31, 1990. This yielded additional workers, resulting in a total cohort of 2,749 individuals. The four exposure groups were similar to those used in earlier studies, with slight changes as follows. The background exposure group (included office utility, warehouse, and transportation workers, N = 347). This group was called the low-exposure group in the previous two studies (Downs et al., 1987; Divine, 1990). The low-exposure group (included workers from operating units, planners and engineers, welders, carpenters, and workers from brick masons, N = 958). This group was a combination of some of the low-exposure and all of the unknown-exposure group from the previous two studies. The nonroutine exposure group (included skilled maintenance workers such as pipefitters, tinsmiths, instrument and electrical workers, and insulators, N = 865). The routine exposure group (included process, lab, storage, and transport workers, N = 1,056). The last two categories appeared to be the same as in the earlier two studies; the change in the number of individuals in these categories was due to the use of complete work histories rather than the last assigned department. For this study, the

investigators reviewed the results of the IH data and information obtained from plant personnel and found that the main difference between the routine and nonroutine exposure groups was in the frequency and not the intensity of exposure.

Monson's computer program (Monson, 1974) was used for analysis of this study also. All the analytical methods included use of white male death rates of the U.S. population (because there were very few blacks in the study, they were assumed to be white for the analysis) and calculation of person-years. The follow-up procedures and acquisition of death certificates were the same as in an earlier study by Divine (1990).

A total of 83,591 person-years was accrued. At the end of the follow-up period, 1,660 individuals were still alive, 38 were lost to follow-up, and 1,051 were deceased (death certificates were obtained for 1,036 individuals).

The overall results observed in this study were similar to the earlier two studies. The statistically significant elevated SMRs were observed for lymphatic and hematopoietic cancers (SMR = 171, 95% CI = 104-264, O = 20) and for lymphosarcoma and reticulosarcoma for workers employed for less than 5 years (SMR = 286, 95% CI = 104-622, O = 6). There were 3 additional leukemia deaths in total cohort, bringing the total to 11 deaths. For workers who were employed for less than 5 years, the SMR for leukemia was 170 (95% CI = 73-335, O = 8). Analysis by wartime and postwar subcohorts found an increase in lymphosarcoma and reticulosarcoma in the wartime employees (SMR = 254, 95% CI = 102-523, O = 7). There were seven leukemia deaths in this group versus four in the postwar subcohort. The analysis by exposure group showed an increase for lymphosarcoma and reticulosarcoma in the routine exposure group (SMR = 452, 95% CI = 165-984, O = 6). There were six leukemia deaths in the nonroutine exposure group. Analysis by latency and duration of employment yielded the largest increase in 0 to 9 years' latency for individuals employed for less than 5 years (possibly wartime employees) for lymphosarcoma and reticulosarcoma. The SMR was 3,333 based on 2 observed cases. No statistical test results were presented for this analysis.

Again, the investigators stipulated that the observed excesses in all lymphatic and hematopoietic cancers, Hodgkin's disease, and leukemia were among those employees who were hired during the war and among the nonroutinely exposed group who had worked for less than 5 years. They observed that this group was employed in both the period and the exposure category in which exposures were highest. They also acknowledged that although the complete work histories were available, the IH data were available only for the last 10 years of the study.

7.1.1.4. *Divine and Hartman (1996): Mortality Update of Butadiene Production Workers*

This recent follow-up of the same cohort added 46 more individuals to the cohort (a total of 2,795) by extending the inclusion criteria and the follow-up period through December 31, 1994. The person-years accrued increased to 85,581. Of the 2,795 individuals, 999 were still alive, 574 were lost to follow-up (all but 28 were known to be alive), and 1,222 were deceased (death certificates were obtained for 1,202 individuals). The follow-up procedures and analytical techniques (for SMR analysis) were the same as for earlier studies. The exposure categories were changed to background-exposure group (same as 1993 follow-up), low-exposure group (same as 1993 follow-up), and varied-exposure group (comprised nonroutine and routine exposure groups of 1993).

Based on IH data available since 1980, each employee's potential exposure to butadiene was estimated by separating the employee's work history by job categories into 1-year segments. Two variables were used to calculate the estimated exposure (job categories and calendar time periods). There were six exposure classes based on job categories: 0, 1, 2, 3, 4, and 5 with 0, 0.1, 0.2, 0.3, 0.4, and 0.5 weights (wt), respectively, and five calendar time periods: <1946 (wt = 10), 1946-59 (wt = 8), 1960-76 (wt = 4), 1977-85 (wt = 2), and 1986-94 (wt = 1). The cumulative exposure was obtained for each individual by summing the scores for all the years of employment. These exposure estimates were used to conduct survival analyses for (1) total lymphohematopoietic cancer, (2) lymphosarcoma, (3) NHL, (4) multiple myeloma, and (5) leukemias.

Three different models were used for the survival analysis: a Cox proportional hazard model with a time-dependent estimate of cumulative exposure, a person-time logistic regression model with a time-dependent estimate of cumulative exposure, and a nested case-control model using conditional logistic regression. Each case had 10 matched controls (by date of birth [$\pm$2 years]). The selection of controls without replacement was from noncases at the time of the occurrence of each case.

The results of the SMR analyses were very similar to the two earlier follow-up studies of this cohort (Divine, 1990; Divine et al., 1993). The SMRs for the all lymphatic and hematopoietic cancers were increased in the total cohort (SMR = 147, 95% CI = 106-198, O = 42) and in the employees who worked for less than 5 years (SMR = 162, 95% CI = 101-245, O = 22). Although the SMR for lymphosarcoma and reticulosarcoma for this group was still elevated (SMR = 261, 95% CI = 95-568, O = 6), it was no longer statistically significant. Similarly, in wartime workers the elevated SMR for lymphosarcoma and reticulosarcoma barely missed statistical significance (SMR = 241, 95% CI = 97-497, O = 7). It is not explained why the number of wartime employees changed from 1,066 in 1990 (Divine) to 1,050 in 1993 (Divine et al.) and to 1,048 in 1996 (Divine and Hartman). The analysis by exposure groups showed

excess SMRs for all lymphatic and hematopoietic cancers (SMR = 172, 95% CI = 117-244) and for lymphosarcoma and reticulosarcoma (SMR = 249, 95% CI = 100-513, O = 7), both in varied exposure group. There were 2 additional deaths from leukemia, bringing the total number of deaths to 13, of which 11 were in the varied-exposure group (SMR = 154, 95% CI = 77-257). All of the leukemia decedents were first employed in the 1940s, and eight had worked for less than 5 years.

Except for the risk factor "Hire-age" in the Cox model, the survival analyses failed to show any significant increase in the risk ratios, in any cause-specific cancer, by any of the three methods. The Cox model, which used time-dependent estimates of exposures, showed significant excesses in relative risks (RR) for Hire-age and occurrence of all lymphatic and hematopoietic cancers (RR = 1.08, 95% CI = 1.05-1.12), leukemia (RR = 1.08, 95% CI = 1.02-1.15), lymphosarcoma (RR-1.07, 95% CI = 1.00-1.14), and NHL (RR = 1.09, 95% CI = 1.04-1.14).

Moreover, the investigators point out that the observed statistically significant excess SMR in the total cohort for all lymphatic and hematopoietic cancers is due to increased SMRs for lymphosarcoma and cancer of other lymphatic tissue. They state that there were no additional deaths from lymphosarcoma since 1981. Of nine lymphosarcoma deaths eight belonged in persons first employed before 1950, six had worked for less than 5 years, seven were hired during WW II, and seven belonged in the varied-exposure group.

In contrast, the investigators found an additional five deaths of cancers of other lymphatic tissue since their last follow-up in 1993. Four of these deaths were due to lymphomas, bringing the total to 15 deaths from this cause. Fourteen of the decedents were employed prior to 1950. The investigators further state that the observed excess SMR for this category in the varied-exposure group increased with increasing length of employment.

The investigators go on to expostulate that per new diagnostic criteria lymphosarcoma is now classified as lymphoma, and thus is included under the category of NHL.[4]

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[4]Under the previous classification (*8th ICD, Adapted)*, lymphohematopoietic cancers comprised the following subcategories: lymphosarcoma and reticular sarcoma, Hodgkin's disease, leukemia, and other lymphatic tissue cancers. In 1994, the International Lymphoma Group's Revised European-American Lymphoma (REAL) classification was proposed for the lymphohematopoietic cancers, and is being adopted into the ICD-O (Berard and Hutchison, 1997). This classification is based on new ideas evolving in the fields of molecular biology, genetics, and immunology, which have rendered the old classification for lymphohematopoietic cancers obsolete. The REAL classification comprises the following subcategories: B-cell neoplasms, T-cell and putative natural killer (NK)-cell neoplasms, Hodgkin's disease, and unclassified lymphomas. It should be noted that both leukemias and lymphomas that are produced by B-cells are included under B-cell neoplasms, and leukemias and lymphomas produced by T-cells and NK-cells are included under T-cell and NK-cell neoplasms. It should also be noted that any lymphoma (such as B-cell, T-cell, and NK-cell) that is not classified as Hodgkin's disease is also included under non-Hodgkin's lymphoma.

Furthermore, the Leukemia Society of America (1999) defines lymphohematopoietic cancers as follows: "Leukemia, Lymphoma, Hodgkin's disease, and Myeloma are cancers of the body's blood forming and immune systems: the bone marrow and lymph nodes. They are considered to be related cancers because they involve the uncontrolled growth of cells with similar functions."

Thus they computed the SMR of 176 (95% CI = 103-282) for the observed 17 deaths from NHL. This was based on the assumption that 50% of the deaths from cancers of other lymphatic tissue were from lymphomas. Out of 17 decedents, 16 were hired before 1950, 6 had worked for more than 5 years, and 12 belonged in the varied-exposure group.

Just as in the earlier two follow-ups (Divine, 1990; Divine et al., 1993), the investigators stipulate that the above findings were consistent with the hypothesis that high exposures to 1,3-butadiene during wartime may be associated with NHL. Since the investigators did not find any association between cumulative exposure and occurrence of non-Hodgkin lymphoma in their survival analysis, they suggest that peak exposures rather than cumulative exposures may be associated with the observed increase. Furthermore, they acknowledge that there are no data or means available to estimate the peak exposures. In addition, they note that extremely high peak exposures did exist during the 1950s and 1960s. Also, there is a lack of any information on other confounding factors.

Although the investigators have done a good job of estimating the exposure and have conducted various analyses, by their own admission they may have used an incorrect category of cumulative exposures. The survival analysis using the Cox model did show statistically significant risk ratios for the risk factor "Hire-age" in four out of five cancer types evaluated. Time-dependent exposure estimates were used for the Cox model. With every update, the cohort inclusion date has been extended, adding more employees. Addition of recent employees, younger in age and with shorter follow-up, may have resulted in dilution of observed risk.

There are a few limitations to this study, some of which are correctly pointed out by the investigators. IH data are available only from 1980. Fifty percent of the cohort worked for less than 5 years. That means they left because they were old (during wartime, mainly the older population was available to work), or sick, or found other jobs and were exposed to other chemicals. The cohort is small, and the cause-specific mortality of NHL is relatively rare. The investigators assert the occurrence of extremely high peak exposures in the 1950s and 1960s has been mentioned, but it is not known how these peak exposures compared with those in wartime. Moreover, since the 1950s, exposure to 1,3-butadiene in the monomer facility has declined. Therefore, considering the very low overall exposure to 1,3-butadiene and the modest size of this cohort, there may not be enough power in the study to detect a leukemia excess.

However, this is still the largest cohort exposed to monomer. Qualitative measures of exposure and IH data since 1980 are available. Upon completion of this study, this cohort will have 52 years of follow-up.

7.1.2. Shell Oil Refinery Cohort

7.1.2.1. *Cowles et al. (1994): Mortality, Morbidity, and Hematological Results From a Cohort of Long-Term Workers Involved in 1,3-Butadiene Monomer Production*

Shell Oil's Deer Park Refinery produced a butadiene monomer from 1941 to 1948 and 1970 to the present. The cohort consisted of male workers who had a minimum of 5 years of employment in jobs with potential exposure to butadiene or at least 50% of their total duration of employment (minimum of 3 months) in these jobs. This facility also had several other refinery operations and chemical production units. Three different analyses were performed on this cohort: (1) mortality, (2) morbidity, and (3) hematological.

1. Mortality Analysis:

A total of 614 employees composed the cohort. The follow-up period was from 1948 to December 31, 1989. Vital status was assessed from company records, SSA, master beneficiary files, and the NDI. Death certificates were obtained for all the deceased workers and coded by a trained nosologist according to the revision of the ICD in effect at the time of death. Mortality rates of Harris County, TX, were used to compute the age-, race-, and calendar year-adjusted SMRs, using the Occupational Cohort Mortality Analysis Program (OCMAP) from the University of Pittsburgh.

A total of 7,232 person-years were accrued. Of 614 employees, 589 were still alive, 1 was lost to follow-up, and 24 were dead. No excess mortality, either for total deaths or total cancers (including cause-specific cancers), was observed.

2. Morbidity Analysis:

Original cohort members who were active at some time between January 1, 1982, and December 31, 1989, qualified for the morbidity study. Morbidity data were obtained from the Shell Health Surveillance System. The follow-up period was from 1982 to 1991. Causes of morbidity were coded according to the 9th revision clinical modification of the ICD. Morbidity ratios (SMbRs) were calculated by using the internal comparison group of employees who were active during the same time period and had no exposure to butadiene.

A total of 438 employees were included in this analysis. No excess morbidity by any cause was observed.

3. Hematological Data Analysis:

Of 438 individuals included in the morbidity study, periodic hematological data were available for 429 individuals. These hematological data reveal that seven hematological outcomes were measured (between 1985 and 1991). The most recent laboratory test results were

used for the analysis. Comparisons were done with similar results from 2,600 nonexposed employees. No differences were observed between butadiene-exposed vs. nonexposed groups.

This study has quite a few methodological limitations. The cohort is small, and deaths are few. No exposure information was available. The number of employees selected for this study from the time period 1941-1948, when exposure was probably higher, is unclear. Over 50% of the cohort was hired in 1970 or later, with an average follow-up of 12 years. This means that the cohort was still young, showing "healthy worker" effect, and enough latent period had not elapsed to show increases in cancers, which usually have a long latent period. Moreover, the power calculations computed by the investigators showed that the study size and duration were sufficient to detect a fivefold excess in lymphohematopoietic cancer. Thus, the absence of positive results in this study does not provide any negative evidence toward the causal association between butadiene and occurrence of cancer.

7.1.3. Union Carbide Cohort

7.1.3.1. *Ward et al. (1995): Mortality Study of Workers in 1,3-Butadiene Production Units Identified From a Chemical Workers Cohort*
Ward et al. (1996c): Mortality Study of Workers Employed in 1,3-Butadiene Production Units Identified From a Large Chemical Workers Cohort

The study cohort was selected from 29,139 workers at three Union Carbide Corporation facilities in the Kanawha Valley, West Virginia. A total of 527 male workers who had worked between 1940 and 1979 were identified from work history records as having ever worked in the departments where there was a potential for butadiene exposure. Only the individuals who worked in these departments during the butadiene production period were selected for the study (i.e., 364 individuals). The vital status was determined through December 31, 1990, using the NDI. Death certificates were obtained for decedents and coded according to the revision of the ICD codes in effect at the time of death. Both U.S. and Kanawha County mortality rates were used for comparison. A modified life table analysis developed by the National Institute for Occupational Safety and Health (NIOSH) was used to compute the SMRs.

Of 364 workers, 176 were alive, 3 were lost to follow-up, and 185 were dead at the end of 1990. The SMR for all causes was 91, while for all cancers it was 105. Neither of them were statistically significant. The only statistically significant increase was observed for lymphosarcoma and reticulosarcoma, based on four cases (SMR = 577, 95% CI = 157-148). A county-based comparison yielded a similar result. By duration of employment and latency, a statistically significant excess of the SMR was observed among workers who were employed for more than 2 years and with more than 30 years of latency (SMR = 1980, 95% CI = 408-5,780, O

= 3). Of these four cases, three had started their employment between 1942 and 1946 (i.e., wartime), while one had started in 1952.

The investigators stated that except for butadiene exposure, there were no common exposures to other chemicals in the four individuals who had died of lymphosarcoma and reticulosarcoma, although two of them had been assigned to an acetaldehyde unit for some time. Investigators considered the possibility that the risk of lymphosarcoma and reticulosarcoma may be attributable to exposure to acetaldehyde. Hence they identified a subcohort of 233 individuals who were ever assigned to an acetaldehyde unit. Of 48 deaths identified from this subcohort, except for the two deaths of lymphosarcoma and reticulosarcoma previously identified among the workers included in the butadiene production cohort, there were no additional deaths found in this subcohort from lymphosarcoma and reticulosarcoma.

This study has a few methodological limitations. The cohort is very small, no adjustments for confounding exposures to other chemicals were done, and no exposure information is available. The qualitative exposure is assumed based on the department coded for potential exposure to butadiene. It is still interesting to note that the exposure in these plants was to butadiene monomer alone, either in the production process or the recovery from the olefin cracking process, and not to styrene-butadiene polymer. The only other cohort exposed to butadiene monomer (Downs et al., 1987; Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996) also found excess in lymphosarcoma and reticulosarcoma in the wartime subcohort. Although by itself this study would not have furnished compelling evidence of positive association between the exposure to 1,3-butadiene monomer and occurrence of lymphosarcoma, it supports the Texaco cohort findings.

Studies in monomer production workers are summarized in Table 7-1.

7.2. POLYMER PRODUCTION

7.2.1. Cohort Identified by Johns Hopkins University (JHU) Investigators

7.2.1.1. *Matanoski and Schwartz (1987): Mortality of Workers in Styrene-Butadiene Polymer Production*

This cohort mortality study of SBR polymer production workers from eight plants (seven U.S. and one Canadian) was reviewed in a 1985 document (U.S. EPA, 1985). At that time, this study was submitted to the U.S. Environmental Protection Agency but was not published. Because the findings of the published study are essentially the same, it will not be reviewed again.

Table 7-1. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—monomer production.

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Downs et al. (1987)	2,586 permanent male employee cohort mortality Worked for at least a minimum of 6 months from January 1, 1943-December 31, 1979 Follow-up from 1943 through 1979 (37 years) Comparison group U.S. population (national) and 7 counties surrounding the plants (local)	Four exposure groups based on qualitative exposure scale: Group I, low (N = 432) Group II, routine (N = 710) Group III, nonroutine (N = 993) Group IV, unknown (N = 451)	SS NSMR = 235 and SNS LSMR = 182 for lymphosarcoma and reticulosarcoma for total cohort SS NSMR R = 1,198 for lymphosarcoma and reticulosarcoma for latency of 0-9 years and <10 years of employment	Cohort of monomer production workers, a major strength Lack of IH data ½ the cohort worked less than 5 years in the plant Relatively small cohort; therefore hard to interpret results after further stratification Lack of adjustment for confounding for people who worked in SBR plant too
Divine (1990)	Update of the cohort from Downs et al. (1987) Cohort reduced to 2,582 Follow-up extended through 1985 Comparison group U.S. population	Same exposure groups as the earlier study	For lymphosarcoma and reticulosarcoma: SS SMR = 229 for total cohort SS SMR = 269 for prewar subcohort SS SMR = 561 for routinely exposed for less than 10 years No pattern with latency or duration of employment	Same methodologic limitations as the earlier study

Table 7-1. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—monomer production (continued)

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Divine et al. (1993)	Update of the cohort from Divine (1990) Cohort increased to 2,749 as the inclusion period extended through December 31, 1990 Follow-up extended through 1990 Comparison group U.S. population	Similar exposure groups as earlier with some redistribution of workers Group I, background (N = 347) Group II, low (N = 958) Group III, nonroutine (N = 865) Group IV, routine (N = 1,056)	For lymphosarcoma and reticulosarcoma SS SMR = 254 for prewar subcohort SS SMR = 286 for workers employed less than 5 years	Same strengths and limitations as earlier study
Divine and Hartman (1996)	Update of the cohort from Divine et al. (1993) Cohort increased to 2,795 as the inclusion period extended through December 31, 1994 Follow-up extended through 1994 Comparison group U.S. population Internal comparison	Based on IH data and work histories using 6 exposure classes 5 calendar periods Individual exposures were estimated for each worker Three different models used for the survival analysis	Results of SMR analysis were similar to earlier studies Analysis by NHL category resulted in SS SMR=176 Except for “Hire-age” in Cox analysis which showed SS RRs for four out of five cancers, survival analysis failed to show any SS excess in any cause-specific cancer by other analytical methods	52 years’ follow-up Exposure estimation useful Major limitation is no peak exposure estimation available in war subcohort, which has the SS lymphosarcoma excess

Table 7-1. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—monomer production (continued).

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Cowles et al. (1994)	Cohort of monomer production workers from 1941-1948 and from 1970-1994 5 years or 50% of total duration worked in jobs with potential to 1,3-butadiene exposure Mortality follow-up from 1948-1989 (614 employees) Morbidity follow-up from 1982-1989 (438 employees) Hematologic data analyses 1985-1991 (429 employees)	None	No excess observed in either mortality or morbidity study No hematologic differences found between the exposed and nonexposed employees	Very small cohort Exposure is not certain Deaths are very few 50% of cohort hired after 1970 when exposures were low Not enough latent period has elapsed
Ward et al. (1995, 1996c)	Cohort of 364 male employees who had worked between 1940 and 1979 Employees who had worked in monomer production during World War II Follow-up through December 31, 1990 U.S. population Kanawha County population	Departments where production of 1,3-butadiene occurred	For lymphosarcoma and reticulosarcoma SS SMR = 577 SS SMR = 1980 for more than 2 years of employment and 30 years of latency	Small cohort Adjustment for work in acetaldehyde unit showed no association with observed lymphosarcoma deaths No adjustments for confounding exposures to other chemicals No exposure information available

SS = Statistically significant.
SNS = Statistically nonsignificant.
NSMR = National standard mortality ratios.
LSMR = Local standard mortality ratios.
IH = Industrial hygiene.

7.2.1.2. *Matanoski et al. (1989): Epidemiologic Data Related to Health Effects of 1,3-Butadiene*
Matanoski et al. (1990): Mortality of a Cohort of Workers in the Styrene-Butadiene Polymer Manufacturing Industry (1943–1982)

These two publications essentially reported the same updated reanalysis of the earlier cohort. In addition, Matanoski et al. (1989) presented the results of the nested case-control study in this population. Three methodological differences in the original analysis (Matanoski et al., 1987) and the reanalysis presented in these two publications should be noted: extension of follow-up through 1982, fewer workers whose vital status was unknown (3.4% vs. 6.6% in the earlier report), and deletion of workers from the Canadian plant who had relatively short-term exposure (i.e., workers who had worked for less than 10 years or who had not reached the age of 45 during employment). Analytical methods were essentially unchanged from the earlier analysis.

In addition to information received from the SSA and the Motor Vehicle Administration, follow-up through local plant beneficiary records and the NDI was done to assess the vital status of the study cohort. Follow-up procedures for Canadian workers were similar to the earlier study. Death certificates were obtained from local health departments. The total cohort was reduced from 13,920 to 13,422 in this study. Of 12,113 workers for whom vital status was successfully traced, 23% (2,784) were still working in the plants, 53.4% (6,472) were alive but not working in the plants, 20.2% (2,441) had died, and vital status was unknown for 3.4% (416). The racial distribution was 75% whites, 10% blacks, 15% unknown (presumed to be white for the analysis), and less than 1% other.[5] Death certificates were obtained for 97.2% of the deceased individuals and were coded by a trained senior nosologist, using the eighth revision of the ICD.

Data analyses were done using age, race, calendar time, and cause-specific U.S. population rates. A modified life-table program by Monson (1974) was used. The person-years were calculated through December 31, 1982. First-year work experience was omitted from person-years because one of the inclusion criteria was that an individual had to have worked for at least 1 year. A total of 251,431 person-years were accrued, of which 226,475 were contributed by whites.

Statistically significant lower SMRs for all causes of death (81) and for all cancers (85) were virtually the same as in earlier studies. The SMRs for all causes of death by 5-year calendar period demonstrated increasing SMRs with increasing time period, indicating a “healthy worker” effect in earlier calendar years. Blacks showed higher SMRs than whites in later years. A statistically significant excess for all causes of deaths was observed for blacks in the last 3 years

[5]The percentages, which are quoted from the paper, add up to 101. This is due to rounding of the numbers by the authors of the paper.

of follow-up (SMR = 134, 95% CI = 101-175, O = 54). Most of the cause-specific cancer SMRs showed deficits in both races. A few cancer sites demonstrated excess mortality in both races. Among whites, excesses were observed for esophageal cancer, kidney cancer, Hodgkin's disease, and other lymphohematopoietic cancers. Among blacks, excesses were observed for stomach, liver, and prostate cancer; all lymphohematopoietic cancers; lymphosarcoma; leukemia; and other lymphohematopoietic cancers. None of the excesses were statistically significant.

Because the risks for kidney, digestive, and lymphohematopoietic system cancers approached those of the reference population, which was unusual for an occupational cohort with low overall risks, investigators further analyzed the data by work areas. For production workers, deaths from lymphohematopoietic cancers, Hodgkin's disease, and leukemia were nonsignificantly increased for the total cohort and among whites (except for leukemia). The only significant excess observed for the total cohort was for other lymphohematopoietic cancers, which included NHL and multiple myeloma (SMR = 260, 95% CI = 119-494, O = 9). Among blacks, however, statistically significant excesses were observed for all lymphohematopoietic cancers (SMR = 507, 95% CI = 187-1,107, O = 6) and leukemia (SMR = 655, 95% CI = 135-1,906, O = 3). The other two excesses observed among blacks for lymphosarcoma and other lymphohematopoietic cancers (including NHL and multiple myeloma) were based on one and two cases, respectively, none being statistically significant (p>0.05).

Among white maintenance workers, no excesses of lymphohematopoietic cancers were found, with the exception of Hodgkin's disease (SMR = 170, 95% CI = 35-495), based on only three deaths. However, rates were nonsignificantly increased for digestive tract malignancies (i.e., esophagus, stomach, and large intestine). Among black maintenance workers, nonsignificant excesses were observed for cancer of the rectum and stomach. For utility workers, the numbers were reported to be too small to reach firm conclusions about risks. For the "other" category of workers (including laboratory workers, management, and administrative workers), excesses were observed for Hodgkin's (SMR = 130, 95% CI = 16-472, O = 2) and leukemia (SMR = 116, 95% CI = 43-252, O = 6) among whites and for leukemia (SMR = 246, 95% CI not given, O = 1) among blacks. Nonsignificant increased SMRs for the digestive system among blacks were also observed for the stomach, liver, and pancreas, all of which were based on fewer than five cases.

Analysis by duration of work or latency for the total cohort did not show an increase in hematopoietic cancers.

This is still the largest cohort of SBR workers. The increased follow-up, better tracing, and exclusion of short-term workers from the Canadian plant have resulted in demonstrating the excess mortality from malignancies of the lymphohematopoietic system, digestive system, and kidney. However, the limitations of the earlier study of this cohort (i.e., lack of exposure data and inclusion of less than 50% of the population in the follow-up cohort) still exist. The

magnitude of the bias introduced by exclusion of workers (2,391) due to missing information on total work history or crucial information such as date of birth could be substantial. Although an attempt was made to correct for race, the race was unknown for 15% of the eligible cohort, and this segment was assumed to be white for the analysis. This would result in an overestimation of rates in blacks and an underestimation of rates in whites. No explanation was given as to how the total eligible population of 13,422 was reduced to 12,113. No data were presented by individual plants, but as indicated in the earlier study, only four plants had follow-up starting from 1943, whereas in the other four plants the starting dates of the follow-up ranged from 1957 to 1970; thus, these latter four plants may not have had long enough follow-up for malignancies to develop.

7.2.1.3. *Matanoski et al. (1989): Epidemiologic Data Related to Health Effects of 1,3-Butadiene*
Santos-Burgoa et al. (1992): Lymphohematopoietic Cancer in Styrene-Butadiene Polymerization Workers

To elucidate the separate contributions of 1,3-butadiene and styrene to the cancers identified in the updated cohort study, a nested case-control study of this cohort of SBR workers was conducted using estimates of exposure to 1,3-butadiene and to styrene for each job. Fifty-nine cases and 193 controls (matched for duration of work) were included in the analysis. Among the case group were 26 cases of leukemia; 18 of other lymphatic cancers, which included 10 multiple myelomas and 7 NHLs; 8 Hodgkin's lymphomas; and 6 lymphosarcomas.

Cases (workers who had lymphohematopoietic cancer as either the underlying or contributory cause of death on death certificates) arose from the original eight plants with the same selection criteria for the eligibility of that cohort (13,422), with the exception of the Canadian plant. For the Canadian plant, the restriction of either 10 years of work or those who had reached age 45 during employment was dropped from the selection of cases, which added two more cases to lymphohematopoietic cancers. Another four cases were added in which individuals had died of another cause of death but had a lymphohematopoietic cancer at the time of their death. Two cases were deleted from the final analysis, one lymphosarcoma due to lack of any controls and one NHL due to lack of job records from which exposure could be identified.

Controls included workers from the same cohort who were alive or had died of any cause other than malignant neoplasms. Controls were individually matched to cases by plant; age; hire year; employment as long as or longer than the case; and, if the control was dead, then survival to the death of the case. Based on these criteria, an average of 3.3 controls per case was selected instead of 4 controls per case as intended by the investigators. This average of 3.3 controls per one case had more than a 90% chance of detecting the twofold risk from exposure to 1,3-

butadiene. Both cases and controls had about 15 years of employment and were hired at 36 to 37 years of age, somewhat older than usually seen in occupational populations.

Exposures to 1,3-butadiene and styrene were calculated from the job records of each subject, the number of months each job was held, and an estimate of the 1,3-butadiene and styrene exposure levels associated with that job. Both the job identification and exposure estimation were done independently and without knowledge of case or control status of the subjects. To estimate 1,3-butadiene and styrene exposures, all jobs within the rubber industry were ranked from 0 to 10 by a group of senior engineers with many years of experience in the industry. One-third of the jobs were determined to have no routine exposure, but almost all jobs were thought to have intermittent exposure. Cumulative dose for both styrene and 1,3-butadiene was calculated using the score and duration for each job in the participants' work history. Because the distribution of exposure scores was skewed to the right, a log transformation of the scores was used in the analyses. As the logarithmic transformation approached normal distribution, only the transformed exposure variables were used for the analyses.

Analyses were done by using "ever/never exposed" categories to both butadiene and styrene and using high-exposure vs. low-exposure groups (based on mean log exposure cumulative rank for each substance determined by combining cases and controls). Both conditional (matched) and unconditional (unmatched) logistic regression analyses were performed. Odds ratios (OR) for matched sets were then calculated based on maximum likelihood estimates of the OR, and test-based confidence limits around the OR were calculated.

Unadjusted for the presence of the other chemicals and unmatched, analyses by "ever/never exposed" to butadiene and styrene found significantly increased relative odds for leukemia for both high and low exposures. Relative odds for butadiene were 6.82 (95% CI = 1.10-42.23) and for styrene were 4.26 (95% CI = 1.02-17.78).

Nonsignificant excesses were also observed for all lymphohematopoietic and other lymphohematopoietic cancers for exposures to both butadiene and styrene. Other excesses were for Hodgkin's disease among workers exposed to butadiene and lymphosarcoma among workers exposed to styrene.

Matched analyses demonstrated that risk for all lymphohematopoietic neoplasms was significantly increased among workers exposed to butadiene (OR = 2.30, 95% CI = 1.13-4.71). Separate evaluation of these neoplasms revealed that most of the association could be explained by a significant excess risk for leukemia (OR = 9.36, 95% CI = 2.05-22.94), but other cancers in this group were not significantly elevated. Leukemia also showed a threefold increase associated with styrene exposure (OR = 3.13, 95% CI = 84-112).

Conditional logistic regression was used to separate the risks associated with each of these substances. Again, there was a significant excess of leukemia associated with butadiene (OR = 7.61, 95% CI = 1.62-35.64) and a nonsignificant excess of leukemia associated with

styrene exposures (OR = 2.92, 95% CI = 0.83-10.27). When exposures to both chemicals were evaluated in the model as dichotomous variables, only butadiene was found to be associated with leukemia (OR = 7.39, 95% CI = 1.32-41.33).

To determine if specific jobs within the SBR industry might explain some of the risk of leukemia, the investigators categorized each worker according to the longest job held. A mixed-job category that combined utilities, operation services, and laboratory jobs was associated with relative odds of 3.78 (95% CI = 1.2-11.9). When butadiene was added to the model, the OR increased to 6.08 for the mixed-job category (95% CI = 1.56-23.72). The relative odds were 13.3 (95% CI = 2.24-78.55) for association between butadiene exposure and risk of leukemia adjusted for mixed jobs in this model. Thus, both the mixed-job category and exposure to butadiene seem to contribute to the risk of leukemia. The trend test for increasing risk of leukemia with increasing exposure levels of butadiene (0 through 8) was statistically significant (trend = 3.76, p=0.05). A similar trend was not found for styrene. The higher risk of leukemia seen in the original cohort for black workers could not be evaluated adequately because race was partially controlled in this nested case-control study.

Unlike the mortality study of this cohort, the case-control study did not show other lymphomas to be associated with production jobs, but the number of cases was small. Interestingly, when each chemical was analyzed by stratification, there was an excess risk for butadiene exposure when exposure to styrene was low (OR = 6.67, 95% CI = 1.06-42.7). A similar nonsignificant increase also was observed for styrene when butadiene exposure was low. This might have resulted from small numbers of NHLs or multiple myelomas included together with potentially different etiologies or correlated exposure data. Thus, investigators suggest that further evaluation of each cancer in this other-lymphoma category should be performed separately.

Investigators also caution that estimated exposures in this study were crude and not substantiated by monitoring data. As they correctly pointed out, the original ordinal rank does not create a perfect exposure scheme. The distribution of ranks was skewed to the right and had to be log-transformed to differentiate between no exposure and low exposure. Matching on duration of work may have overmatched the dose and resulted in underestimation of the risk. Validation of diagnosis of lymphohematopoietic malignancies was not done in this study. The panel ranked 71% of the jobs in ranks of two or less; thus misclassification of exposure based on the estimated exposure by job as judged by the panel members is quite possible. Because the panel members were blind concerning the status of the individual being the case or control, the distribution of misclassification should be the same in cases and controls.

7.2.1.4. *Matanoski et al. (1993): Cancer Epidemiology Among Styrene-Butadiene Rubber Workers*

This was an effort to verify the findings of the investigators' earlier nested case-control study among styrene-butadiene production workers (Santos-Burgoa et al., 1992). This study had shown statistically significant elevated relative odds for leukemias. The results from the analysis conducted with a new set of three controls per case were similar to the results from the earlier study. The new controls were matched to all the variables except duration of work with the case. Comparability between the previous and new controls was checked by reviewing the information on cases and controls from the earlier study. To verify that the cause of death was correctly coded on the death certificates, hospital records for cases were obtained. Of their initial 59 cases, diagnosis was verified for 55 cases by hospital records review. Records were available for 54 cases and the diagnosis was correct in all cases. Two cases were omitted for incorrect diagnosis and one was added that was identified by hospital record review. These omissions and additions were in the categories of NHL (ICD 202) and lymphosarcoma (ICD 200). Records were available for 25 out of 26 leukemia cases and were found to be correctly coded on the death certificates.

Exposure estimation was done based on measurements provided by seven rubber plants, the International Institute of Synthetic Rubber Producers, and NIOSH. Although there was variability among plants, a significant correlation was observed between the log transformed data provided by the company and the ranks of 464 job- and area-specific titles. Of the seven plants that provided exposure measurements for butadiene, three had geometric means. Thus, using the geometric means, the cohort data were reanalyzed for these three plants. The workers who were hired before 1960 and had 10 or more years of service showed excesses for all lymphohematopoietic cancers (SMR = 163, 95% CI = 113-227, O = 34) and leukemia and aleukemia (SMR = 181, 95% CI = 101-299, O = 15).

This reanalysis of earlier data with new information on exposure estimation validates the earlier results of these investigators.

7.2.1.5. *Matanoski et al. (1997): Lymphohematopoietic Cancers and Butadiene and Styrene Exposure in Synthetic Rubber Manufacture*

This is a further analysis of exposures (in synthetic rubber production plants) to the case-control population that was studied earlier by Matanoski et al. (1993). Selection of 1,242 controls was based on the frequency distribution of population sizes across the eight plants. Potential for exposure for controls was slightly longer since they had to live at least as long as or longer than the cases. Thus the variable of "duration worked" was included in the model.

Of eight plants, exposure measurements were obtained from seven plants. Several exposure measurements for 1,3-butadiene (3,952) and styrene (3,649) were obtained, most being

personal monitoring data. Based on these, job exposures were calculated, assuming that relative exposures of jobs were similar across the plants. Using z-score transformation, exposure estimation was carried out for all jobs with missing measurements, for each plant. Thus, using these job- and plant-specific exposure levels, as well as job histories of cases and controls, cumulative exposures were calculated in ppm multiplied by number of months exposed (ppm-months). Time-weighted average (TWA) exposures in ppm were also calculated based on total cumulative exposures in ppm-months divided by the total time employed for both styrene and 1,3-butadiene for each individual.

Using SPSS unconditional logistic regression, analysis was done. Beta values were converted into corresponding odds ratios for TWA exposure of log normal (ln) ppm for each chemical. The variables used in the multivariate models included birth year, year of hire (prior to or after 1950), age at hire, race, and duration worked. Step-down analysis was done.

TWA exposure of ln (ppm + 1) of 1,3-butadiene was associated with leukemia (OR = 1.5, 95% CI = 1.07-2.1) and Hodgkin's lymphoma (OR = 1.73, 95% CI = 0.99-3.02). On the other hand, average time-weighted exposure of ln (ppm + 1) of styrene was significantly associated with all lymphohematopoietic cancers (OR = 2.2, 95% CI = 1.46-3.33): NHLs, which included ICD codes 200 and 202 (OR = 2.67, 95% CI = 1.22-5.84); lymphosarcoma (OR = 3.88, 95% CI = 1.57-9.59); and myeloma (OR = 3.04, 95% CI = 1.33-6.96).

The category of all leukemias was significantly associated with 1,3-butadiene TWA exposure of ln (ppm + 1) as well as longest job in "labor, or laboratory," with ORs of 1.49 (95% CI = 1.05-2.11) and 2.64 (95% CI = 1.21-5.30), respectively. The subtype lymphoid leukemia was associated with 1,3-butadiene TWA exposure of ln (ppm + 1), while myeloid leukemia was associated with the category of longest job in "services, labor, or laboratory." Both were statistically significant. The analysis by cumulative exposure found higher risks for workers who had their cumulative dose in a shorter period (i.e., higher intensity) than for workers who had the same cumulative dose in a longer period of time.

This analysis confirms the association between TWA exposure and cumulative exposure to 1,3-butadiene and occurrence of leukemia. In this study, an association between styrene and NHL is found for the first time. Investigators are unable to explain this association successfully, but they qualify it by stating that probably both styrene and 1,3-butadiene are associated with lymphohematopoietic cancers. Since exposures to these two chemicals are closely related in the SBR industry, it is difficult to tease out the differences between these two; thus styrene may be masking the effect of 1,3-butadiene and its association with NHL. The authors correctly point out that exposure measures represent the more recent exposures in the industry, the IH data being available only for the past 15 to 20 years. There may have been misclassification due to individual recollection in assigning the job exposures. Furthermore, the authors assert that

relative exposure differences for specific jobs have changed greatly over time and that further work is required to correct these differences.

7.2.2. Cohort Identified by University of Alabama (UAB) Investigators

7.2.2.1. *Delzell et al. (1996): A Follow-Up Study of Synthetic Rubber Workers*

A retrospective cohort mortality study was conducted by Delzell et al. (1996) of synthetic rubber workers employed in seven U.S. and one Canadian plant. Of the eight plants, seven plants (including the Canadian plant) were studied by JHU (Matanoski and Schwartz, 1987; Matanoski et al., 1989, 1990, 1993; Santos-Burgoa et al., 1992) and one (two initial plants combined into one) by Meinhardt et al. (1982). Of the seven plants studied by JHU, one located in Texas that opened in 1970 was not included in UAB study. The cohort comprised all the male workers who had worked for at least 1 year between January 1, 1943, and January 1, 1992 (49 years), which was the end of the follow-up period. The follow-up period was shorter for plants 1, 2, and 6 because the complete records of employees from these plants were available much later than 1943. The Canadian plant (plant 8) also had a shorter follow-up period because follow-up of men who had left employment before 1950 was not feasible.

Since the inclusion criteria for this study were different, there were some additions and deletions to the earlier study cohort. The vital status was assessed by using plant records; the SSA's death master file; the NDI; DMV records of Texas, Louisiana, and Kentucky for the U.S. plants; and plant records and record linkage with the Canadian Mortality Data Base for the Canadian plant.

Death certificates were acquired from plant and corporate offices and from state vital records. The underlying cause of death was coded by a trained nosologist using the ninth revision of the ICD. Any cancer was coded as a contributory cause of death. For the Canadian decedents, the underlying cause of death was used from Canadian death registration and coded according to the ICD revision in effect at the time of death. All ICD codes were converted to eighth revision codes for analysis. The Ontario Cancer Registry provided the information on incident cancer cases (including the date of diagnosis, primary site, ninth revision ICD code, and histologic classification) for the study period.

Mortality analysis included computation of SMRs using the U.S. male general and state population rates and Ontario male rates; SMRs by quantitative exposure (cumulative ppm-years and peak ppm-years) to 1,3-butadiene, styrene, and benzene; and stratified internal comparisons. Various within-cohort analyses were conducted using Poisson regression models.

This study included exposure estimation for each individual. A detailed description of this estimation appears in Section 7.2.2.2, Macaluso et al., 1996. Complete work histories were available for 97% of the cohort. Analysis for process group was conducted on the workers from

all the plants. Subgroup analyses were restricted to 6 plants (1,354 workers from 2 plants were excluded from the analyses due to the lack of information on specific work areas).

Of 15,649 males who had worked in SBR and related processes, 13,586 were white and 2,063 were black. Vital status assessment indicated that 10,939 (70%) workers were alive, 3,976 (25%) were dead, and 734 (5%) were lost to follow-up. Death certificates were acquired for 3,853 (97%) individuals. A total of 386,172 person-years (336,532 for whites and 49,640 for blacks) was accrued.

Total cohort analysis found SMRs of 87 and 93 for all causes and all cancers, respectively. The SMR for leukemia was 131 based on 48 observed deaths (95% CI = 97-174). The SMRs for lymphosarcoma and other lymphopoietic cancers were close to null. Subcohorts of whites, blacks, ever-hourly, and never-hourly showed a similar pattern of below-null results for both all causes and all cancer deaths.

Ever-hourly was the only subcohort in which statistically significant excesses were found for leukemia. The SMR was 143 (95% CI = 104-191, O = 36) for this subcohort. For white ever-hourly workers, the SMR was 130 (95% CI = 91-181, O = 36), while for blacks the SMR was 227 (95% CI = 104-431, O = 9). Further analyses of this ever-hourly subcohort by year of death (<1975, 1975-84, 1985+), year of hire (<1950, 1950-59, 1960+), and age at death (<55 years, 55-64 years, 65+ years) showed statistically significant leukemia excess SMRs for 1985+ year of death (SMR = 187, 95% CI = 111-296, O = 18), 1950-59 year of hire (SMR = 200, 95% CI = 122-310, O = 20), and <55 years at death (SMR = 179, 95% CI = 104-287, O = 17). The lymphosarcoma SMR for this subcohort was 102 based on 4 cases, while the SMR for other lymphopoietic cancers was 106 based on 17 cases. Neither of these excesses was statistically significant.

When this subcohort of ever-hourly workers was further restricted to >10 years of employment and >20 years since hire, leukemia SMRs of 224 (95% CI = 149-323, O = 28) for all workers, 192 (95% CI = 119-294, O = 21) for whites, and 436 (95% CI = 176-901, O = 7) for blacks were observed. Analysis by presence of solution polymerization versus absence of such process in this restricted cohort resulted in leukemia SMRs of 209 (95% CI = 100-385) and 228 (95% CI = 135-160), respectively.

When analyses were done by various process groups, more than twofold increases were observed for leukemia in polymerization process, SMR = 251 (95% CI = 140-414, O = 15); coagulation process, SMR = 248 (95% CI = 100-511, O = 7); maintenance labor, SMR = 265 (95% CI = 141-453, O = 13); and laboratory workers, SMR = 431 (95% CI = 207-793, O = 10). Analysis by further restricting the process groups by 5+ years of employment and 20+ years since hire in each group showed excesses in leukemia SMRs in the same processes as above.

Analyses by mutually exclusive process groups showed excesses for ever in polymerization and never in maintenance labor or laboratories (O/E = 8/4.7), ever in maintenance

labor and never in polymerization or laboratories (O/E = 6/3.7), and ever in laboratories and never in polymerization or maintenance labor (O/E = 8/1.6). Within the labor group, leukemia increase was observed for workers ever in maintenance labor and never in production labor (O/E = 11/3.8). On the other hand, for workers in production labor and never in maintenance labor, the leukemia excess was negligible (O/E = 2/1.4). No excess mortality from leukemia was observed among ever in finishing and never in polymerization process workers (O/E = 4/4.5).

In six out of eight plants, increased SMRs for leukemia were observed in ever-hourly workers, exhibiting an internal consistency among plants. None of the excess was statistically significant.

A statistically nonsignificant deficit was observed for lymphosarcoma in total cohort based on 11 cases (SMR=80). Although some subcohort groups showed SMR excesses for this specific cause, i.e., in ever-hourly white men during 1985-1991 (SMR=284, O=4), in field maintenance workers (SMR=219, O=7), in production laborers (SMR=263, O=2), and in maintenance laborers (SMR=188, O=3), none was statistically significant and most were based on small numbers. Among the 42 deaths observed from other lymphohematopoietic cancers, 17 were NHL. As per International Classification of Diseases for Oncology (ICD-O) guidelines (see footnote 4), the diagnosis of lymphosarcoma is no longer in use; instead they are classified as lymphomas (Berard and Hutchinson, 1997). Thus, addition of 17 cases of NHL observed in this study with 11 cases of lymphosarcoma results in 28 cases of lymphomas. Furthermore, it should be noted that survival rates for NHL are high. A mean 5-year survival rate for males diagnosed in 1974-1989 was reported to be 48% by Miller et al. (1993).

An unpublished report by the same authors (Delzell et al., 1996) submitted to the International Institute of Synthetic Rubber Producers (IISRP) in October 1995 (Delzell et al., 1995) included many more results of analyses of this cohort that are relevant to this assessment. A review of the unpublished results is presented in the following paragraphs.

Various analyses by estimated 1,3-butadiene and styrene exposures were conducted. The RRs calculated by Poisson regression for 1,3-butadiene ppm-years adjusted for styrene ppm-years, age, years since hire, calendar period, and race for 0, >0-19, 20-99, 100-199, and 200+ ppm-years were 1, 1.1, 1.8, 2.1, and 3.6, respectively. When analysis was restricted to leukemia as the underlying cause of death and person-years 20+ years since hire, the results were similar. Analysis restricted to ever-hourly also showed positive results for butadiene. Various analyses were conducted by using alternate ppm-years categories of exposure. All the analyses consistently showed similar results, strengthening the association between 1,3-butadiene and occurrence of leukemias. It is interesting to note that all the leukemia subjects who were exposed to 1,3-butadiene were also exposed to styrene. Only two leukemia cases had exposure to styrene but not to 1,3-butadiene.

Analysis by 1,3-butadiene peak-years and styrene peak-years demonstrated an association with 1,3-butadiene peak-years and occurrence of leukemia when adjusted for styrene peak-years, 1,3-butadiene and styrene ppm-years, and other covariates. The association, however, was irregular. A similar analysis for styrene peak-years was weak and imprecise.

The investigators also conducted a cancer incidence study in the Canadian plant. Information was obtained from the Ontario Cancer Registry from 1965 to 1992. Standard incidence ratios (SIRs) were calculated by using the male general population of Ontario. No increased incidence was found for any cancer in this study.

This is a well-designed, -conducted, and -analyzed study. The main strengths of the study are large cohort size; long follow-up period (49 years); availability of exposure estimations on each individual, processes, and tasks; and in-depth analyses using both the general population and internal comparison groups.

There are a few limitations, as correctly pointed out by the investigators. The cause of death on death certificates was not confirmed by medical records. Histologic typing was not available for leukemias. These limitations may have led to misclassification. Furthermore, as pointed out in the Macaluso et al. (1996) study, there may have been misclassification of exposure, but this was thought to be nondifferential by investigators. Two plants were eliminated from the final analysis due to the lack of detailed work histories. Although this may have resulted in fewer uncertainties, valuable data may have been lost. Nevertheless, the association between exposure to butadiene and occurrence of leukemia was present among both white and black workers and was fairly consistent across plants.

7.2.2.2. *Macaluso et al. (1996): Leukemia and Cumulative Exposure to Butadiene, Styrene, and Benzene Among Workers in the Synthetic Rubber Industry*

A cohort mortality study conducted in synthetic rubber workers by Delzell et al. (1996) (Section 7.2.2.1) had a component of exposure estimation. The exposures to 1,3-butadiene, styrene, and benzene were estimated by Macaluso et al. (1996).

An exposure estimation was conducted on each and every worker based on detailed work histories, work area/job specification, IH monitoring survey records, IH recommendations, various records from the plants, historical aerial pictures, use of protective and safety equipment, walk-through surveys, and interviews with plant management as well as long-term employees in specific areas/jobs. The quantitative exposure estimation was based on process analysis, job analysis, and exposure estimation for specific tasks in different time periods. The job-exposure matrices (JEMs) were computed for 1,3-butadiene, styrene, and benzene, which were linked to work histories of each employee to compute the cumulative exposure indices.

Quantitative estimates of exposure to 1,3-butadiene and styrene were based on background exposure plus task-specific exposure, using multiple exposure and point source

models, respectively. Input variables for these models were derived from several information sources described earlier. Limited validation of exposure estimates was attempted by comparing the available IH data from the 1970s and 1980s as well as actually measuring the air concentrations of 1,3-butadiene and styrene under controlled conditions. The latter method showed good agreement among the methods of sampling, whereas the comparison of IH data indicated overestimations of 1,3-butadiene exposure.

For each job, 8-h TWA intensities and the number of peak exposures (15-min exposures over 100 ppm) were calculated. Based on job exposures, a JEM database was developed that was linked with individual work histories to develop individual quantitative work exposure estimates. For each individual, the exposure indices were multiplied by the length of employment in that particular process or job and were added up for the total employment period in various jobs to estimate the cumulative exposure.

Mortality analysis was done by calculating the SMRs and RRs using estimated quantitative exposures to 1,3-butadiene, styrene, and benzene. Both cumulative ppm-years and peak-years were calculated for each individual in the study. Person-year data were grouped by 1,3-butadiene, styrene, and benzene ppm-years for both SMR analyses as well as RR analyses. Comparability between cohort mortality rates and general population reference mortality rates was assured by limiting the SMR analysis to the individuals whose underlying cause of death was listed as leukemia (51 people). Risk ratios were computed by the Mantel-Haenszel method and the 95% CI were computed by the Breslow method. Poisson regression models were used for adjustment of multiple confounders and to compute within-cohort mortality rates, and the X^2 test for linear trend was used to examine the dose response.

Work histories were available for 97% of the population. Fifty-two in-depth interviews with plant management and long-term employees identified 446 specific tasks/work areas with potential for 1,3-butadiene, styrene, and benzene (3 plants only) exposure. Eight-hour TWAs for 1,3-butadiene, styrene, and benzene were 0 to 64 ppm, 0 to 7.7 ppm, and <1 ppm, respectively, the median exposures being <2 ppm for 1,3-butadiene and 0.5 to 1.1 ppm for styrene.

Exposure analysis found that 75% of the cohort was exposed to 1,3-butadiene, 83% was exposed to styrene, while only 25% was exposed to benzene. The median cumulative exposure to 1,3-butadiene, styrene, and benzene was 11.2, 7.4, and 2.9 ppm-years, respectively. The exposure prevalence as well as median cumulative exposure were higher in individuals who had died of leukemia. Among the leukemia decedents, 85% had exposure to 1,3-butadiene, with their median cumulative exposure being 36.4 ppm-years. This exposure was two times higher compared with all decedents and three times higher compared with all other employees. The exposure to styrene was present in 90% of leukemia decedents, with median cumulative exposure being 22.4 ppm-years, two times and three times higher compared with all the decedents and all other employees, respectively. Benzene exposure was found to be less frequent among leukemia

decedents compared with all other employees. Analysis by benzene exposure showed no association with the occurrence of leukemia after adjustment for 1,3-butadiene and styrene.

Leukemia SMRs increased with increasing cumulative exposure to 1,3-butadiene as well as styrene. Mortality RRs computed for cumulative 1,3-butadiene exposure adjusted for race, age, and cumulative styrene exposure also showed increasing RRs for increasing cumulative exposure to 1,3-butadiene. The adjusted RRs for cumulative exposures of butadiene of 0, <1, 1 to 19, 20 to 79, and 80+ ppm-years were 1, 2.0, 2.1, 2.4, and 4.5, respectively. The linear X^2 test for trend was statistically significant (p=0.01). When similar RRs were computed for styrene exposure, neither showed a consistent pattern nor a trend of increasing risk with increasing exposure. A similar trend test was statistically not significant.

Analysis by exclusion of the nonexposed population resulted in RRs of 1, 1.5, and 1.7 for 0.1 to 19, 20 to 79, and 80+ ppm-years of the cumulative exposures of 1,3-butadiene. The linear trend test was statistically significant (p=0.03), substantiating the earlier finding of increasing risk of leukemia with increasing cumulative exposure to 1,3-butadiene. Although the same analysis suggested increasing risk of leukemia with increasing cumulative exposure to styrene after adjustment for 1,3-butadiene and other covariates, the results were imprecise and statistically nonsignificant.

Neither a positive nor negative interaction was found between the cumulative exposures to 1,3-butadiene and styrene.

For the last decade or so, epidemiologists have been including exposure estimation in their studies. The methods used and efforts made to do exposure estimations are improving but variable. This study is one of the best efforts of exposure estimations to date. The investigators have used many available methods to come up with best estimates of exposures of 1,3-butadiene, styrene, and benzene. They also have validated these estimates on a smaller scale. Although this is considered the best effort, it should be noted that these are estimates and not actual measurements. Two plants were eliminated from the analysis because detailed work histories were lacking. Thus it is possible that individuals may have been misclassified with respect to process or job, resulting in either over- or underestimations of exposure. However, there is no reason to believe that the misclassification of exposure occurred only in individuals who had died of leukemia.

7.2.2.3. *Sathiakumar et al. (1998): Mortality from Cancer and Other Causes of Death Among Synthetic Rubber Workers*

This paper presented further analysis of the cohort studied by Delzell et al. (1996) and Macaluso et al. (1996). Thus, the follow-up period, methods of data collection for exposure, and health endpoints are the same as Delzell et al. (1996). Statistically significant deficits were observed for all causes of deaths, all cancers, and quite a few other cause-specific cancer and

noncancer deaths in the total cohort. Except for statistically significant elevated SMR for leukemia in the subcohort of ever-hourly workers, no other excess was observed for any other cancer or noncancer endpoint. When this ever-hourly subcohort was further restricted to worked for $\geq$ 10 years and $\geq$ 20 years since hire by race, in addition to excess leukemia in both whites and blacks, statistically significant increased SMR was observed for cancer in the large intestine among blacks (SMR = 231, 95% CI = 119-402, O = 12). Investigators examined the cause-specific deaths for cancers of the lung and large intestine in the ever-hourly subcohort by different characteristics such as year of death, years since hire and years worked, and year of hire. The only statistically significant excess was found for lung cancer among whites who had died between 1975 and 1984. Among blacks, excesses for lung cancer were found in workers who had died after 1985 and workers who had more than 20 years since hire and had worked for less than 10 years. Excess for cancer of the large intestine was found in black workers who had more than 20 years since hire and had worked for more than 10 years. When analysis was done by process group, excess lung cancer was observed for the maintenance group among both whites and blacks while most of the other groups had deficit for this cancer. Laryngeal cancer was increased only in whites among production, maintenance, and other operation workers.

The limitations listed in Delzell et al. (1996) and Macaluso et al. (1996) about misclassification apply to this study too. Adjustment for smoking was not performed; thus, it is difficult to know whether the observed excesses in some subgroups for lung and laryngeal cancer had any causal association with 1,3-butadiene.

Please note that the results for lymphohematopoietic cancer reported in this paper are not included in this writeup to avoid repetition.

Studies in polymer production workers are summarized in Table 7-2.

7.3. POPULATION STUDY

7.3.1. Loughlin et al. (1999): Lymphatic and Haematopoietic Cancer Mortality in a Population Attending School Adjacent to Styrene-Butadiene Facilities, 1963–1993

This study was conducted in the Port Neches-Groves High School, southeastern Texas, to address the communities' concern for the students' health. The Texas Department of Health had conducted a retrospective cohort mortality study of female graduates of the same high school in 1980 (unpublished report authored by R. K. Donelson) in which no excess of lymphhematopoietic cancer was observed. The current investigation by Loughlin et al. (1999) is a follow-up of the earlier Health Department study. The investigators included students who graduated between 1963 and 1993, including males, and followed them through 1995.

The cohort was assembled by including all the seniors pictured or listed in the high school yearbooks for the school years 1962-63 to 1992-93. Any student who attended the school

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—polymer production.

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Matanoski et al. (1989 and 1990)	Update of the cohort from Matanoski and Schwartz (1987) Cohort mortality of 8 SBR polymer production plant workers Reduced cohort of 13,422 followed through 1982 Worked for at least 1 year Comparison group U.S. population	Divided in four major areas based on the longest job held: • Production workers • Utility workers • Maintenance workers • All other work sites	Among production workers: • SS SMR = 260 for other lymphohematopoietic cancers in whites • SS SMR = 507 for all lymphohematopoietic cancers • SS SMR = 655 for leukemia in blacks No relation observed with latency or duration of employment	Largest cohort mortality study of SBR workers Lack of exposure data Exclusion of 50% of the population in the follow-up Four plants had follow-up ranging from 12 years to 25 years; may not be enough time for malignancies to develop
Matanoski et al. (1989) Santos-Burgoa et al. (1992)	Nested case-control study Cases: • Of leukemia 26 • Of other lymphatic cancers 18 Controls matched on: • Plant, age, hire year, employment duration, survival to the death of the case • An average 3.3 controls (instead of intended 4) were selected	Exposure to 1,3-butadiene and styrene was done by job identification and levels associated with that job Estimations of job and exposure levels were done independently of the status of the case or control The jobs were ranked from 0 to 10 Cumulative dose was calculated using the score and duration for each job	For 1,3-butadiene: • Ever/never exposure - SS OR = 6.82 (high) and 4.26 (low) were found for leukemia • Matched analyses - SS OR = 2.3 for all lymphohematopoietic cancer - SS OR = 9.36 for leukemia • Conditional analyses • SS OR = 7.61 for leukemia	One of the strengths is that an attempt was made to estimate actual exposure Matching may have overmatched the dose Lack of validation of diagnosis of hematopoietic malignancies may have resulted in misclassification Misclassification of exposure based on job categories

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—polymer production (continued)

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Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Matanoski et al. (1989), Santos-Burgoa et al. (1992) (continued)		Log transformation of scores used in analyses Analyses were done by: • Ever/never exposed • High vs. low exposure • Both matched (conditional) and unmatched (unconditional)	• SS OR = 6.67 for other lymphoma when styrene exposure was low • SS trend of 3.76 was found for increased risk of leukemia with increasing exposure levels of butadiene	
Matanoski et al. (1993)	Same as nested case-control study A new set of 3 controls per case Cause of death verified by hospital records Cohort data reanalysis	Exposure estimation done based on measurements provided by seven plants, IISRP, and NIOSH	• Similar results with new controls • Reanalysis of cohort data for three plants -SS SMR = 163 for all LHC -SS SMR = 181 for leukemia and aleukemia	Verification of cause of death New set of controls validates earlier results

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—polymer production (continued).

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Matanoski et al. (1997)	Case-control population of Matanoski et al. (1993)	Based on personal monitoring exposure measurements: 1,3-butadiene - 3,952 styrene - 3,649 Time-weighted average (TWA) exposures in log normal (ln) ppm calculated for each chemical	TWA of ln (ppm+1) was associated with: • SS OR = 1.5 for leukemia • SS OR = 2.64 leukemia among labor or laboratory SS associations were found with styrene TWA of ln (ppm+1) for: • OR = 2.2 lymphohemato-poietic cancer • OR = 2.67 non-Hodgkin's lymphoma • OR = 3.88 lymphosarcoma • OR = 3.04 myeloma	- Confirms the association between 1,3-butadiene and leukemia excess - association between styrene and non-Hodgkin's lymphoma found for the first time is not explained fully

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—polymer production (continued)

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Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Macaluso et al. (1996)	Cohort of seven U.S. and one Canadian SBR workers mortality study Worked for at least 1 year between January 1, 1943, and January 1, 1992 Follow-up period through January 1, 1992 15,649 male workers U.S. population Respective State populations where the plants were located Ontario male rates for Canadian plant Internal comparison using Poisson regression	Exposure estimation based on several information sources including IH Quantitative exposure estimates on background, task-specific, multiple exposure, and point sources models for 1,3-butadiene, styrene, and benzene Peak exposures 8-h time-weighted intensities Cumulative exposures Exposures estimated for each individual	• Adjusted RRs for cumulative exposure to 1,3-butadiene of 0, <1, 1-19, 20-79, and 80+ ppm-years were 1, 2.0, 2.1, 2.4, and 4.5. Trend test was SS • Exclusion of nonexposed population also had similar results with SS trend test	Methods used and efforts made for exposure estimation are best efforts to date Misclassification with respect to job may be possible but unlikely to be only in leukemia deaths

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene—polymer production (continued).

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Delzell et al. (1996)	Same as Macaluso et al. (1996)	Same as Macaluso et al. (1996) Analysis by ever-hourly and never-hourly Analysis by process groups	• Ever-hourly workers showed for leukemia -SS SMR = 143 for all ever-hourly workers -SS SMR = 227 for blacks -SS SMR = 187 for 1985+ year of death -SS SMR = 200 for 1950-59, year of hire -SS SMR = 179 for <55 years age at death -SS SMR = 224 for >10 years of employment and >20 years since hire (SMR = 192 for whites and SMR = 436 for blacks, both SS) • Various process groups showed for leukemia -SS SMR = 251 for polymerization process -SS SMR = 265 for maintenance labor -SS SSMR = 431 for laboratory worker • Cancer incidence study in Canadian plant did not show any increased incidence for any cancer	Same as Macaluso et al. (1996) Cause of death not verified Histologic typing of leukemia not available, thus leading to misclassification

SS= Statistically significant, SMR=Standard mortality ratio.
IH=Industrial hygiene, RR=Risk ratios.
OR=Odds ratio, LHC=Lymphohematopoietic cancers.

for 3 months was included and was considered to have attended the school for that academic year. The school records were abstracted for the students' names, dates of birth, and years they attended the school. The name changes were noted if there were any due to adoption or marriage. A total of 15,553 students was identified. Sex and name change were verified by accessing several ancillary data sources. Vital status was ascertained by using the NDI, SSA death master files, and records of the Texas Department of Health. The name changes were checked by using the marriage databases from 1966 to 1994 and creating an algorithm to match with one or more marriages resulting in name change. A total of 15,722 possible last names was identified for the 7,591 women who had qualified for the cohort. The final cohort consisted of 15,403 students after the elimination of 145 students with unknown date of birth, 2 with incongruous date of birth, and 3 with unknown sex.

A total of 310,254 person years was accrued by 7,882 males and 7,521 females. Deficits in SMRs were observed for all causes in both males and females. For all cancers, females had a deficit, while excess was observed in males (SMR = 1.22). Neither the deficits nor the excesses were statistically significant A subcohort of males was examined by years of attendance at Port Neches High School. Males who had attended the high school for $\leq$ 2 years showed increases for all causes (SMR = 1.42, 95% CI = 1.11-1.80, O = 71), for all cancers (SMR = 2.11, 95% CI = 0.96-4.00, O = 9), for leukemia/aleukemia (SMR = 5.39, 95% CI = 1.09-15.64, O = 3), and for all external causes of death (SMR = 1.58, 95% CI = 1.17-2.10, O = 48). For males who had attended the high school for $\geq$3 years the only statistically significant excess was observed for benign neoplasms (SMR = 6.05, 95% CI = 1.65-15.48, O = 4). Similar analysis in females showed excess in all causes of deaths among females who attended the school for $\leq$ 2 years (SMR = 1.64, 95% CI = 1.14-2.29).[6]

Analysis by year of graduation, graduation either before 1980 or after 1980, did not show any clear pattern. Use of the Texas population for comparison ensued in similar results.

This study has quite a few methodologic limitations. First, no environmental measurements of either styrene or 1,3-butadiene for the school are available. Second, the cohort is young; a person who entered the high school at the age of 14 in 1963 would be age 46 in 1995. Since students up to 1992-93 are included, some will be barely 14 to 18 years old. Still, it is interesting to note that males who had attended the high school for $\leq$ 2 years had excesses for all causes, all cancers, and leukemias. Third, as correctly pointed out by the authors, an incidence study would have been more appropriate because the survival for leukemias is high; thus a mortality study may result in underestimating the problem. Fourth, no other information such as occupation, lifestyle, etc., was available. Therefore, it is difficult to establish a causal link

[6]The paper does not provide the number of observed deaths on which this SMR was based.

between the environmental exposure to 1,3-butadiene and observed excess in leukemias in men who had attended the high school for $\leq$ 2 years.

A comment on this paper submitted to the editors by Hanks and Townsley (2000), the lawyers representing 14 cases, points out that only four of their clients were dead by the cutoff date and were counted in the study. Since the end of the follow-up date of this study, there have been two deaths from leukemia and two deaths from Hodgkin's disease in males (one died from small-cell carcinoma of the lung presumed to be secondary to extensive chemotherapy of Hodgkin's disease). Among the six who are still alive, two males have Hodgkin's disease and one has NHL, while two females have leukemias and one has Hodgkin's disease. Furthermore, they mention that they are aware of several other students who died from lymphohematopoietic cancers who were not included in the study cohort because they attended the high school prior to 1963.

7.4. SUMMARY AND DISCUSSION

1,3-Butadiene is a colorless, odorless gas and due to its extremely volatile nature exposure to this gas is entirely via inhalation. 1,3-Butadiene becomes diluted in ambient air and is eliminated by photooxidation. Occupational populations are exposed to 1,3-butadiene in the production/recovery of 1,3-butadiene monomer and the production of synthetic rubber (polymer), resins, and plastics. The general population is exposed to butadiene in ambient air, the major sources of its release being automotive exhaust and cigarette smoke. 1,3-Butadiene has been shown to be mutagenic as well as carcinogenic in animals and humans. Data in animals, particularly in mice, show that 1,3-butadiene is a multisite carcinogen even at the lowest dose of 6.25 ppm (NTP, 1993). Therefore, its potential to cause cancer in humans has become an important public health issue.

It is difficult to study the health effects of exposure to 1,3-butadiene in the general population because exposure to 1,3-butadiene is ubiquitous, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are exposed to 1,3-butadiene nonoccupationally. Furthermore, the issue of health measurement is complicated by the fact that occupational cohorts tend to be healthier than the overall general population and have below-average mortality, which is referred to as the "healthy worker effect." Thus, the SMRs observed in occupational cohorts, computed using the general population as the reference group, are underestimations of real risk.

7.4.1. Monomer Production

To evaluate the carcinogenicity of 1,3-butadiene, cohorts from monomer and polymer production plants were studied by several investigators. The largest cohort of monomer

production workers was initially studied by Downs et al. (1987) with three follow-ups by Divine (1990), Divine et al. (1993), and Divine and Hartman (1996). The cohort initially included 2,586 workers, which increased to 2,795 individuals in the last follow-up due to an extended time period for the inclusion criteria. The four exposure groups were identified by Downs et al. (1987) based on a qualitative exposure scale. The groups remained the same in Divine's (1990) follow-up and were similar but slightly changed in Divine et al. (1993). In their last follow-up, based on IH data, Divine and Hartman (1996) estimated the potential exposure to 1,3-butadiene for each employee by their work histories (in 1-year segments), using job categories and calendar time periods. Cumulative exposures were obtained by summing the scores for all the years of employment.

The findings of all four investigations were essentially the same even after 52 years of follow-up. Deficits were observed for mortality from all causes and all cancers. The only statistically significant excess observed was for lymphosarcoma (ICD code 200). Downs et al. (1987) observed this excess for the total cohort and for the subcohort of workers who had worked for less than 10 years and latency of 0 to 9 years. This excess was seen in the wartime subcohort in all three follow-up studies (SMR = 269 [Divine, 1990], and SMR = 254 in two latter follow-ups: Divine et al. [1993]; Divine and Hartman [1996]). Furthermore, in the last follow-up, the SMR of 176 (95% CI=103-282) was computed for NHL, using new diagnostic criteria that classified lymphosarcoma as lymphoma and thus included it in NHL. Of 17 cases on which this excess was based, 16 were hired prior to 1950. Survival analysis using estimated cumulative exposures failed to show any association between the exposure and occurrence of NHL.

Hence, the investigators thought that peak exposures rather than cumulative exposures may be associated with the observed increase in NHL. No information on peak exposure levels was available for this period, but it was believed that the peak exposures were high during the war period. When analyses were done by years of employment and latency excess for lymphosarcoma, mortality was always found to be in individuals employed for less than 10 years with a latency of 0 to 9 years. It should be noted that after 52 years of follow-up, except in the survival analysis using the Cox model (which showed statistically significant excess in leukemia for the risk factor "Hire-age"), no statistically significant elevated mortality was observed for leukemia in this cohort (because the study population may not have enough power to detect a leukemia excess), which was the main finding in the SBR workers.

A small cohort of 364 individuals was identified from 29,139 workers at three Union Carbide Corporation plants who had potential exposure to 1,3-butadiene (Ward et al., 1995, 1996c). The exposure to 1,3-butadiene was assumed based on department codes, and no adjustments for confounding by other chemicals were done. Analysis of the subcohort of workers who had also worked in the acetaldehyde unit did not show any excess in deaths from

lymphosarcoma independent of exposure to 1,3-butadiene. As observed in the Divine studies (Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996), a statistically significant excess for lymphosarcoma (SMR = 577) was observed in this cohort based on four cases. Of these four cases, three had started their employment between 1942 and 1946, that is, during wartime.

A third cohort of 614 workers exposed to monomer was studied by Cowles et al. (1994), but the study failed to show any excess mortality or morbidity. Due to several methodologic limitations, this study failed to provide any negative evidence to support the causal association between exposure to 1,3-butadiene and the occurrence of lymphosarcoma that was observed in the other two cohorts.

7.4.2. Polymer Production

A further follow-up and reanalysis of a large SBR polymer production workers' cohort study (Matanoski and Schwartz, 1987) was conducted by Matanoski et al. (1989, 1990). This follow-up added 3 years to the earlier study, but the findings of this follow-up were essentially the same as those of the earlier study. The only statistically significant excesses were among production workers. Among whites, the excess was for other lymphohematopoietic cancers (SMR = 260), and among blacks the excesses were for all lymphohematopoietic cancers (SMR = 507) and leukemia (SMR = 655). Analyses by duration of work and latency did not show any increases in hematopoietic cancers. There were no exposure measurements or estimations done in this study.

A nested case-control study from this cohort (Matanoski et al., 1989, 1990) was conducted by the same investigators and reported by Matanoski et al. (1989) and Santos-Burgoa et al. (1992). Fifty-nine cases of lymphohematopoietic cancers and 193 matched controls were identified. Exposures to 1,3-butadiene and styrene were estimated for these individuals using job records and levels of exposure to 1,3-butadiene and styrene associated with those jobs independent of the case or control status. The jobs were ranked and cumulative dose was calculated for each case and control. Analyses were conducted using log transformed scores. The relative odds were increased for high (OR = 6.82) and low (OR = 4.26) exposures in the ever/never exposed analysis, matched analysis (OR = 9.36), and conditional analysis (OR = 7.61) for leukemia. All the increases were statistically significant. A statistically significant trend also was observed for increasing risk of leukemia with increasing levels of exposure to 1,3-butadiene.

In 1993, Matanoski et al. (1993) reevaluated the analysis of the nested case-control study because their findings had been questioned by Acquavella (1989) and Cole et al. (1993), particularly with respect to the base cohort study. Even though the investigators chose a new set of three controls per case, and verified the cause of death by obtaining hospital records, the findings of the new analysis were similar to those of the earlier analysis. Furthermore, the

investigators estimated the exposures to the cohort based on measurements provided by seven rubber plants, IISRP, and NIOSH. In an analysis of the subcohort from the three plants, for which they had geometric means of exposure, statistically significant excesses were observed for all lymphohematopoietic cancers (SMR = 163) as well as for leukemia and aleukemia (SMR = 181).

In Matanoski et al. (1997), the exposure refinement was done by the authors using several exposure measurements for 1,3-butadiene and styrene. These included personal monitoring, job- and plant-specific exposure levels, job histories, and time spent in each job to calculate the cumulative exposure in ppm-months. Statistically significant excesses were observed for a TWA of 1 ppm of 1,3-butadiene and leukemia (OR =1.5) and Hodgkin's lymphoma (OR = 1.73). Statistically significant associations also were observed for all leukemias as well as lymphoid leukemia for a TWA of 1 ppm and for all leukemias and myeloid leukemia for the longest job in "service, labor, or laboratory." The TWA of 1 ppm of styrene was significantly associated with all lymphohematopoietic cancers (OR=2.2), NHL (OR=2.67), lymphosarcoma (OR=3.88), and myeloma (OR=3.04). The investigators were unable to explain the observed association between NHL and styrene.

Delzell et al. (1996) and Macaluso et al. (1996) reported separately the two components of the follow-up study of synthetic rubber workers. These investigators studied the seven plants studied by Matanoski and Schwartz (1987), Matanoski et al. (1989, 1990, 1993), and Santos-Burgoa et al. (1992) and one plant (two initial plants combined into one) by Meinhardt et al. (1982). The follow-up period was 49 years. Investigators estimated the exposures to 1,3-butadiene, styrene, and benzene for each worker. This was done by using various means such as job histories, work areas, IH data, historical plant data, aerial pictures, interviews with long-term employees and managers, walk-through surveys, etc. Quantitative exposures were calculated, and limited validation of exposure estimates were attempted using available 1970s and 1980s IH data. Cumulative and peak exposures were calculated for each worker. Comparison with the U.S. population resulted in statistically significant excesses for leukemia in ever-hourly workers (SMR = 143) and its subcohort of black workers (SMR = 227). The excesses also were found in the ever-hourly cohort for year of death (SMR = 187 for 1985+), year of hire (SMR = 200 for 1950-59), age at death (SMR = 179 for <55 years), and for more than 10 years employment and more than 20 years since hire (SMR = 192 for whites and SMR = 436 for blacks). Laboratory workers, maintenance workers, and polymerization workers also showed increased SMRs of 431, 265, and 251, respectively. All these analyses were done adjusting for styrene and benzene. When internal comparison was done using the estimated ppm-years exposure data, relative ratios increased with increasing exposures. The trend test was statistically significant.

The incidence study conducted in the Canadian plant employees did not show any increases in any cause-specific cancers.

These investigators found a nonsignificant deficit in lymphosarcoma in the total cohort for the specific site observed in monomer workers. However, they did find nonsignificant excesses in ever-hourly workers during the time period 1985-1991 (field maintenance workers, in production laborers, and maintenance laborers). In Sathiakumar et al. (1998), these investigators reported the results for NHL, which includes lymphosarcoma per ICD-O (Berard and Huchinson, 1997). Of 28 NHLs observed in the cohort, 26 occurred in ever-hourly workers (SMR = 99). Although the SMR was just below 100 and statistically nonsignificant, the authors noted that due to high survival rates for NHL, the association between the employment in SBR industry and NHL may be obscured.

7.4.3. Relevant Methodologic Issues and Discussion

Throughout this chapter, various methodologic issues, including strengths and limitations, are discussed. Except for a morbidity analysis in one monomer study (Cowles et al., 1994) and an incidence analysis in the Canadian plant of the cohort identified by UAB, all of the studies reviewed here are mortality studies. The 5-year relative survival rates for leukemia by year of diagnosis between 1989 and 1994, among whites were 44% and among blacks were 31%. For NHL the survival rates were 52% for whites and 41% for blacks for the same calendar period (Landis et al., 1999). Thus, these mortality studies may not be the appropriate measure to evaluate the carcinogenicity of 1,3-butadiene. The major concerns of the mortality studies reviewed here are small cohort sizes in monomer studies, lack of data on confounding variables, and latency analysis in one study. Furthermore, death certificates were used by all the investigators, which could lead to misclassification bias. Validation of diagnosis of lymphohematopoietic cancer was not done in any of the studies except Matanoski et al. (1993). Although it is a minor limitation, it is a methodologic concern given the fact that recording of lymphohematopoietic cancer, particularly leukemia, on death certificates is unreliable (Percy et al., 1981).

Lack of exposure information is another major limitation of Cowles et al. (1994) and Ward et al. (1995, 1996c). Cowles et al. (1994) did not attempt any exposure estimation. The cohort was very small, there were very few deaths, and more than 50% of the cohort had an average follow-up of 12 years.

Ward et al. (1995, 1996c) did not attempt any exposure estimation either. This cohort also was very small and was restricted to workers who had worked in 1,3-butadiene production. The high SMR for lymphosarcoma and reticulosarcoma observed in this study was based on only four cases. They used employment of 2+ years as surrogate for exposure and stated that there

were no other common exposures to other chemicals. Two cases also had exposure to acetaldehyde, which has never shown any lymphohematopoietic carcinogenesis before. Furthermore, it should be noted that only the morbidity study involving acetaldehyde exposure, by Bittersohl (1974), reported nine cancer cases: five squamous cell carcinomas of the bronchi, two squamous cell carcinomas of the mouth cavity, and one adenocarcinoma of the stomach and cecum each. No lymphosarcoma or reticulosarcoma was observed in this study. Thus, it is unlikely that lymphosarcoma observed in the Union Carbide cohort is due to the confounding effect of the acetaldehyde exposure. In animal studies the only statistically significant excesses observed were for nasal tumors in male and female rats and laryngeal tumors in male and female hamsters after inhalation exposure to acetaldehyde (U.S. EPA, 1987).

Considering that the cohort was small and only four deaths occurred from lymphosarcoma and reticulosarcoma, it should be noted that this finding is consistent with the finding of the other monomer facility studied by Divine (1990), Divine et al. (1993), and Divine and Hartman (1996).

A monomer cohort study conducted by Downs et al. (1987) and followed by Divine (1990) and Divine et al. (1993) also lacked exposure information, although the surrogate exposure grouping was done by qualitative exposure information based on job descriptions/work areas. The investigators estimated the exposures in their last follow-up (Divine and Hartman, 1996) and found that except for an excess observed for lymphosarcoma and reticulosarcoma in the wartime subcohort, there were no excesses in any cause-specific cancer mortality. They go on to discuss that, per new diagnostic criteria, lymphosarcoma is now classified as lymphoma and thus is included under the category of NHL with other lymphomas. Thus they computed the SMR of 176 (95% CI = 103-282) for the observed 17 deaths from NHL. This was based on the assumption that 50% deaths in cancers of other lymphatic tissue were from lymphomas. Out of 17 decedents, 16 were hired before 1950, 6 had worked for more than 5 years, and 12 belonged in the varied-exposure group.

Just as in the earlier two follow-ups (Divine, 1990; Divine et al., 1993) the investigators stipulate that the above findings were consistent with the hypothesis that high exposures to 1,3-butadiene in wartime may be associated with NHL. Since they did not find any association between cumulative exposure and occurrence of non-Hodgkin lymphoma in their survival analysis, they suggest that peak exposures rather than cumulative exposures may be associated with the observed increase. Furthermore, they acknowledge that there are no data or means available to estimate the peak exposures. In addition, they note that extremely high peak exposures did exist during the 1950s and 1960s. They also assert that the lack of association may be due to a high survival rate for NHL. Also, there is a lack of any information on other confounding factors. After 52 years of follow-up and extensive analyses, this cohort has not

observed any statistically significant excess in mortality from leukemia except in survival analysis by Cox model, for the risk factor "Hire-age," that was observed in SBR workers. Nonetheless, the finding of excess mortality from lymphosarcoma and reticulosarcoma is consistent with the findings of Ward et al. (1995, 1996c), and, although not statistically significant (SMR = 224), of Meinhardt et al. (1982).

Matanoski and Schwartz (1987) and Matanoski et al. (1989, 1990) did not have any exposure information available. The cohort was distributed in four major areas based on longest jobs held and the qualitative exposure information used as surrogate. When the nested case-control study was undertaken by these investigators (Matanoski et al., 1989; Santos-Burgoa et al., 1992), exposure estimation was done by using various sources only for selected cases and controls. They observed a statistically significant high excess from leukemia mortality, which the authors concluded as being causally associated with exposure to 1,3-butadiene.

Matanoski et al. (1993) validated their earlier results of the nested case-control study by using a new set of three controls per case. They also verified the cause of death noted on the death certificates and the diagnosis noted on the hospital charts. They found that the diagnosis noted on 25 out of 26 charts agreed with the cause of death noted on the death certificates. The results of this study were similar to the earlier nested case-control study.

This finding of a high excess of leukemia mortality in the case-control study was questioned by Acquavella (1989) and Cole et al. (1993) because no excess leukemia mortality was found in the base cohort study from which the cases and controls were selected. Their argument that the results of the case-control study were statistically incompatible with the results of the cohort study was based on the calculations of number of leukemias that should have been seen in the cohort study, based on the relative odds observed in the case-control study. When Cole et al. (1993) applied the relative odds of 7.6 observed by Santos-Burgoa et al. (1992) to the 60% of the cohort exposed to 1,3-butadiene, their calculation resulted in approximately 104 leukemia cases. When the leukemia rates of the general population were applied to the remaining 40% of the cohort that was not exposed to 1,3-butadiene, an additional 9.2 leukemia cases were expected. Thus, a total of 113 leukemia cases should have been observed for this cohort instead of the 22 leukemia cases that actually were found by the investigators. Variability in both the prevalence of exposure and the relative odds was looked at by these authors (Cole et al., 1993), and they concluded that there was no reasonable combination that resolved the incompatibility between the findings of the cohort and case-control studies.

Matanoski and Santos-Burgoa (1994) disagreed with this criticism. They asserted that the 60% exposure observed among the controls in the case-control study overestimated the prevalence of exposure for the cohort population and that the matching criteria may have skewed the control selection and produced controls who were not representative of the base cohort.

The disagreement about the cohort and case-control study is still not resolved. Nonetheless, it should be noted that the nested case-control study was the first to demonstrate a strong association between exposure to 1,3-butadiene and occurrence of leukemias.

The main limitations of the cohort study were that more than 50% of the population was excluded due to lack of work histories or start date and lack of exposure data. The follow-up for four plants where the starting date was 1957 to 1970 may not have been long enough for malignancies to develop. As far as the nested case-control study is concerned, as pointed out by the authors, the estimated exposures were crude and not substantiated by IH data. The exposure misclassification may have occurred based on the estimated exposure by job if the jobs were incorrectly identified for higher or lower exposure. However, the panel members were blind toward the status of cases and controls; thus the distribution of misclassification should be the same in cases and controls.

In their latest study, Matanoski et al. (1997) estimated the exposures for each case and the control. Leukemia was found to be associated at a TWA of ln (ppm + 1) of butadiene. The other associations observed in this study were Hodgkin's lymphoma with exposure to 1,3-butadiene and NHL with styrene.

The Delzell et al. (1996) and Macaluso et al. (1996) cohort study is one of the best efforts of exposure estimation to date. Some misclassification of exposure may have occurred with respect to certain jobs, but it is unlikely to have occurred only in leukemia cases. The investigators also did some validation of exposure estimates based on IH data. They pointed out correctly that the excess mortality observed for leukemia was based on death certificates and was not verified by medical records. Histologic typing of leukemia was also not available. This may have resulted in misclassification. Two plants were eliminated from the final analysis due to the lack of work histories, which may have resulted in the loss of valuable data.

In a commentary paper, Irons and Pyatt (1998) raised an issue of potential confounding exposure to dithiocarbamates (DTC), which include compounds such as dimethyldithiocarbamate (DMDTC) and diethyldithiocarbamate (DEDTC) and their respective oxidized dithiuram counterparts such as thiram and disulfiram, in SBR production workers. When SBR production was changed from hot process to cold process, DMDTC was used as a stopping agent in cold process for SBR production from 1950 to 1965. No exposure data for DMDTC have been presented in the Irons and Pyatt (1998) paper. Based on circumstantial evidence Irons and Pyatt claim that since 87% of the polymerization, coagulation, and laboratory workers who developed leukemia were employed during the 1950 to 1965 time period (Delzell et al., 1996), there was more likelihood that exposure to DMDTC could be associated with the occurrence of leukemia in this cohort.

It is arguable whether DTCs are really confounding the results of Delzell et al. (1996). It should be noted that to be a confounding factor the agent should be associated with exposure as well as outcome. Dithiocarbamates have been in use since the 1940s as fungicides and to treat parasitical skin diseases. Disulfiram has been in use for treatment of alcoholism. So far there is not even a case report of leukemia in the literature in reference to any of the DTCs. Hence, at this time it is conjecture on the authors' part that DTCs are causally associated with leukemia. The main exposure route for DMDTC in SBR production facilities was dermal. Therefore, it is inconceivable that workers could have had constant exposure to DMDTC 8 hours a day, 5 days a week. Furthermore, since it was not known whether the workers who developed the leukemia were actually exposed to DMDTC, the circumstantial evidence presented by the authors may be an ecological fallacy. No data are available for DMDTC in animals. Pyatt et al. (1998) found DMDTC to be a potent immunosuppressive compound in vitro. Relevance of this finding in vivo is not known at this time.

When data on other related compounds, i.e., the oxidized dithiuram counterparts, were reviewed, it was found that rats developed tumors when disulfiram was administered simultaneously with nitrite via ingestion, but when it was administered alone, they did not develop any tumors (PDR, 1998). An inhibitory effect of disulfiram on carcinogenesis has been demonstrated in rats and mice for tumors induced by other chemicals at different sites (IARC, 1976). Moreover, both DEDTC and disulfiram, when administered to animals, inhibit the cytochrome P-450 containing monooxygeneses and decrease the level of cytochrome P-450 detectable as its carbon monoxide complex. These two compounds also inhibit the cytochrome P-450 when incubated with hepatic microsomes in vitro in the presence (but not absence) of NADPH. This suggests that the inhibition reflects a primary effect of metabolism of the compound, rather than secondary effect of liver damage (Neal and Halpert, 1982).

In their review of thiram, IARC (1991) reported on the only human data available to them from the USSR. In a group of 223 workers who were employed in the manufacture of thiram for more than 3 years, 1 case of thyroid cancer was reported among 105 workers examined. It should be noted that this study was published in 1971. There are no other human data available on this compound since then.

Thiram was tested via diet for 104 weeks (all the animals were killed at 112 weeks) in SPF Fischer rats. The incidence of leukemia was significantly decreased in both male (10/50 control, 4/49 low dose - $p<0.05$, and 2/50 high dose - $p<0.05$) and female (14/49 control, 6/50 low dose - $p<0.05$, and 2/50 high dose - $p<0.01$) rats. In another diet study for 104 weeks (experiment terminated at 130 weeks) in Fischer 344 rats, there was a significant decrease in monocytic leukemia in both male and female treated rats (IARC, 1991).

Villani et al. (1999), in their experiment with oral administration of thiram to $B6C3F_1$ male mice, found no significant increase of micronucleated splenocytes. A borderline increase of DNA damage was found by Comet assay on circulating lymphocytes, but a negative result was observed for splenocytes. On the other hand, in vitro, positive results with both genetic endpoints were reported in assays with human lymphocytes in the dose ranges of 0.5 to 24 μ/mL and 0.1 to 8 μ/mL for micronucleus and Come assays, respectively. Based on these results, the authors concluded that despite its established genotoxicity in vitro, thiram is devoid of appreciable clastogenic and/or aneugenic activity in vivo after oral administration to mice at the maximum tolerated dose.

Therefore, the current data exhibit that DTCs are probably protective against causation of cancer rather than the other way around.

Based on these monomer and polymer production worker cohorts, it is observed that an increased number of lymphohematopoietic cancers occur in these populations. However, a clear difference is becoming apparent. Increased NHLs develop in workers exposed to the monomer (Downs et al., 1987; Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996; Ward et al., 1995, 1996c), while excess leukemias occur in workers exposed to the polymer (Matanoski et al., 1990, 1993; Santos-Burgoa et al., 1992; Delzell et al., 1996; Macaluso et al., 1996, Sathiakumar et al., 1998). Furthermore, in the monomer workers, probably the high exposures over a shorter period of time (wartime) rather than low exposures over a longer period of time were associated with observed excess of NHLs. As stipulated in their last follow-up (Divine and Hartman, 1996), since most of the workers who developed NHLs were hired prior to the 1950s, when very high peak exposures occurred, the peak exposures and not the cumulative exposures may be associated with the observed excess of NHLs. The investigators also asserted that very high peak exposures existed in the 1950s and 1960s, but neither any information about the frequency of these peak exposures (compared to prior to the 1950s) nor any information about the variations in intensity of peak exposures for these different time periods is available.

In the case of 1,3-butadiene, the scientific community is divided in two schools of thought. One school believes that both NHLs and leukemias should be considered as tumors of the same organ system since they both are mesodermic in origin, i.e., in the lymphohematopoietic system, whereas the other school believes that they should be considered as two separate site-specific tumors. This second group proposes that the excess observed for leukemia in polymer production workers is due to the SBR process, and therefore the SBR process and not 1,3-butadiene should be considered carcinogenic to humans. The Leukemia Society of America defines “Leukemia, Lymphoma, Hodgkin’s disease, and Myeloma are cancers of the body’s blood-forming and immune systems: the bone marrow and lymph nodes. They are considered to be related cancers because they involve the uncontrolled growth of cells

with similar function." Recently the International Lymphoma Group revised the classification of the lymphohematopoietic system. The Revised European American Lymphoma (REAL) was proposed in 1994 and is being adapted into the *International Classification of Disease of Oncology* (ICD-O) (Berard and Hutchison, 1997). This classification is based on new ideas evolving in the fields of molecular biology, genetics, and immunology that have rendered the old classifications for lymphohematopoietic neoplasms obsolete (see footnote 4).

These monomer and polymer production worker cohorts demonstrate an excess number of lymphohematopoietic cancers in the occupationally exposed workers. Increased NHLs are reported for monomer production workers, whereas excess leukemias occur predominantly in polymer production workers. There are several possible explanations for this apparent discrepancy between the monomer and polymer workers. It has been hypothesized that the observed excess of NHLs in the monomer production workers may be related to exposure intensity, i.e., the excess risk may result from the high (peak) exposures during wartime rather than the much lower exposures currently encountered by monomer production workers or the likewise comparatively lower exposures encountered by polymer production workers. The absence of a significant leukemia excess in these same monomer workers may be attributable to low statistical power in the monomer studies. It should be noted that there is some suggestion of excess leukemias in the monomer production workers, although these were not statistically significant. The Union Carbide cohort had a leukemia excess of 23% based on 2 cases, and the Texaco cohort has had several follow-ups since it was first studied by Downs et al. (1987). Downs et al. studied the monomer workers cohort from 1943 through 1979 and reported a total of seven leukemia cases. In a subsequent follow-up through 1986, eight deaths from leukemia were reported by Divine (1990). In a further follow-up of this cohort through 1990, Divine et al. (1993) observed 11 leukemia deaths, while Divine and Hartman (1996) found an elevated risk of leukemias of 13% based on 13 cases through 1994. Though the Texaco cohort is a relatively large monomer production cohort, it has low power to detect a statistically significant excess for leukemias. Furthermore, with every follow-up, the investigators of the Texaco cohort increased the calendar period for the worker inclusion criteria, hence adding many younger workers with little cumulative exposure, shorter follow-up periods, and inadequate latency periods, thereby diluting the risk. In addition, 1,3-butadiene is produced at the end of the monomer production process, and current 1,3-butadiene exposures are very low in these workers. Finally, the workers may lack exposure to a necessary co- or modifying factor that may be present in the polymer production, resulting in the development of leukemias, although the findings of Delzell et al. (1996) and Macaluso et al. (1996) show no evidence of confounding by exposure to other chemicals.

In fact, the apparent discrepancy may be largely an artifact. Under the latest classification system for lymphohematopoietic cancers, all lymphomas not classified as Hodgkin's disease are now included under NHL (see footnote # 4). Using this classification, an excess of NHL of 37% (based on 15 cases; not statistically significant) was reported for workers who had worked $\geq$10 years and with $\geq$20 years since hire in the UAB (polymer) cohort (Sathiakumar et al., 1998; previously lymphosarcomas and NHL were reported separately for this cohort). Furthermore, as these investigators report, their evaluation of NHL relations was limited by their reliance on death certificates. NHL has high survival rates and may, in later clinical stages, transform into leukemia; therefore leukemia may be reported on the death certificates. In addition, as discussed above, nonsignificant excesses of leukemia were observed in two monomer studies. Thus, excesses of both leukemia and NHL have been observed for both monomer and polymer workers, and it may be that the increased risk of NHL is primarily observed among workers exposed to high concentrations of 1,3-butadiene (mostly wartime monomer workers), whereas the polymer production studies have greater power to detect a significant leukemia excess among SBR workers who have modest to low exposures. In any event, leukemias and lymphosarcomas are related tumor types and can both be classified as lymphohematopoietic cancers (see footnote # 4).

Finally, an alternate explanation is that the monomer workers may lack exposure to a necessary co- or modifying factor that may be present in polymer production, resulting in the development of leukemias, although the findings of Delzell et al. (1996) and Macaluso et al. (1996) show no evidence of confounding by exposure to other chemicals.

7.4.4. Criteria of Causal Inference

In most situations, epidemiologic data are used to delineate the causality of certain health effects. Several cancers have been causally associated with exposure to agents for which there is no direct biological evidence. Insufficient knowledge about the biological bases for diseases in humans makes it difficult to identify exposure to an agent as causal, particularly for malignant diseases when the exposure was in the distant past. Consequently, epidemiologists and biologists have provided a set of criteria that define a causal relationship between exposure and health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually prove causality; actual proof is rarely attainable when dealing with environmental carcinogens. None of these criteria should be considered either necessary (except temporality of exposure) or sufficient in itself. The absence of any one or even several of these criteria does not prevent a causal interpretation. However, if more criteria apply, they provide credible evidence for causality.

Thus, applying the criteria of causal inference to the monomer and polymer cohort mortality studies and one nested case-control study, in which an increased risk of lymphohematopoietic cancers was demonstrated, resulted in the following:

- **Temporality**. There is temporality of exposure to 1,3-butadiene prior to the occurrence of NHLs in the monomer workers and leukemias in the SBR workers.

- **Strength of association**. Strength of association between exposure and the occurrence of NHLs in the wartime workers ranged from 154% to 477% higher risk among workers exposed to the monomer as compared with the nonexposed general population (Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996; Ward et al., 1995, 1996c). The excess risk of leukemia ranged from 43% to 127% higher among workers exposed to the SBR in ever-hourly workers compared with the general population (Delzell et al., 1996). Internal comparison of the SBR worker population resulted in a 4.5-fold increased leukemia risk among the highest exposure group in the same cohort (Macaluso et al., 1996). The nested case-control study from the SBR cohort showed a 7.6-fold increase in the risk of leukemia (Matanoski et al., 1989, 1993; Santos-Burgoa et al., 1992).

- **Consistency**. Two cohort studies in monomer workers showed an increased risk of NHLs (Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996; Ward et al., 1995, 1996c), while one cohort study (Delzell et al., 1996; Macaluso et al., 1996) (with a cohort derived from seven U.S. plants and one Canadian plant) and one nested case-control study (Matanoski et al., 1989, 1993; Santos-Burgoa et al., 1995) showed an excess risk of leukemia in SBR workers. The SBR workers cohort defined by Delzell et al. (1996) showed a fairly consistent association between exposure to butadiene and occurrence of leukemia across the plants included in the study. Furthermore, there is a major population overlap between the SBR cohorts identified by JHU and by UAB. These two groups of investigators have independently found excess leukemias in the SBR workers.

- **Specificity**. Increased lymphohematopoietic cancers are observed in both the monomer (NHLs) and polymer (leukemias) production workers. It should be noted that exposure to a particular chemical (or drug or radiation) may cause more than one type of leukemia or another type of hematopoietic cancer (Linet, 1985). Hays et al. (1997), in

their Chinese cohort exposed to benzene, found that development of leukemia and NHL was dose related.

- **Biological gradient**. The biological gradient, which refers to the dose-response relationship, was observed only in SBR workers. Both the nested case-control study and the cohort study showed increasing risk of leukemia with increasing exposures. Such a relationship was not observed in monomer workers. The reason may be that a very small number of people were exposed to high peak levels of 1,3-butadiene for a shorter period of time who showed the occurrence of NHL. Due to a lack of information on intensity and frequency of exposure levels they could not be further stratified to evaluate the dose response. As noted earlier, the monomer studies did not have enough power to detect a statistically significant excess of leukemia.

- **Biological plausibility**. As described in Chapter 4, hemoglobin adducts have been detected in humans exposed to 1,3-butadiene (Osterman-Golkar et al., 1993; Sorsa et al., 1996). Significantly increased frequencies of *hprt* mutant lymphocytes were observed in high-exposure groups by Legator et al. (1993) and Ward et al. (1994). Mutations, chromosomal aberrations, and cell transformations, all well-established steps in the process of carcinogenesis, were observed in human and animal studies. This makes a convincing argument for the biological plausibility of occurrence of leukemia in SBR workers and lymphosarcoma in monomer workers.

In summary, the findings of excess lymphohematopoietic cancers in polymer and monomer production workers are consistent with a causal association with exposure to 1,3-butadiene. As demonstrated above, the causality criteria of *temporality*, *strength of association*, *consistency*, *specificity*, and *biological gradient* are satisfied. In addition, as discussed in the earlier sections, 1,3-butadiene is metabolized by humans and other species to genotoxic metabolites and is carcinogenic in mice and rats, thus fulfilling the criterion of *biological plausibility* as well. Thus, the human evidence is considered sufficient.

8. HAZARD CHARACTERIZATION

8.1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) first published a health assessment of 1,3-butadiene in 1985 (U.S. EPA, 1985). The 1985 assessment concluded that 1,3-butadiene was a probable human carcinogen and calculated an upper bound cancer potency estimate of 0.25/ppm based on mouse data. Since then, a number of new studies on 1,3-butadiene have been completed in various disciplines such as epidemiology, toxicology, and pharmacokinetics. The purpose of this document is to review the new information and determine if any changes are needed to the earlier conclusions.

The current reassessment focuses primarily on the major health effects, as requested by EPA's Office of Transportation and Air Quality (formerly the Office of Mobile Sources): carcinogenicity, mutagenicity, and reproductive/developmental toxicity. A detailed exposure assessment was not requested nor conducted.

The major findings of this report are based on extensive carcinogenicity, toxicology, mutagenicity, and pharmacokinetic data. Epidemiologic data consist of studies in monomer and polymer production workers and include estimated exposures to 1,3-butadiene, styrene, and benzene in some studies. Animal studies reviewed for the report consist of acute, subchronic, and chronic assays, primarily in rodents.

This chapter briefly summarizes and integrates the critical data on which these hazard findings are based and discusses the strengths and weaknesses of those data and the resulting confidence in the findings. In addition, this chapter discusses the limited data on potentially sensitive or more highly exposed subpopulations and suggests future research needs. With the exception of the section on special subpopulations, all of the sections in this chapter discuss material presented in the earlier chapters of this assessment.

1,3-Butadiene (CH_2=CH-CH=CH_2, CAS No. 106-99-0) is a colorless, odorless, noncorrosive gas with a boiling point of −4.4°C and a vapor pressure of 1,900 mm/Hg at 20°C (Kirshenbaum, 1978).

Approximately 12 billion pounds of 1,3-butadiene are produced annually worldwide, with 3 billion pounds produced in the United States (Morrow, 1990; USITC, 1990). It is used as an intermediate in the production of polymers, elastomers, and other chemicals. The major uses of 1,3-butadiene are in the manufacture of styrene-butadiene rubber (SBR)—that is, synthetic rubber—and of thermoplastic resins. 1,3-Butadiene is not a component of gasoline or diesel fuel, it is formed as a by-product of incomplete combustion. The primary way the 1,3-butadiene is released in the environment is via emissions from gasoline- and diesel-powered vehicles and equipment. 1,3-Butadiene emissions from vehicles are reduced by catalytic converters; total

emissions may decline as older cars without converters are removed from service. Other releases occur from the combustion of other fossil fuels and biomass. Minor releases occur in production processes, tobacco smoke, and vapors from the burning of plastics as well as rubber (Miller, 1978).

1,3-Butadiene is highly volatile and has a low solubility in water; thus environmental release results in atmospheric contamination. Atmospheric destruction of 1,3-butadiene occurs primarily by photoinitiated reactions. A significant amount of destruction also occurs by the gas phase reaction with ozone and reaction with nitrate radicals at nighttime, particularly in urban areas (U.S. DHHS, 1992). The major photooxidation products of 1,3-butadiene are acrolein and formaldehyde (Maldotti et al., 1980). Although it is degraded rapidly in the atmosphere, 1,3-butadiene is almost always present at low concentrations in urban and suburban areas. 1,3-Butadiene is not found in significant amounts in food, soil, water, plants, fish, or sediment. Therefore, the predominant pathway of exposure is via inhalation.

Monitoring done from 1987 to 1994 by Aerometric Information Retrieval System at more than 20 different urban and suburban locations detected ambient air levels of 1,3-butadiene ranging from 0.22 to 1.02 $\mu g/m^3$ (0.10 to 0.46 ppb). Indoor air levels are likely to be higher than ambient levels when smoking occurs. 1,3-Butadiene emissions from cigarettes have been measured to be 200 to 400 μg/cigarette, and levels in smoke-filled bars have been found to range from 2.7 to 19 $\mu g/m^3$ (1.2 to 8.4 ppb) (Löfroth et al., 1989; Brunnemann et al., 1990).

8.2. HAZARD ASSESSMENT

8.2.1. Metabolism and Pharmacokinetics

1,3-Butadiene is metabolized into several genotoxic metabolites by experimental animals and humans. Metabolic activation is required for 1,3-butadiene carcinogenicity, and there is evidence that 1,3-butadiene is metabolized to at least three genotoxic metabolites: a monoepoxide (1,2-epoxy-3-butene, EB), a diepoxide (1,2:3,4-diepoxybutane, DEB), and an epoxydiol (3,4-epoxy-1,2-butanediol, EBD) (see Figure 3-1 for a schematic of the 1,3-butadiene metabolic pathways). Qualitatively, the metabolism of 1,3-butadiene is similar among species. The enzymes responsible for the metabolic activation of 1,3-butadiene to these epoxide metabolites, as well as the enzymes responsible for the detoxification of these reactive metabolites, exist in humans as well as in mice and rats. EB and DEB have been measured in the blood of rats, mice, and monkeys after 1,3-butadiene exposure, and their production by human tissues has been observed in vitro. Formation of EBD has been observed in vitro using tissues from mice, rats, and humans. Recently, EBD has also been detected indirectly in vivo in rats, mice, and humans by the analysis of DNA and hemoglobin adducts.

Although the metabolic pathways of 1,3-butadiene biotransformation are similar across species, there are quantitative differences in the metabolic rates for various pathways between different species. These rate differences result in quantitative differences in the blood and tissue concentrations of the various metabolites across species that, in turn, likely account for some of the differences in carcinogenicity and other toxicity observed between the mouse and rat. Activation rates for 1,3-butadiene to EB and EB to DEB are higher in the mouse than in the rat. In the limited number of human tissue samples that have been examined, activation rates in humans exhibit a high degree of variability and appear to nearly span the range between mice and rats, although most of the rates are well below those in mice. Detoxification pathways also differ quantitatively, with glutathione conjugation being the predominant pathway for the removal of EB and DEB in the mouse and hydrolysis being the major detoxification pathway in humans. In the rat, EB and DEB in the liver are removed at a slightly greater rate by glutathione transferase than by epoxide hydrolase. Overall, the greater rates of activation compared with detoxication for biotransformation of 1,3-butadiene in mice versus rats is reflected by higher blood and tissue concentrations of EB and DEB in vivo.

Hemoglobin adducts, which can serve as a measure of tissue dose, have been detected in exposed workers (Osterman-Golkar et al., 1993; Sorsa et al., 1996) as well as in the blood of exposed mice and rats.

8.2.2. Mutagenicity

1,3-Butadiene, through its metabolism, has exhibited mutagenic and genotoxic activity in a wide variety of in vitro and in vivo assay systems. The genetic toxicology literature on 1,3-butadiene, EB, and DEB consists of more than 450 publications with positive genotoxic findings in viruses, bacteria, plants, and animals. Consistent with (more accurately, as a consequence of) the differences in metabolism described in Chapter 3, mutagenic responses in mice are consistently greater than the responses in rats when animals were exposed to 1,3-butadiene. The observation that the species difference in *hprt* response was greater after 4 weeks exposure than after 2 weeks (Meng et al., 1999a) suggests that the differences would continue to diverge with the length of exposure. A range of studies, both in vitro and in vivo, on two major metabolites indicate that EB primarily induces point mutations and small deletions, while DEB, as a bifunctional alkylating agent, also results in larger deletions. Additionally, DEB is mutagenically active in in vitro systems at concentrations that are similar to those found in the blood of mice exposed to 1,3-butadiene by inhalation. EB requires concentrations 10-fold to 100-fold greater in vitro than those found in the blood of 1,3-butadiene-exposed mice. However, a recent study with EB and DEB measuring *hprt* mutations in female mice and rats revealed that DEB was about threefold more mutagenic in the Fischer 344 rats than in C3B6F1 mice (Meng et al., 1999b).

EBD has been less extensively studied, but recent evidence suggests that most of the trihydroxybutyl-guanine adducts in mice and rats exposed to 1,3-butadiene are derived from EBD, and that this is also a major metabolite in vivo. Positive dominant lethal and heritable translocation studies in mice indicate that exposure to 1,3-butadiene can result in germ cell mutation and heritable risk.

Significantly increased frequencies of *hprt* mutant lymphocytes were observed in high-exposure workers in a butadiene production plant by Ward et al. (1994, 1996b), but not in rubber plant workers in China (Hayes et al., 1996) or in a Czech 1,3-butadiene plant (Tates et al., 1996). Both positive (Sram et al., 1998) and negative (Sorsa et al., 1994) findings for chromosome damage have also been reported in exposed workers. Although the reasons for the differences in these findings are not clear, several in vitro studies indicate that genetic polymorphisms for enzymes involved in the metabolism of 1,3-butadiene affect the induction of genetic damage (Uuskula et al., 1995; Norppa et al., 1995; Bernardini et al., 1998).

These mutagenicity and molecular data suggest that 1,3-butadiene is both a somatic and germ cell mutagen in mammals, possibly including humans.

8.2.3. Characterization of Reproductive and Developmental Effects

A variety of reproductive and developmental effects have been observed in mice exposed to 1,3-butadiene by inhalation. There are no human data on reproductive or developmental effects. The most sensitive short-term developmental endpoint was decreased fetal weight in the mouse. Decreases were observed at the lowest exposure concentration (40 ppm, 6 h/day, gestation days 6-15); thus there was not a no-observed-adverse-effect level (NOAEL) for this effect. No developmental toxicity was observed in rats.

The most sensitive reproductive endpoint observed in subchronic exposure studies was fetal deaths in dominant lethal studies of mice (i.e., male mice were exposed to 1,3-butadiene and effects on litters were measured after mating to unexposed females). The dominant lethal responses are believed to represent a genotoxic effect. Dominant lethal effects in humans would likely be manifested as infertility (due to reduced fertility or very early deaths) or spontaneous abortions.

From chronic exposure studies (2-year bioassays), the most sensitive reproductive effects were ovarian atrophy in female mice and testicular atrophy in male mice. Testicular atrophy was primarily a high-exposure effect. Ovarian atrophy, on the other hand, was observed at the lowest exposure level (6.25 ppm, 6 h/day, 5 days/week for 2 years). Uterine atrophy was also observed in the highest exposure groups; however, this is likely to be a secondary effect of the ovarian atrophy. The mechanisms of ovarian atrophy are unknown, although there is strong evidence that the effect is mediated by the diepoxide metabolite (see Chapter 5). It is further expected, based

on metabolic data, that humans would produce lower concentrations of this metabolite than do mice (see Chapter 3). Thus, it is likely that humans are less sensitive to 1,3-butadiene-induced ovarian atrophy than are mice.

8.2.4. Cancer Characterization

Cancer characterization is a synthesis of all pertinent information addressing the question of how likely an agent is to be a human carcinogen. EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986) provided a classification system for the characterization of the overall weight of evidence for potential human carcinogenicity based on human evidence, animal evidence, and other supportive data. This system included Group A: *Human Carcinogen*; Group B: *Probable Human Carcinogen*; Group C: *Possible Human Carcinogen*; Group D: *Not Classifiable as to Human Carcinogenicity*; and Group E: *Evidence for Noncarcinogenicity to Humans*.

As part of the guidelines development and updating process, the Agency has developed revisions to the 1986 guidelines to take into account knowledge gained in recent years about the carcinogenic processes. With regard to the weight-of-evidence evaluation for potential human carcinogenicity, EPA's 1996 *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996) and the subsequent revised external review draft *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999b) emphasize the need for characterizing cancer hazard, in addition to hazard identification. Accordingly, the question to be addressed in hazard characterization is expanded to how likely an agent is to be a human carcinogen, and under what exposure conditions a cancer hazard may be expressed. The revised guidelines also stress the importance of considering the mode(s) of action information for making an inference about potential cancer hazard beyond the range of observation, typically encountered at levels of exposure in the general environment. "Mode of action" refers to a series of key biological events and processes that are critical to the development of cancer. This is contrasted with "mechanisms of action," which is defined as a more detailed description of the complete sequence of biological events at the molecular level that must occur to produce a carcinogenic response. Furthermore, the 1999 guidelines (U.S. EPA, 1999b) have extensive discussion on children's health and sensitive populations and will serve as EPA's interim guidance to risk assessors (Federal Register, 2001).

To express the weight of evidence for potential human carcinogenicity, EPA's proposed guidelines utilize a hazard narrative in place of the classification system. However, in order to provide some measure of consistency, standard hazard descriptors are used as part of the hazard narrative to express the conclusion regarding the weight of evidence for potential human carcinogenicity.

The sections to follow evaluate and weigh the individual lines of evidence and combine all evidence to make an informed judgment about the potential human carcinogenicity of 1,3-butadiene. A conclusion regarding carcinogenicity in accordance with EPA's 1999 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999b) is provided, which also includes a hazard narrative along with an appropriate hazard descriptor.

8.2.4.1. *Cancer Evidence From Experimental Animals*

1,3-Butadiene is an animal carcinogen. Several chronic inhalation bioassay studies have been conducted with 1,3-butadiene: a 2-year rat study (HLE, 1981b); two lifetime mouse studies (NTP 1984, 1993)—the first terminated early because of excessive mortality and the second one using lower exposure concentrations; a 2-year stop-exposure study with male mice (NTP, 1993); and a 1-year study comparing the induction of thymic lymphomas in two different strains of male mice (Irons et al., 1989). These studies provide unequivocal evidence that 1,3-butadiene is a multisite carcinogen in both rats and mice (see Chapter 6). These studies also demonstrate that the mouse is more sensitive than the rat to 1,3-butadiene-induced carcinogenicity and develops tumors at different sites, although the reasons for these interspecies differences are not fully understood at this time. The most sensitive site was the female mouse lung, which exhibited significantly increased tumor incidence at the lowest exposure concentration tested (6.25 ppm). Other tumors that exhibited increased incidences in the mouse include lymphomas, heart hemangiosarcomas, histiocytic sarcomas, and tumors of the lung, forestomach, Harderian gland, liver, preputial gland, ovary, and mammary gland. In the rat, increases were observed in tumors of the brain, testis, pancreas, Zymbal gland, thyroid gland, uterus, and mammary gland. The mammary gland (female) is the only site with significant tumor increases common to both mice and rats.

8.2.4.2. *Cancer Evidence in Humans*

Sufficient evidence exists of increased lymphohematopoietic cancer in workers exposed to 1,3-butadiene to consider it carcinogenic to occupationally exposed populations. The conclusion of "sufficient evidence" to consider 1,3-butadiene carcinogenic to occupationally exposed populations is based mainly on epidemiologic studies examining workers exposed to 1,3-butadiene. An excess of lymphohematopoietic cancers was observed in 1,3-butadiene polymer production workers (studied by two different investigators) and monomer production workers in North America. An excess of leukemias was observed in polymer production workers while an excess of non-Hodgkin's lymphomas (NHL) (previously diagnosed as lymphosarcoma and reticular sarcoma, but now included in NHL per the new classification in the *International Classification of Diseases of Oncology [ICD-O]*) was observed in monomer workers. In light of

the revised classification adopted by the International Lymphoma Group's Revised European-American Lymphoma (REAL) classification and the Leukemia Society of America, these cancers are included in the category of lymphohematopoietic cancers, and thus will be so used by the authors of this assessment document.[1]

The epidemiologic studies in polymer and monomer production workers reviewed in Chapter 7 are summarized in Table 8-1.

The strongest evidence comes from a retrospective cohort study of 15,000 synthetic rubber workers (UAB cohort) with 49 years of follow-up Delzell et al. (1996). Quantitative exposures (cumulative and peak) to 1,3-butadiene, styrene, and benzene were estimated for each worker (Macaluso et al., 1996). Limited validations of exposure estimates were attempted by various means. Significant excesses ranging from 43% to 336% were found for leukemia in ever-hourly workers as compared with the general population, after adjusting for styrene and benzene. An internal comparison, using estimated ppm-years of 1,3-butadiene exposure resulted in increasing risk ratios for leukemia with increasing exposures. This trend was statistically significant. A fairly consistent association between exposure to 1,3-butadiene and occurrence of leukemia across the six plants was also found.

The major strengths of this study are as follows. First, the study had detailed and comprehensive quantitative exposure estimations for 1,3-butadiene, styrene, and benzene for each individual. Second, the cohort was large, with a long follow-up period of 49 years. Third, both external and internal comparison showed similar results. Fourth, adjustments for potential confounding factors were carried out. Fifth, analyses by duration of employment and for latency were conducted.

The study had some limitations. First, some misclassifications of exposure may have occurred with respect to certain jobs, but it is unlikely to have occurred only in leukemia cases because the exposures were calculated *a priori*. Second, the excess mortality observed for leukemia was based on death certificates and was not verified by medical records. This may have resulted in misclassification. Third, histologic typing of leukemia was also not available, so currently it is not known whether a single cell type or more than one cell type is associated with

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[1]Under the previous classification (8th ICD, Adapted), lymphohematopoietic cancers comprised the following subcategories: lymphosarcoma and reticular sarcoma, Hodgkin's disease, leukemia, and other lymphatic tissue cancers. In 1994, the International Lymphoma Group's REAL classification was proposed for the lymphohematopoietic cancers and is being adopted into the ICD-O (Berard and Hutchison, 1997). This classification is based on new ideas evolving in the fields of molecular biology, genetics, and immunology, which have rendered the old classification for lymphohematopoietic cancers obsolete. The REAL classification comprises the following subcategories: B-cell neoplasms, T-cell and putative natural killer (NK)-cell neoplasms, Hodgkin's disease, and unclassified lymphomas. It should be noted: Both leukemias and lymphomas that are produced by B-cells are included under B-cell neoplasms, and leukemias and lymphomas produced by T-cells and NK-cells are included under T-cell and NK-cell neoplasms. Please note that any lymphoma (such as B-cell, T-cell, and NK-cell) that is not classified as Hodgkin's disease is also included under NHL.

Furthermore, the Leukemia Society of America defines lymphohematopoietic cancers as follows: "Leukemia, Lymphoma, Hodgkin's disease, and Myeloma are cancers of the body's blood forming and immune systems: the bone marrow and lymph nodes. They are considered to be related cancers because they involve the uncontrolled growth of cells with similar functions."

Table 8-1. Summary of key epidemiologic studies.

Plants	Number of workers, dates studied	Authors	Approach	Significant findings
7 U.S. and 1 Canadian polymer production plants (UAB cohort)[a]	15,000, 1943-1994	Delzell et al., 1996 Macaluso et al., 1996	Cohort study using quantitative exposure estimates for 1,3-butadiene, styrene, and benzene for each worker	—Excess mortality for leukemia observed in ever-hourly workers, SMR = 143 to 436 —4.5-fold increased leukemia risk among the highest exposure group with internal comparison —Excess leukemia observed consistently across the plants —Leukemia risk increased with increasing exposure level
7 U.S. and 1 Canadian polymer production plants (JHU cohort)[a]	13,500, 1943 - 1985	Matanoski and Schwartz, 1987 Matanoski et al., 1989, 1990, 1993, 1997 Santos-Burgoa et al., 1992	Cohort study using qualitative exposures; case-control study using estimated quantitative exposures for each case and control	—Excess mortality due to lymphohematopoietic cancers observed in cohort study —7-fold increase for leukemia observed in case-control study —Leukemia risk increased with increasing exposure level in case-control study
1 U.S. monomer production plant (Texaco cohort)	2,800, 1943-1994	Downs et al., 1987 Divine, 1990 Divine et al., 1993 Divine and Hartman, 1996	Cohort study using qualitative exposures, last follow-up includes quantitative exposure estimates	—Excess mortality due to NHL, SMR = 176 in wartime workers —Earlier studies report excess mortality due to lymphosarcoma, SMR = 254 to 269 in wartime workers
3 U.S. monomer production plants (Union Carbide cohort)	364, 1940-1990	Ward et al., 1995, 1996a	Cohort study using qualitative exposures	—Excess mortality due to lymphosarcoma[b] —SMR = 577 in workers hired between 1942 and 1952
1 U.S. monomer production plant (Shell Oil Deer Park cohort)	614, 1948-1989	Cowles et al., 1994	Cohort study using qualitative exposures	—No increase in cancer mortality or cancer morbidity observed

[a] Six U.S. plants and one Canadian plant were common in Johns Hopkins University (JHU) and University of Alabama, Birmingham (UAB), studies.
[b] Now included under NHL.
SMR: standard mortality ratio.

the exposure to 1,3-butadiene. Fourth, two plants were eliminated from the final analysis due to the lack of work histories, which may have resulted in the loss of valuable data. Finally, Irons and Pyatt (1998) have recently raised an issue of potential confounding exposure to dithiocarbamates (DTCs). The DTCs comprise thiono-sulfur compounds. The representative compounds include dimethyldithiocarbamate (DMDTC) and diethyldithiocarbamate (DEDTC) and their oxidized counterparts thiram and disulfiram, respectively. DTCs have been in use since the early 1940s as fungicides and treatments for parasitic skin diseases. The only human study in thiram manufacturing workers (one of the DTC) reported a single case of thyroid cancer (IARC, 1991). Available animal studies have not provided any evidence that DTC causes carcinogenesis. As a matter of fact, the studies in animals observed decreased incidence of leukemia that was dose related (higher the dose, lower the incidence) as compared with control animals. Two different diet studies where thiram was administered for 104 weeks (SPF Fischer rats and Fischer 344 rats) observed significantly decreased incidence of leukemia in both male and female treated rats (IARC, 1991). Disulfiram has been in use for the treatment of alcoholism. So far there is not even a case report of leukemia in the literature in reference to any of the DTCs. Therefore, at this time it is conjecture that DTCs are causally associated with leukemia, hence confounding the results of Delzell et al. (1996). A detailed discussion on DTCs can be found in Section 7.4.3.

Additional evidence is provided by the studies conducted earlier by Matanoski and Schwartz (1987), Matanoski et al. (1989, 1990), Santos-Burgoa et al. (1992), and Matanoski et al. (1993) in many of these same polymer workers (13,500 individuals; JHU cohort).[2] A significant excess of lymphohematopoietic cancer but not leukemia was observed in the cohort studies, while a significantly increased odds ratio of 7 for leukemia was observed in a nested case-control study of this cohort (Santos-Burgoa et al., 1992; Matanoski et al., 1993). A significant trend of increasing risk of leukemia with increasing exposure level of 1,3-butadiene was also observed. The finding of a high excess of leukemia mortality in the case-control study was questioned by Acquavella (1989) and Cole et al. (1993) because no excess leukemia mortality was found in the base cohort study from which the cases and controls were selected. These authors asserted that the results of the case-control study were statistically incompatible with the results of the cohort study. Matanoski and Santos-Burgoa (1994) disagreed with this assertion. The debate about the findings of the cohort study and the case-control study is still unresolved. Nonetheless, it should be noted that this nested case-control study was the first to demonstrate a strong association between exposure to 1,3-butadiene and occurrence of

[2]One Canadian plant and six U.S. plants were common in the JHU and the UAB cohorts.

leukemias. A detailed discussion of the differing views about the findings of the cohort study and the case-control study can be found in Section 7.4.3.

Three different cohorts of monomer production workers have been studied. The strongest evidence of human carcinogenicity is provided by the largest cohort of approximately 2,800 workers in a Texaco plant studied by several investigators (Downs et al., 1987; Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996). The only significant excess mortality observed was for lymphosarcoma (now included in NHL) in the wartime subcohort of workers (154% to 169% higher than the general population). The investigators estimated exposures for each individual in their last follow-up (Divine and Hartman, 1996) and found that except for an excess observed for NHL (76% higher than the general population) in the wartime subcohort, there were no excesses in any cause-specific cancer mortality. Since they did not find any association between cumulative exposure and occurrence of NHL in their survival analysis, they indicated that it may be peak exposures rather than cumulative exposures that are associated with the observed increase. No information about peak exposure levels was available for the wartime period that was found to be associated with the occurrence of NHL; however, it is believed that exposures were high.

The major strengths of this study are, first, it is a relatively large cohort of monomer workers. Second, it had a long follow-up period of 52 years. Third, analyses by duration of employment and for latency, as well as adjustment for potential confounding factors, were conducted. Fourth, the exposures in each individual were estimated in the last follow-up. Except for "Hire-age" in survival analysis using Cox model, after 52 years of follow-up this study did not find any statistically significant excess in leukemia (observed in SBR workers); however, this study may not have enough power to detect the leukemia increase.

Some of the limitations of the study are as follows. First, although the exposures were estimated in their last follow-up, there were no data or means available to the investigators to estimate the peak exposures that were hypothesized to be associated with the observed increase in NHL in wartime workers. Second, although the authors state that extremely high peak exposures did exist during the 1950s and 1960s, neither any information about the frequency of these peak exposures (as compared with prior to the 1950s) nor any information about the variations in intensity of peak exposures for these different time periods was available. Third, there is a lack of information on other confounding factors. Fourth, although the cohort is relatively large, it had low power to detect excess leukemias (power calculation resulted in 25% power to detect the observed 13% increase). Nonetheless, the finding of excess mortality from lymphosarcoma (now included in NHL) is consistent with findings of Ward et al. (1995, 1996c) and, although not statistically significant, of Meinhardt et al. (1982).

A small cohort of 364 individuals who had potential exposure to 1,3-butadiene at three Union Carbide plants was studied by Ward et al. (1995, 1996a). A statistically significant excess for lymphosarcoma (477% higher than the general population) was found based on four cases. The main limitations of this study are that the cohort was small and that exposures were assumed based on department codes. In addition, there was no analysis for latency or adjustments for potential confounding by exposure to other chemicals.

Cowles et al. (1994) studied the third cohort of 614 workers. No excess cancer was observed either in mortality or morbidity studies. Due to several methodologic limitations (such as small cohort size, short follow-up, lack of exposure information), the absence of positive results in this study does not provide any negative evidence towards the causal association between exposure to 1,3-butadiene and occurrence of cancer.

All the epidemiologic studies, cohort and nested case-control, evaluated for this assessment are observational studies in occupationally exposed populations. As such, they have various methodologic strengths and limitations as discussed above. A common limitation to all the studies is the use of death certificates, which could lead to misclassification bias. Validation of diagnosis of lymphohematopoietic cancer was not done in any of the studies except in Matanoski et al. (1993).

The scientific community is divided into two schools of thought about the evaluation of carcinogenicity of 1,3-butadiene. One school believes that both NHL and leukemias should be considered as tumors of the same organ system since they are both mesodermic in origin, that is, lymphohematopoietic system (see footnote 1 on p. 8-6), while the other school believes that they should be considered as two separate site-specific tumors. This second group proposes that the excess observed for leukemia in polymer production workers is due to the SBR process, and therefore the SBR process and not 1,3-butadiene should be considered carcinogenic to humans.

These monomer and polymer production worker cohorts demonstrate an excess number of lymphohematopoietic cancers in these occupationally exposed workers. Increased lymphosarcomas are reported for monomer production workers, whereas excess leukemias occur predominantly in polymer production workers. There are several possible explanations for this apparent difference between the monomer and polymer workers. It has been hypothesized that the observed excess of lymphosarcomas in the monomer production workers may be related to exposure intensity, i.e., the excess risk may result from the high (peak) exposures during wartime rather than the much lower exposures currently encountered by monomer production workers or the likewise comparatively lower exposures encountered by the polymer production workers. The absence of a significant leukemia excess in these same monomer workers may be attributable to low statistical power in the monomer studies. There is some suggestion of excess leukemias in the monomer production workers, although these were not statistically significant.

The Union Carbide cohort had a leukemia excess of 23% based on 2 cases, and the Texaco cohort had an elevated risk of leukemias of 13% based on 13 cases. Even the Texaco cohort, which is a relatively large monomer production cohort, has low power to detect a statistically significant excess for leukemias, and with every follow-up, the investigators of the Texaco cohort increased the calendar period for the worker inclusion criteria, hence adding many younger workers with little cumulative exposure, shorter follow-up periods, and inadequate latency periods, thereby diluting the risk. Furthermore, it should be noted that in every follow-up since the initial study by Downs et al. (1987) additional cases of leukemia (7 to 13) have been reported by investigators. In addition, 1,3-butadiene is produced at the end of the monomer production process and current 1,3-butadiene exposures are very low in these workers. Finally, the workers may lack exposure to a necessary co- or modifying factor that may be present in the polymer production, resulting in the development of leukemias, although the findings of Delzell et al. (1996) and Macaluso et al. (1996) show no evidence of confounding by exposure to other chemicals.

In fact, the apparent difference may be largely an artifact. Under the latest classification system for lymphohematopoietic cancers, all lymphomas not classified as Hodgkin's disease are now included under NHL (see footnote #1). Using this classification, an excess of NHL of 37% (based on 15 cases; not statistically significant) was reported for workers who had worked $\geq$ 10 years and with $\geq$ 20 years since hire in the UAB (polymer) cohort (Sathiakumar et al., 1998; previously lymphosarcomas and NHL were reported separately for this cohort). Furthermore, as these investigators report, their evaluation of NHL relations was limited by their reliance on death certificates. NHL has high survival rates and may, in later clinical stages, transform into leukemia, therefore leukemia may be reported on the death certificates. In addition, as discussed above, nonsignificant excesses of leukemia were observed in two monomer studies. Thus, excesses of both leukemia and NHL have been observed for both monomer and polymer workers, and it may be that the increased risk of NHL is primarily observed among workers exposed to high concentrations of 1,3-butadiene (mostly wartime monomer workers), whereas the polymer production studies have greater power to detect a significant leukemia excess among SBR workers who have modest to low exposures. In any event, leukemias and lymphosarcomas are related tumor types and can both be classified as lymphohematopoietic cancers (see footnote #1).

Thus, applying the modified "Hill criteria" of causal inference to the monomer and polymer cohort mortality studies and nested case-control study, in which an increased risk of lymphohematopoietic cancers was demonstrated, results in the following:

- **Temporality.** There is temporality of exposure to 1,3-butadiene prior to the occurrence of NHL in the monomer workers and leukemias in the SBR workers.

- **Strength of association.** Strength of association between exposure and the occurrence of NHL in the wartime workers ranged from 154% to 477% higher risk among workers exposed during monomer production as compared with the nonexposed general population (Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996; Ward et al., 1995, 1996c). The excess risk of leukemia ranged from 43% to 336% higher among ever-hourly workers exposed during SBR production as compared with the general population (Delzell et al., 1996). Internal comparison of the SBR worker population resulted in a 4.5-fold increased leukemia risk among the highest exposure group in the same cohort (Macaluso et al., 1996). The nested case-control study from the SBR cohort showed a sevenfold increase in the risk of leukemia (Matanoski et al., 1989, 1993; Santos-Burgoa et al., 1992).

- **Consistency.** Two cohort studies in monomer workers showed an increased risk of NHL (Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996; Ward et al., 1995, 1996c), while one cohort study (Delzell et al., 1996; Macaluso et al., 1996; with a cohort derived from seven U.S. plants and one Canadian plant) and one nested case-control study (Matanoski et al., 1989, 1993; Santos-Burgoa et al., 1992) showed an excess risk of leukemia in SBR workers. The SBR workers cohort defined by Delzell et al. (1996) showed a fairly consistent association between exposure to 1,3-butadiene and occurrence of leukemia across the plants included in the study. Furthermore, there is a major population overlap between the SBR cohorts identified by JHU and by UAB. These two groups of investigators have independently found excess leukemias in the SBR workers.

- **Specificity.** Increased lymphohematopoietic cancers are observed in both the monomer (NHL) and polymer (leukemias) production workers. It should be noted that exposure to a particular chemical (or drug or radiation) may cause more than one type of leukemia or another type of lymphohematopoietic cancer (Linet, 1985). Hayes et al. (1997) in their Chinese cohort exposed to benzene found that development of leukemia and NHL were dose related.

- **Biological gradient.** The biological gradient, which refers to the dose-response relationship, was observed only in SBR workers. Both the nested case-control study and the cohort study showed increasing risk of leukemia with increasing exposures. Such a relationship was not observed in monomer workers. The reason may be because only a small number of people who were exposed to high peak levels of 1,3-butadiene for a short period of time were probably at increased risk of NHL. Due to a lack of information of intensity and frequency of exposure levels they could not be further stratified to evaluate the dose response. As noted earlier, the monomer studies did not have enough power to detect statistically significant excess of leukemia.

- **Biological plausibility**. Extensive data suggest that the metabolism of 1,3-butadiene is qualitatively similar across species investigated, although there are differences in the amounts of the active metabolites (detailed in Chapter 3). Hemoglobin adducts have been detected in exposed workers. In addition, some studies have reported significantly increased frequencies of *hprt* mutant lymphocytes and chromosome damage in exposed workers.

In summary, the findings of excess lymphohematopoietic cancers in polymer and monomer production workers are consistent with a causal association with exposure to 1,3-butadiene. As demonstrated above, the causality criteria of *temporality*, *strength of association*, *specificity*, *biological gradient* (in SBR workers only), and *consistency* are satisfied. In addition, as discussed in the earlier sections, 1,3-butadiene is metabolized by humans and other species to genotoxic metabolites and is carcinogenic in mice and rats, thus fulfilling the criterion of *biological plausibility* as well. Thus, the human evidence is considered sufficient.

8.2.4.3. *Cancer Characterization Conclusion*

1,3-Butadiene is carcinogenic to humans by inhalation. This characterization is supported by the total weight of evidence provided by the findings discussed above: (1) epidemiologic studies showing increased lymphohematopoietic cancers in workers occupationally exposed to 1,3-butadiene (by inhalation), (2) laboratory studies showing that 1,3-butadiene causes a variety of tumors in mice and rats by inhalation, and (3) studies demonstrating that 1,3-butadiene is metabolized into genotoxic metabolites by experimental animals and humans. The specific mechanisms of 1,3-butadiene-induced carcinogenesis are unknown; however, it is virtually certain that the carcinogenic effects are mediated by genotoxic

metabolites of 1,3-butadiene. Under EPA's 1999 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999b), 1,3-butadiene is characterized as "carcinogenic to humans" by inhalation.

8.2.4.4. *Recent Evaluations of 1,3-Butadiene by Other Organizations*

In recent years, various organizations have arrived at different classifications of carcinogenicity or hazard characterizations for 1,3-butadiene. The conclusions of the evaluations conducted over the past 5 years are as follows:

1. 9th Report on Carcinogens, 2000, by the National Toxicology Program (NTP): In their most recent report (NTP, 2000), mandated by the U.S. Congress, 1,3-butadiene is listed as ***known to be a human carcinogen***. This listing was based on sufficient evidence of carcinogenicity from studies in humans, including epidemiologic and mechanistic information, to indicate a causal relationship between occupational exposure to 1,3-butadiene and excess mortality from lymphatic and hematopoietic cancers.

2. International Agency for Research on Cancer (IARC): In their re-evaluation of some organic chemicals (IARC, 1999), 1,3-butadiene was classified as ***probably carcinogenic to humans*** **- Group2A**. This classification was based on limited evidence in humans and sufficient evidence in animals for the carcinogenicity of 1,3-butadiene, and sufficient evidence in experimental animals for the carcinogenicity of 1,2:3,4-diepoxybutane.

3. List of MAK and BAT Values of Germany: In their maximum concentrations and biological tolerance values at the workplace, Germany's Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (Deutsche Forschungsgemeinschaft, 1999) has classified 1,3-butadiene as ***Category 1***, a substance that causes cancer in humans and that can be assumed to make a significant contribution to cancer risk. The basis for this classification was that epidemiologic studies provide adequate evidence of an association between the exposure of persons and the development of cancer. Alternatively, inadequate epidemiologic data can be substituted with information about the mechanism of action in humans.

4. Occupational Safety and Health Administration (OSHA), 1996: OSHA, part of the U.S. Department of Labor, in its final 1,3-butadiene rule (OSHA, 1996), listed 1,3-butadiene as ***a potential human carcinogen***. OSHA concluded that on the basis of its analysis of animal studies and epidemiologic studies, there is strong evidence that workplace

exposure to 1,3-butadiene poses increased risk of death from cancers of the lymphohematopoietic system. The epidemiologic findings supplemented the findings from the animal studies, which demonstrated a dose response for multiple tumors, particularly for lymphomas in mice exposed to 1,3-butadiene.

5. Canadian Environmental Protection Act, 1998: Health Canada (1998) stated that 1,3-butadiene is considered ***highly likely to be carcinogenic in humans***. This characterization was based on the evidence of an association between exposure in the occupational environment and leukemia that fulfills several of the traditional criteria for causality of association observed in epidemiologic studies, supporting limited data on genotoxicity in human populations and the overwhelming weight of evidence of carcinogenicity and genotoxicity at relatively low concentrations in some species of experimental animals.

All of the evaluations described here were based on the same database. Some of the interpretation differences were because of various uncertainties and data gaps in the current 1,3-butadiene literature. These evaluations are summarized in Table 8-2.

8.3. SPECIAL SUBPOPULATIONS

8.3.1. Sensitive Subpopulations

It is uncertain whether children or other subpopulations have greater susceptibility to exposure to 1,3-butadiene than does the general population. There is no information available in humans on the potential carcinogenicity in children from exposure to 1,3-butadiene at this time. Occurrence of leukemia is causally associated with exposure to 1,3-butadiene in adults, and leukemia is one of the most common cancers in children. Furthermore, leukemia risk in children has been shown to increase with simultaneous exposure to multiple risk factors (Gibson et al., 1968). Thus, exposure to 1,3-butadiene may be an additional risk factor increasing the leukemia risk further in children.

Both the rat and mouse studies suggested that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males, and the female mammary gland was the only 1,3-butadiene-related tumor site common to both species. The underlying mechanism for the observed gender differences in rodents is unknown. Breast cancer rates are increasing in women of all ages. The known risk factors explain only a small proportion of the occurrence of breast

Table 8-2. Carcinogenicity assessments of 1,3-butadiene.

Agency (year)	Cancer classification	Remarks
NTP (2000)	"Known to be a human carcinogen" —based on sufficient evidence of carcinogenicity in humans, including epidemiologic and mechanistic information to indicate causal relationship	Using NTP classification system
IARC (1999)	"2A-probably carcinogenic to humans"—based on limited evidence in humans, sufficient evidence in animals, and sufficient evidence in experimental animals for the carcinogenicity of 1,2:3,4-diepoxybutane	Cancer classification using IARC system.
Deutsche Forschungsgemeinschaft (1999)	"Category 1" —based on epidemiologic studies provide adequate evidence between exposure and the development of cancer	MAK and BAT guidance for classification
OSHA (1996)	"Potential occupational carcinogen" —based on strong evidence in workers exposed at workplace poses increased risk of death from cancer and dose-response for multiple tumors in animals	Using OSHA standards of carcinogen characterization
Health Canada (1998)	Proposed "highly likely to be carcinogenic to humans" —based on fulfillment of several causality criteria in humans, limited data on genotoxicity in humans, and overwhelming evidence of carcinogenicity and genotoxicity in animals at low doses	Cancer characterization system of Canadian Environmental Protection Act

cancer. If exposure to 1,3-butadiene is causally associated with breast cancer in women, then it is an additional breast cancer risk factor, the risk from which could be reduced by reducing exposure to 1,3-butadiene.

Tobacco smoke contains 1,3-butadiene as well as other carcinogens, and a few studies suggest that parental smoking increases the risk of leukemia or lymphoma in children (John et al., 1991; Stjernfeldt et al., 1986). The overall evidence, however, is inconclusive because other studies observed no increased risk. Furthermore, if there is an effect in children from parental smoking, it is unclear whether it is attributable to preconception effects on fathers' sperm, in utero exposure of the fetus, and/or postnatal exposure to environmental tobacco smoke.

Because metabolic activation of 1,3-butadiene to epoxide metabolites is believed to be necessary for carcinogenicity, it is possible that genetic differences in activation or detoxification

enzymes could result in different risks to different human subpopulations. For example, investigators have observed that polymorphism in glutathione-S-transferase genes confers differential susceptibility to the induction of sister chromatid exchanges by 1,3-butadiene metabolites in cultured human lymphocytes. However, the critical/rate-limiting mechanistic steps are unknown at present; thus, it is unknown whether actual human subpopulations may have notably different susceptibility to 1,3-butadiene.

There is no information available in humans on the developmental effects of 1,3-butadiene exposure at this time. The animal data, particularly in the mouse, indicate an effect from prenatal exposure on growth and an effect from paternal exposure on prenatal death. Only the Anderson et al. (1993, 1995, 1996) study followed animals postnatally after paternal exposure, and no significant effects were detected. The postnatal effects of prenatal exposures have not been evaluated, but the fact that fetal weight is the critical effect for this type of exposure suggests the possibility of greater sensitivity of the developing animal than the adult, and the long-term consequences of 1,3-butadiene exposure may indicate that they are at even greater risk. Thus, exposure to 1,3-butadiene may be an additional risk factor for developmental disorders in children.

The effects of chronic exposure on ovarian, uterine, and testicular atrophy in mice suggest that females may be at greater risk than males because of the greater sensitivity of the ovary to the effects of 1,3-butadiene exposure. The ovarian atrophy effects suggest that exposure to 1,3-butadiene may increase the risk for women of early menopause. Early menopause has been shown to result from cigarette smoking, of which 1,3-butadiene is a component. Further elucidation of species differences in the levels of the diepoxide metabolite, which has been shown to be a major factor in ovarian atrophy, is important in determining the risks of early menopause in women.

8.3.2. Highly Exposed Subpopulations

Some subpopulations may be at greater risk than the general population as a result of higher exposure to 1,3-butadiene. Heavy smokers may be highly exposed to 1,3-butadiene due to its formation in tobacco smoke. Cigarette smoke has been shown to be a risk factor for various types of leukemias. It should be noted, however, that known and suspected leukemogenic constituents of tobacco smoke include benzene, polonium-210, nitrosamines, and hydrocarbons in addition to 1,3-butadiene (Schottenfeld and Fraumeni, 1996).

8.4. FUTURE RESEARCH NEEDS

Although there is sufficient evidence to characterize 1,3-butadiene as carcinogenic to humans in this assessment, there are some data gaps in various areas that, if filled, would refine the assessment. The specific research needs are as follows:

8.4.1. Epidemiology

- Exposures in the monomer production workers should be estimated using the same methodology that was used for the SBR production workers by the University of Alabama investigators. If it turns out that the exposures to 1,3-butadiene in the monomer and SBR production are fairly comparable, then that would suggest that co-exposures to other factor(s) in the SBR production (not 1,3-butadiene alone) are associated with the occurrence of leukemia. If, on the other hand, the exposures to 1,3-butadiene in the monomer production workers are considerably lower than in the SBR production workers, then the lack of a statistically significant excess leukemia in the monomer cohort (13) would not nullify the interpretation of the causal association between exposure to 1,3-butadiene and excess leukemia in the SBR production workers.

- Dithiocarbamate exposure should be estimated for the UAB cohort and its relationship to leukemias analyzed.

- The medical records for the leukemia cases in the studies by Delzell et al. (1995, 1996) and Macaluso et al. (1996) should be reviewed to verify the cell types of leukemias.

- Further followup of the SBR cohort will serve two goals: (1) to determine whether any excesses in cardiovascular disease or other site-specific malignancies with a longer latency period are associated with exposure to 1,3-butadiene, and (2) to examine the subcohort hired after 1965 when the DMDTC exposures were drastically reduced and use of protective gear became mandatory. The investigators also will have to consider whether the exposures to 1,3-butadiene were also reduced at that time. Only then can the association between DMDTC and occurrence of leukemia deaths be interpreted.

- Mortality studies should be conducted in other monomer and SBR production worker cohorts to examine whether the results of the North American studies can be duplicated.

- All epidemiologic studies to date have examined male cohorts. Some 1,3-butadiene production facilities around the world (e.g., China) employ women in their laboratories. If the number of women in these facilities is large enough, a cancer study might help determine whether women have an increased risk of getting breast or ovarian cancers, as observed in rodents. Similarly, a reproductive/developmental study might help determine whether there is an increased risk of any other reproductive effects or of developmental effects in exposed fetuses.

- A reproductive study of exposed males is also needed to examine potential dominant lethal effects in humans.

- Biomarkers of exposure have been recently identified in a transitional study. A health outcome study in these workers would be valuable.

8.4.2. Toxicology

- A bioassay should be performed to observe the effects of combined exposures to 1,3-butadiene and DMDTC.

- Bioassays should be performed to evaluate if there are any neurological effects, especially developmental neurotoxicity effects, associated with exposure to 1,3-butadiene.

- Further research is needed on the three active metabolites of 1,3-butadiene to elucidate the mechanism of action for testicular atrophy, abnormal sperm heads, ovarian atrophy to ovarian neoplasms, fetal weight loss, and dominant effects.

- Elucidation of the mechanisms responsible for the interspecies differences in sensitivity to 1,3-butadiene could assist in resolving questions about the human risk for reproductive effects and for cancer at sites for which the Delzell et al. (1995, 1996) study may have had insufficient power to detect an effect.

- Postnatal evaluation of pre- and perinatal exposure to 1,3-butadiene and multigeneration studies might elucidate the longer term consequences of developmental exposures, as well as the reproductive toxicity in both males and females, including early ovarian atrophy in females.

8.4.3. Molecular Biology

- Once the mechanisms of 1,3-butadiene-induced health effects are better understood, information on polymorphisms in human metabolic enzymes (or DNA repair enzymes, etc.) could help define sensitive subpopulations.

8.5. SUMMARY AND CONCLUSIONS

The purpose of this assessment is to review the new information that has become available since EPA's 1985 health assessment of 1,3-butadiene (U.S. EPA, 1985) and to determine if any changes were needed to the earlier conclusions.

1,3-Butadiene is a gas used commercially in the production of styrene-butadiene rubber, plastics, and thermoplastic resins. The major environmental source of 1,3-butadiene is the incomplete combustion of fuels from mobile sources (e.g., automobile exhaust). Tobacco smoke can be a significant source of 1,3-butadiene in indoor air.

This assessment concludes that 1,3-butadiene is carcinogenic to humans by inhalation, based on the total weight of evidence: (a) epidemiologic studies showing increased lymphohematopoietic cancers (leukemias in polymer and NHL in monomer workers) in workers occupationally exposed to 1,3-butadiene (by inhalation), (b) studies showing that 1,3-butadiene causes a variety of tumors in mice and rats by inhalation, and (c) studies demonstrating that 1,3-butadiene is metabolized into genotoxic metabolites by experimental animals and humans. The specific mechanisms of 1,3-butadiene-induced carcinogenesis are unknown; however, it is virtually certain that the carcinogenic effects are mediated by genotoxic metabolites of 1,3-butadiene.

Animal data suggest that females may be more sensitive than males for cancer effects; nevertheless, there are insufficient data from which to draw any conclusions on potentially sensitive subpopulations.

In summary, the primary change in EPA's conclusions about the health effects of 1,3-butadiene from the 1985 document is that the cancer characterization has been changed from probable human carcinogen to carcinogenic to humans by inhalation.

9. PHARMACOKINETIC MODELING

9.1. INTRODUCTION

Several physiologically based pharmacokinetic (PBPK) models of 1,3-butadiene metabolism and disposition have been developed to attempt to explain the interspecies differences in the potency and site specificity of the carcinogenic response between mice and rats and to provide a corresponding dosimetric basis for quantitatively extrapolating carcinogenic potency from rodents to humans. PBPK models use species-specific physiological parameters such as alveolar ventilation rates and blood flow rates, chemical-specific distribution parameters such as blood:air and tissue:blood partition coefficients, and species- and chemical-specific metabolic rates to elucidate the pharmacokinetics (i.e., the uptake, distribution, metabolism, and excretion) of a chemical.

Ideally, such models provide species-specific target tissue doses of the toxicologically active form(s) of the chemical. Carcinogenic risks from bioassay data can then be extrapolated to humans on the basis of equivalent effective doses, reducing some of the uncertainties that occur when interspecies extrapolation is based simply on exposure to the parent compound, especially when nonlinear physiological processes are involved. Assumptions must still be made to the effect that the mechanisms of action of the active form(s) of the compound at the target tissue(s) are the same across species and that the tissues of different species are equally sensitive. If these assumptions are not valid, pharmacodynamic data and modeling would be required for more precise risk assessment.

PBPK models that fall short of describing target tissue doses of the active form(s) of a chemical may still be useful for improving the dosimetric basis of interspecies extrapolation for quantitative risk assessment. For example, it is well established that metabolic activation of 1,3-butadiene is necessary for its carcinogenic action (Chapter 4). Therefore, a PBPK model describing the production and disposition of 1,2-epoxy-3-butene (EB), the first product of metabolic activation of 1,3-butadiene, may be able to provide a better dose metric than the default methodology of using exposure to 1,3-butadiene itself.

This chapter reviews and analyzes the PBPK models for 1,3-butadiene that are currently available and assesses their usefulness for quantitative risk assessment of 1,3-butadiene based on interspecies extrapolation. These models generally assume, for simplicity, that the transfer of 1,3-butadiene to tissues is blood flow-limited and that each tissue compartment is “well mixed.”

9.2. PBPK MODELS FOR 1,3-BUTADIENE

9.2.1. Hattis and Wasson (1987)

The first PBPK model for 1,3-butadiene was the unpublished model of Hattis and Wasson (1987). They defined the effective dose of 1,3-butadiene as the amount that is metabolically converted to EB and used this dose as a basis for a risk assessment of occupational 1,3-butadiene exposure. Their model consists of three compartments: a fat compartment; a muscle compartment; and a liver and vessel-rich compartment, which includes the brain, heart, kidneys, and other small visceral organs. The transfer of 1,3-butadiene between blood and tissues is assumed to be blood flow-limited. Metabolism to the monoepoxide is ascribed to the entire liver and vessel-rich compartment and is assumed to follow simple Michaelis-Menten kinetics. No further metabolism of EB is considered.

The only chemical-specific parameter values then available were whole-body maximal metabolic rates for mice and rats inferred from the chamber study data of Kreiling et al. (1986b). These data provided the V_{max} and preliminary K_M estimates for the liver and vessel-rich compartment. Tissue:blood and blood:air partition coefficients were estimated from chemical structure and solubility data using empirical relationships (e.g., Fiserova-Bergerova and Diaz, 1986). Model simulations were then run, adjusting K_M and the blood:air partition coefficient to fit the blood 1,3-butadiene concentration data of Bond et al. (1986), to derive "best estimates" for these parameters. Human metabolic rates were estimated by allometric scaling of the mouse and rat rates because no PBPK data were available for human metabolism of 1,3-butadiene. The parameter values used by Hattis and Wasson (1987) are summarized in Table 9-1.

No additional data were available at that time for an independent validation of this model. A minimal sensitivity analysis was conducted by varying K_M and the blood:air partition coefficient among a few values and observing the effect on the ultimate risk estimates. Hattis and Wasson (1987) claimed that their model is not very sensitive to reasonable differences in partition coefficients. Similarly, the model is insensitive to the precise value of the metabolic parameters because, given the blood:air partition coefficient values that were used, metabolic conversion in their model is limited by blood flow to the liver and vessel-rich compartment.

The Hattis and Wasson (1987) model is not discussed further here because it has been superseded by new data and other modeling efforts.

9.2.2. Hallenbeck (1992)

Hallenbeck (1992) reported having done a PBPK-based cancer risk assessment for 1,3-butadiene; however, he provided no details of the PBPK model that he used. Furthermore, he used the area under the 1,3-butadiene concentration-versus-time curve for the lung as his

.

Table 9-1. Parameter values used in the Hattis and Wasson (1987) PBPK model.

Parameter	Rat	Mouse	Human
Physiological parameters			
Alveolar ventilation (L/min)	0.15	0.0233	11.38[a] 4.8[b]
Weight (kg)	0.40	0.028	70
Q_f (L/min)	0.0136	0.00192	0.69[a] 0.35[b]
Q_m (L/min)	0.0226	0.00319	2.61[a] 1.1[b]
Q_{lvr} (L/min)	0.1042	0.01617	5.09[a] 4.35[b]
V_f (L)	0.028	0.0028	14.024
V_m (L)	0.300	0.0196	34.756
V_{lvr} (L)	0.036	0.00308	8.513
Partition coefficients[c]			
Blood:air	0.35	0.35	0.35
P_f	118.2	118.2	118.2
P_m	5.26	5.26	5.26
P_{lvr}	5.4	5.4	5.4
Metabolic parameters			
V_{max} (mol/min)	1.47E-6[d]	1.87E-7[d]	8.0E-5[e]
K_M (mol/L)[f]	5E-6[f]	5E-6[f]	5E-6[f]

[a]Awake.
[b]Asleep.
[c]The blood:air partition coefficient of 0.35 is the "best estimate" value from "fitting" the model. The tissue:blood partition coefficients (P) are from functions of the blood:air partition coefficient for which the "best estimate" value of 0.35 was used. Partition coefficients are assumed to be the same across species.
[d]From Kreiling et al. (1986b).
[e]From allometric scaling of the rodent values.
[f]"Best estimate" from "fitting" the model.

Subscripts f, m, and lvr designate the fat, muscle, and liver and vessel-rich compartments (tissues), respectively.
Q: tissue blood flow rate.
V: tissue volume.
P: tissue:blood partition coefficient.

tissue-dose surrogate, taking no account of metabolic activation. As presented, this model contributes nothing to the current state of knowledge regarding the pharmacokinetic modeling of 1,3-butadiene.

9.2.3. Kohn and Melnick (1993)

The PBPK model of Kohn and Melnick (1993) focuses on the disposition of EB in the mouse, rat, and human. This model incorporates additional tissues (compartments) and metabolic reactions based on experimental data that were not available at the time of the Hattis and Wasson (1987) model; however, it also relies on theoretically derived partition coefficients. The Kohn and Melnick model is blood flow-limited and consists of six compartments: lung, blood, fat, liver, other rapidly perfused tissues (viscera), and slowly perfused tissues (muscle). Metabolism occurs in the liver, lung, and viscera compartments. The metabolic reactions include conversion of 1,3-butadiene to EB, the conversion of EB to 1,2:3,4-diepoxybutane (DEB), the enzymatic hydrolysis of EB, and the enzymatic conjugation of EB with glutathione.

With the exception of the partition coefficients, which were derived theoretically from published methodologies, all of the mouse, rat, and human parameter estimates were from experimental data in the literature. None of the parameter values were obtained by optimization. The parameter values used by Kohn and Melnick (1993) are summarized in Table 9-2. Blood:tissue partition coefficients for 1,3-butadiene were from Hattis and Wasson (1987). The blood:air partition coefficients reported by Csanády et al. (1992) for 1,3-butadiene and EB were used as lung:air partition coefficients. The fat:blood partition coefficient for EB was calculated using an empirical relationship from Lyman et al. (1990), whereas the tissue:blood partition coefficients of EB for the other tissues were derived using the method of Fiserova-Bergerova and Diaz (1986). These are essentially the same procedures used by Hattis and Wasson (1987).

Michaelis-Menten kinetics were used to describe the oxidation of 1,3-butadiene and EB by the cytochrome P-450 isozyme CYP2E1, the hydrolysis of EB by epoxide hydrolase, and the glutathione S-transferase-catalyzed conjugation of EB with glutathione. K_M and V_{max} values for each of these reactions in the liver and lung of the mouse, rat, and human were taken from the in vitro data of Csanády et al. (1992). The lung values were also assumed to apply to the viscera compartment. Csanády et al. reported kinetic constants for the oxidation of EB to DEB only for mouse liver preparations. Therefore, Kohn and Melnick (1993) included DEB production only in the mouse liver compartment and only as a disappearance route for EB; the distribution of DEB was not further modeled. 1,3-Butadiene and EB were treated as competitive inhibitors of each other in the rate equations for mouse liver CYP2E1. Finally, although glutathione was treated as saturating for glutathione S-transferase in the mouse, rat, and human liver, glutathione conjugation with EB in human lung and viscera was assumed to be first order.

To validate their model, Kohn and Melnick (1993) compared predicted 1,3-butadiene absorption and blood concentrations for mice and rats with the measurements of Bond et al. (1986). They also modified the model to include a chamber compartment and compared predicted EB concentrations in the chamber and maximum metabolic elimination rates with the

Table 9-2. Parameter values used in the Kohn and Melnick (1993) PBPK model.

Parameter	Mouse	Rat	Human
Physiological parameters[a]			
Body weight (kg)	0.028	0.4	70
Cardiac output (L/h)	1.044	7.32	660[b]
Ventilation rate (L/h)	2.64	15.6	1,200[b]
Fraction blood	0.05	0.054	0.077
Fraction fat	0.04	0.08	0.144
Fraction liver	0.062	0.05	0.025
Fraction viscera	0.05	0.083	0.037
Fraction muscle	0.78	0.59	0.547
Fat flow fraction	0.05	0.07	0.036
Liver flow fraction	0.16	0.16	0.16
Viscera flow fraction	0.52	0.40	0.446
Muscle flow fraction	0.19	0.36	0.361
Partition coefficients[c]			
Air partition BD		1.5	
Fat partition BD		118.2	
Liver partition BD		5.49	
Viscera partition BD		5.34	
Muscle partition BD		5.26	
Air partition EB		60	
Fat partition EB		1.8083	
Liver partition EB		0.6545	
Viscera partition EB		0.6348	
Muscle partition EB		0.6533	
Biochemical parameters for 1,3-butadiene oxidation[d]			
Liver V cyt1 (nmol/h/mg)	155.4	35.4	70.8
Liver Km cyt1 (mM)	0.002	0.00375	0.00514
Lung V cyt1 (nmol/h/mg)	138.6	9.6	9
Lung Km cyt1 (mM)	0.00501	0.00775	0.002

Table 9-2. Parameter values used in the Kohn and Melnick (1993) PBPK model (continued).

Parameter	Mouse	Rat	Human
Biochemical parameters[d]			
Liver V cyt2 (nmol/h/mg)	12		
Liver Km cyt2 (mM)	0.0156		
Liver V EH (nmol/h/mg)	347.4	148.8	1,110
Liver Km EH (mM)	1.59	0.26	0.58
Liver V GST (nmol/h/mg)	30,000	14,460	2,706
Liver Km GST (mM)	35.3	13.8	10.4
Lung k hydr (h^{-1}/mg)	0.1116	0.0792	0.1914
Lung V GST (nmol/h/mg)	6,380	2,652	
Lung Km GST (mM)	36.5	17.4	
Lung k GST (h^{-1}/mg)			0.1536
Protein concentrations			
Liver micro prot (mg/L)[d]	11,600	16,800	14,500
Liver cyto prot (mg/L)[d]	82,800	108,000	58,000
Lung micro prot (mg/L)[e]	3,000	3,000	3,000
Lung cyto prot (mg/L)[f]	82,800	108,000	58,000

[a]Compartment volumes are given as fractions of body weight; compartment blood flow rates are given as fractions of cardiac output.
[b]Human cardiac output at rest: 336 L/h; human ventilation rate at rest: 240 L/h.
[c]Lung:air and tissue:blood; assumed same for all species.
[d]Data from Csanády et al. (1992).
[e]Personal communication from R. Philpot, NIEHS.
[f]Assumed same as for liver.

BD: 1,3-butadiene; EB: 1,2-epoxy-3-butene.
V: V_{max}; Km: K_M.
cyt1 denotes oxidative metabolism of butadiene to EB; cyt2 denotes oxidative metabolism of EB.
EH: epoxide hydrolase.
GST: glutathione S-transferase.
micro prot: microsomal protein; cyto prot: cytoplasmic protein.
k hydr: apparent first-order rate constant for EB hydrolysis; k gst: apparent first-order rate constant for glutathione conjugation.

Laib et al. (1990) results for mice and rats. Kohn and Melnick claimed that their model predictions are comparable to the experimental results except for overestimates in the blood 1,3-butadiene concentrations, which they ascribed to inadequacies in the model or experimental sources of error in the blood concentration measurements.

To assess the sensitivity of the model to the values of various parameters, relative sensitivity coefficients for different model variables were estimated by finite differences, as given by Frank (1978). The physiological parameters to which the model was the most sensitive were the lung:air partition coefficient and the cardiac output. Because the ventilation rate is greater than the rate of 1,3-butadiene absorption, the lung:air partition coefficient and the cardiac output are the major parameters governing 1,3-butadiene uptake. Predicted 1,3-butadiene concentrations were not very sensitive to variations in the biochemical parameters; however, EB levels were somewhat more sensitive to the parameters describing hepatic glutathione S-transferase and epoxide hydrolase kinetics.

Based on their model simulations, Kohn and Melnick (1993) reported that 1,3-butadiene uptake and the disposition of EB are controlled to a greater extent by physiological parameters than by biochemical parameters. The model further suggests that storage in fat is a significant fraction of retained 1,3-butadiene, especially in rats and humans. Kohn and Melnick also found that predicted EB tissue concentrations do not correlate with tumor incidences in mice and rats, and they concluded that other factors are crucial in 1,3-butadiene-induced carcinogenesis. These other factors may include pharmacokinetic variables that were not part of the model, such as accumulation of the diepoxide or formation of other metabolites or mechanistic (pharmacodynamic) phenomena, such as formation of DNA adducts or efficiency of DNA repair.

The Kohn and Melnick (1993) model appears to have a reasonable basic structure, in terms of the compartments and metabolic reactions included, given the biochemical parameters that were available at the time. A major strength of their model is that none of the parameter estimates is adjusted to fit experimental data. Two important drawbacks of the model are the use of empirically derived partition coefficients and the lumping of various tissues with different metabolic capabilities (Chapter 3) into a viscera compartment, which is assumed to have the same metabolic activity as the lung. Partition coefficients for 1,3-butadiene and EB have since been measured by Johanson and Filser (1993) and Medinsky et al. (1994), and experimental values for the 1,3-butadiene partition coefficients are substantially less than the empirically derived estimates, which suggests that the specific results reported by Kohn and Melnick may not be relevant. For example, the role of physiological parameters in controlling 1,3-butadiene uptake and the amount of 1,3-butadiene storage in fat may not, in fact, be as great as the Kohn and Melnick model predicts (Medinsky et al., 1994). More recent revisions to this model are discussed in Sections 9.2.7.1 and 9.2.10.

9.2.4. Johanson and Filser (1993)

Johanson and Filser (1993) developed a PBPK model for 1,3-butadiene and EB disposition in rats and mice. Their model is blood flow-limited and consists of four main physiological compartments—lungs and arterial blood, muscle and vessel-rich tissues, fat, and liver—as well as a chamber compartment and an intrahepatic subcompartment. Metabolism is assumed to take place exclusively in the liver. The metabolic reactions include oxidation of 1,3-butadiene to EB; hydrolysis of EB; intrahepatic first-pass hydrolysis of EB; conjugation of EB with glutathione, which is described by a "ping-pong" mechanism; and the turnover and depletion of hepatic glutathione.

In contrast with the previous PBPK modeling efforts for 1,3-butadiene, Johanson and Filser (1993) conducted in vitro studies of rat homogenates to obtain empirical values for the tissue:air partition coefficients for 1,3-butadiene and EB. All physiological parameters were taken from Arms and Travis (1988), except the alveolar ventilation rates, which were reduced to 60% of those suggested by Arms and Travis on the basis of generalized observations of uptake rates of various gases in closed-chamber experiments (Johanson and Filser, 1992).

For the oxidative metabolism of 1,3-butadiene, the model uses the V_{max} values from the in vitro studies of Filser et al. (1992). A K_M value was derived by fitting the model to the in vivo data of Lieser (1983) for the rat and Kreiling (1986b) for the mouse because the model could not reproduce the results observed in these closed-chamber studies using the K_M values of either Filser et al. (1992) or Csanády et al. (1992). Values for the metabolic parameters pertaining to the conjugation of EB with glutathione and to the hydrolysis of EB were taken from the in vitro data of Kreuzer et al. (1991). The value of the "intrinsic K_M" for the intrahepatic hydrolysis of EB (see below) was set to 20% of the "apparent K_M" value of Kreuzer et al. because the model then fit various in vivo data. The flow rate between the hepatic and intrahepatic compartments was estimated from the kinetic parameters. The physiological and biochemical parameter values used by Johanson and Filser (1993) are summarized in Table 9-3.

In terms of the metabolic reactions involved, the Johanson and Filser (1993) model differs from the Kohn and Melnick (1993) model in that further oxidation of EB to DEB is not included, conjugation of EB with glutathione is described by the two-substrate ordered sequential ping-pong mechanism (reviewed by Mannervik, 1985) rather than by Michaelis-Menten kinetics, and glutathione turnover and the intrahepatic first-pass hydrolysis of EB are incorporated. Given the K_M values for glutathione conjugation used in the model, the conjugation of EB becomes rate-limited by glutathione only when glutathione is almost completely depleted. Cytosolic glutathione turnover is depicted by zero-order production and first-order elimination. Intrahepatic first-pass hydrolysis of EB is hypothesized to occur, based on the observations of

Table 9-3. Parameter values used in the Johanson and Filser (1993) PBPK model.

Parameter		Mouse	Rat
Physiological data			
Body weight (g)	Standard animal Simulations	25 27.5	250 157.5-217.5[a]
Alveolar ventilation (mL/min)	Standard animal Simulations	15 proportional to $bw^{2/3}$	70.2
Cardiac output (mL/min)	Standard animal Simulations	17 proportional to $bw^{2/3}$	83
Blood flows (% of cardiac output)	Muscle and VRG Fat Liver	66 9 25	66 9 25
Compartment volumes (% of body weight)	Lung and arterial Muscle and VRG Fat Liver	1 75 10 5.5	1 80 7 4
Partition coefficients[b]			
1,3-Butadiene	Lung and arterial, muscle and VRG, liver Fat Blood	0.25 7.23 3.03	
1,2-Epoxy-3-butene	Lung and arterial, muscle and VRG, liver Fat Blood	0.706 1.89 83.4	

Table 9-3. Parameter values used in the Johanson and Filser (1993) PBPK model (continued).

Parameter		Mouse	Rat
Metabolic constants			
1,3-Butadiene oxidation	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·min^{-1}·mg^{-1})[c]	3.22	2.17
	K_M (μmol/L air)[d]	5	5
EB hydrolysis	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·min^{-1}·mg^{-1})[e]	19	17
	Apparent K_M (mmol/L)[e]	1.5	0.7
	Intrinsic K_M (% of apparent K_M)[d]	20%	20%
EB conjugation	Cytosolic protein (mg/g liver)	95	95
	V_{max}/K_M of EB (μL·min^{-1}·mg^{-1})[e]	15	11
	K_M toward EB (mmol/L)[e]	100	100
	K_M toward glutathione (mmol/L)[f]	0.1	0.1
Glutathione kinetics	Initial steady-state concentration (mmol/L)	8.31[g] 5.5[h]	5.56[g] 4.2[h]
	Elimination rate constant (h^{-1})[f]	0.15	0.15

[a]Depending on experiment simulated.
[b]Tissue:blood and blood:air. measured for rats; assumed same for mice.
[c]From Filser (1992).
[d]Obtained by best fit.
[e]Kreuzer et al. (1991).
[f]Average of literature data.
[g]Deutschmann and Laib (1989).
[h]Kreiling et al. (1988).

VRG: vessel-rich tissue group.
EB: 1,2-epoxy-3-butene.
$bw^{2/3}$ = (body weight)$^{2/3}$.

Filser and Bolt (1984), because of proximity of the monooxygenase to the epoxide hydrolase in the endoplasmic reticulum. Newly formed EB within this intrahepatic compartment will be more readily hydrolyzed than EB that must diffuse in from outside the compartment, as reflected by a lower K_M in the intrahepatic compartment.

To attempt to validate the model, Johanson and Filser (1993) compared simulated results with the data from various in vivo experiments. In addition to the 1,3-butadiene kinetics data used to fit the K_M for 1,3-butadiene oxidation and the EB kinetics data of Filser and Bolt (1984) for the rat and Kreiling (1987) for the mouse that were used to fit the intrinsic K_M for intrahepatic first-pass hydrolysis, the model apparently reproduces the EB concentrations appearing in chamber air as a result of 1,3-butadiene exposure in the experiments of Rolzhäuser (1985) for the rat and Kreiling (1987) for the mouse. However, it is not clear from the text whether these experimental data were also used to fit the intrinsic K_M. The model also reproduces the glutathione concentrations observed by Deutschmann (1988) in rat and mouse liver after 1,3-butadiene exposure, and Johanson and Filser claimed that no model parameters were fitted to these data. Finally, simulated blood concentrations of EB approximate those observed by Bond et al. (1986) in the mouse but are slightly higher than those observed in the rat.

No sensitivity analysis for the model parameters was reported.

The results of Johanson and Filser's (1993) model simulations suggest that the internal dose of EB, expressed as the concentration of EB or the area under the concentration-time curve in the venous blood, the other compartments, or the whole body, is at most about three times greater in the mouse than in the rat for a given exposure concentration. The greatest differences in internal dose of EB between the two species result from 1,3-butadiene exposure concentrations of above 1000 ppm, when glutathione depletion occurs in the mouse but not in the rat after 6 to 9 h of exposure. Once again, the relatively small interspecies differences in body burden of EB indicated by PBPK modeling cannot explain the striking differences in cancer response between mice and rats exposed to 1,3-butadiene. Johanson and Filser suggested that differences in the kinetics of DEB or nonmetabolic factors, such as differences in immune response or in the expression of oncogenes, may be responsible for the interspecies differences in cancer response.

A major advancement found in the PBPK model of Johanson and Filser (1993) is the use of experimentally derived partition coefficients, especially because these values differ substantially from the theoretically estimated values. A further strength of their analysis is that they compared the simulation results with data from several different experiments. The Johanson and Filser model also incorporates hepatic glutathione turnover and depletion as well as intrahepatic first-pass hydrolysis of EB, although the significance of these refinements is unknown. Some of the limitations of the model include the exclusion of extrahepatic metabolism and of further metabolism of EB to DEB. In addition, the values of the K_M for 1,3-butadiene

oxidation and of the intrinsic K_M for intrahepatic first-pass hydrolysis of EB were obtained by fitting in vivo data. Finally, no sensitivity analysis was reported, although, for example, it was acknowledged that wide ranges of glutathione concentrations and turnover rates have been observed. Therefore, it is unknown how sensitive the model is to changes in these and other parameters. More recent modifications to this model are discussed in Sections 9.2.7.2 and 9.2.7.3.

9.2.5. Evelo et al. (1993)

Evelo et al. (1993) present a PBPK model for the uptake, distribution, and metabolic clearance of 1,3-butadiene in mice and rats. Their stated objective was to investigate the relative importance of liver and lung metabolism at different 1,3-butadiene exposure concentrations. The Evelo et al. model has six physiological compartments: liver, fat, muscle, a vessel-rich group, the bronchial area of the lung, and the alveolar area of the lung. A chamber compartment is also included for validation against the data from closed-chamber experiments. 1,3-Butadiene metabolism is assigned to both the alveolar and bronchial areas of the lung and to the liver. Gas exchange occurs in the alveolar area of the lung.

Values for the standard physiological parameters were allometrically scaled from the data of Travis (1988). Volumes and blood flows for the two separate lung compartments were taken from Greep and Weis (1977). Tissue:blood and blood:air partition coefficients were theoretically estimated using the regression analysis method of Fiserova-Bergerova and Diaz (1986), as was done previously by Hattis and Wasson (1987).

To describe the oxidation of 1,3-butadiene to EB, Evelo et al. (1993) calculated the ratios of the maximum metabolic activity between the liver and the lung from the in vitro data of Schmidt and Loeser (1985) for the mouse and the rat. Then, the total (whole-body) maximum metabolic activities, the K_Ms, and the "most probable distribution" of metabolic activity between the alveolar and bronchial areas of the lung were derived by optimizing the model against the closed-chamber data of Kreiling et al. (1986b) for the mouse and Bolt et al. (1984) for the rat. The only options considered for the distribution of the metabolic activity of the lung were that all the metabolism took place in either one of the two areas, that it was equal in each area, or that it was distributed relative to the volumes of each area; the best fit was found using the latter distribution. The values of the physiological and metabolic parameters used in the Evelo et al. model are summarized in Table 9-4.

The only independent validation of the model was against the whole-body extraction ratios reported by Dahl et al. (1990). Evelo et al. (1993) calculated extraction ratios of 8.4% for the mouse and 5.2% for the rat, whereas Dahl et al. found ratios of 12.8% for the mouse and 4.3% for the rat. Evelo et al. also noted that the whole-body V_{max} value obtained for the rat by

Table 9-4. Parameter values used in the Evelo et al. (1993) PBPK model.

Parameter	Mice	Rats
Physiological parameters		
Body mass (kg)	0.0275	0.215
Cardiac output (mL/min)	24.83	75.93
Alveolar ventilation (mL/min)	24.5	118.7
Blood flows (mL/min):		
Liver		
Fat	6.14	19.17
Muscle	2.34	6.52
Vessel-rich tissue	3.81	11.13
Bronchial lung area	10.75	33.60
Alveolar lung area	1.79	5.514
	23.04	70.42
Volumes (mL):		
Liver	1.65	8.63
Fat	2.94	14.0
Muscle	19.09	162.7
Vessel-rich tissue	1.17	9.49
Bronchial lung area	0.2	1.29
Alveolar lung area	0.18	1.63
Partition coefficients[a]		
Blood:air	0.894	
Fat:blood	32.362	
Liver:blood	2.675	
Muscle:blood	1.871	
Kidney:blood	1.690	
Lung:blood	1.272	
Brain:blood	2.355	
Vessel rich:blood[b]	2.02	
Metabolic parameters		
$V_{max,total}$ ($\mu mol \cdot h^{-1} \cdot kg^{-1}$)	465	200
$V_{max,liver}$ ($\mu mol \cdot h^{-1} \cdot kg^{-1}$)	318	171
$V_{max,bronchial}$ ($\mu mol \cdot h^{-1} \cdot kg^{-1}$)	77	13
$V_{max,alveolar}$ ($\mu mol \cdot h^{-1} \cdot kg^{-1}$)	70	16
K_M (μM)	8	5

[a]Same for all species.
[b]Mean value of kidney:blood and brain:blood.

fitting the model to the data of Bolt et al. (1984) does not fall within the range of values allowed by experimental error based on the gas-uptake studies of Laib et al. (1992).

Evelo et al. (1993) stated that sensitivity analyses found the model optimization to be relatively insensitive to variability in the value of K_M. No other sensitivity analysis results are reported.

The model simulations of Evelo et al. (1993) suggest that the relative importance of 1,3-butadiene metabolism in the mouse lung is greater than the distribution of metabolic activity would imply, especially at exposure concentrations of less than 200 ppm and for K_M values of less than the "best fit" value. Evelo et al. concluded that there is a strong first-pass effect in the mouse lung. At higher concentrations, alveolar metabolism is saturated, and liver metabolism becomes relatively more important. The relative importance of lung metabolism also increases with decreasing exposure concentration for the rat and human, especially with lower values of K_M; however, unlike for the mouse, the lung metabolism never exceeds the liver metabolism. Evelo et al. suggested that the higher rate of metabolic activation in the mouse lung could be responsible for the mouse's greater sensitivity to developing lung carcinomas and heart hemangiosarcomas from exposure to 1,3-butadiene.

The Evelo et al. (1993) model suffers from a number of serious weaknesses. Several important parameters are not empirically derived. The partition coefficients are estimated theoretically, and the whole-body V_{max} and K_M are optimized. For the rat, this exercise generated a V_{max} value that was inconsistent with other in vivo data. Furthermore, sensitivity analyses revealed that the optimization was insensitive to variability in the value of K_M, so there is considerable uncertainty in the actual value of this parameter. The results pertaining to the relative importance of lung metabolism, however, are highly sensitive to the value of K_M. The separation of the lung into alveolar and bronchial areas and the "optimized" distribution of lung metabolism between the two areas also appear tenuous. Other limitations of the model are that metabolism is limited to the lung and liver and that further metabolism of EB is not incorporated. In addition, the model was not adequately validated, and only limited sensitivity analyses are described. Finally, results for humans are discussed; however, the parameters used for the human model are not fully reported.

9.2.6. Medinsky et al. (1994)

The model of Medinsky et al. (1994) describes 1,3-butadiene and EB uptake and metabolism in mice and rats. The Medinsky et al. model is a venous equilibration, flow-limited model with six physiological compartments—liver, lung, fat, slowly perfused tissue group, rapidly perfused tissue group, and blood—and a compartment representing the air in closed-chamber experiments. The model describes the oxidative metabolism of 1,3-butadiene in the

liver and lung, as well as hydrolysis and glutathione conjugation of EB in the liver. In the mouse, hepatic oxidation of EB is also included. In addition to measuring actual partition coefficients, Medinsky et al. conducted closed-chamber experiments of 1,3-butadiene uptake with both mice and rats to test the predictions of their model.

Medinsky et al. (1994) measured partition coefficients for 1,3-butadiene and EB experimentally in vitro for both mouse and rat tissues. They found no significant differences between the two species, except for the muscle:air partition coefficient for 1,3-butadiene and the fat:air coefficient for EB (although the ultimate fat:blood coefficient was not significantly different). Organ and body weights were taken from specific experiments on 1,3-butadiene. The remaining physiological parameters were based on average literature values, with the exception of alveolar ventilation rate. Alveolar ventilation rates, conventionally defined as 70% of measured total ventilation rates, yielded overestimates of 1,3-butadiene uptake at low concentrations, consistent with observations by Johanson and Filser (1992) for other volatile organic chemicals. Therefore, "apparent" alveolar ventilation rates were obtained by optimization to provide rates that yielded the best fit of the model to the EB uptake data. The optimized rates represented 63% of alveolar ventilation for both rats and mice.

Oxidation of 1,3-butadiene and EB (the latter in mouse liver only) and hydrolysis of EB were described using Michaelis-Menten kinetics. Glutathione conjugation of EB was assumed to be first order, based on the large K_M value reported by Csanády et al. (1992).

Rate constants for the metabolism of 1,3-butadiene and EB were taken from the in vitro data of Csanády et al. (1992). Apparent enzyme affinities (K_M) measured in vitro were used directly, whereas maximum metabolic rates (V_{max}) were scaled to the whole organs. However, when the organ microsomal concentrations reported by Csanády et al. are used to scale the metabolic rates similarly reported by Csanády et al., "[1,3-butadiene] uptake from the closed chamber is underestimated." Therefore, Medinsky et al. (1994) used literature values that were two to six times greater for microsomal concentrations in the liver and lung in order to successfully simulate the chamber study results. The parameter values used in the Medinsky et al. model are summarized in Table 9-5.

For validation of the model components pertaining to EB uptake and metabolism, model predictions were compared with the EB uptake data from the closed-chamber experiments of Filser and Bolt (1984) for rats and Kreiling et al. (1987) for mice, although these were the same data used to optimize the alveolar ventilation rates. The model predictions were deemed "adequate," although EB uptake was overestimated at the highest exposure concentration, especially for the rats (3,000 ppm). Medinsky et al. (1994) then compared model simulations of 1,3-butadiene uptake to their own closed-chamber data for mice and rats exposed to 1,3-butadiene and to data from the closed-chamber experiments of Bolt et al. (1984) for rats and

Table 9-5. Parameter values used in the Medinsky et al. (1994) PBPK model.

Parameter	Rat	Mouse
Physiological parameters		
Alveolar ventilation (L/hr/kg)[a]	17	41
Cardiac output (L/hr/kg)[b]	17	41
Body weight (kg)[c]	0.215-0.475	0.028-0.035
Blood flows (fraction of cardiac output)		
Liver	0.25	0.25
Fat	0.09	0.09
Lung	1.0	1.0
Slowly perfused tissues	0.15	0.15
Rapidly perfused tissues	0.51	0.51
Organ volumes (fraction of body weight)		
Liver	0.05	0.0624
Fat	0.09	0.10
Lung	0.0053	0.005
Slowly perfused tissues	0.71	0.70
Rapidly perfused tissues	0.0347	0.0226
Partition coefficients for 1,3-butadiene		
Blood:air	1.49	1.34
Liver:blood	0.799	1.01
Lung(& rapid):blood	0.617	1.10
Muscle(& slow):blood	0.987	2.99
Fat:blood	14.9	14.3
Partition coefficients for EB		
Blood:air	50.4	36.6
Liver:blood	1.43	1.15
Lung(& rapid):blood	1.09	1.54
Muscle(& slow):blood	0.393	0.645
Fat:blood	2.74	2.49
Tissue concentrations		
Liver microsomal concentration (mg/g liver)	35	35
Lung microsomal concentration (mg/g lung)	20	20
Liver cytosolic concentration (mg/g liver)[d]	108	82.8

Table 9-5. Parameter values used in the Medinsky et al. (1994) PBPK model (continued).

Parameter	Rat	Mouse
Rate constants for oxidative metabolism of 1,3-butadiene[d]		
Liver V_{max} (μmol/kg/h)	62	338
K_M (μmol/L)	3.75	2.00
Lung V_{max} (μmol/kg/h)	1.01	21.6
K_M (μmol/L)	7.75	5.01
Rate constants for EB metabolism in the liver[d]		
Oxidation V_{max} (μmol/kg/h)		26
K_M (μmol/L)		15.6
Hydrolysis V_{max} (μmol/kg/h)	260	754
K_M (μmol/L)	260	1590
Glutathione conjugation K (L/kg/h)	5.66	4.36

[a]Obtained by optimization.
[b]Ventilation/perfusion = 1.
[c]Depending on experiment simulated.
[d]From Csanády et al. (1992), with V_{max} values scaled to whole organ using above microsomal concentrations.

EB: 1,2-epoxy-3-butene.

Kreiling et al. (1986b) for mice, and concluded that the model adequately predicted the in vivo uptake results. Medinsky et al. also compared model predictions with the 1,3-butadiene retention data of Bond et al. (1986) and found the results similar for exposure concentrations up to about 100 ppm. At higher concentrations, the model overestimated 1,3-butadiene retention observed in mice. Furthermore, the blood concentrations of EB following 1,3-butadiene exposure, as reported by Bond et al., were overestimated by the model for both mice (except at the lowest exposure) and rats by about two- to fourfold, although Medinsky et al. suggested that the discrepancy might be attributable to EB loss from the blood during sampling.

No comprehensive sensitivity analysis for the model parameters was reported. Medinsky et al. (1994) did note that use of the microsomal concentrations reported by Csanády et al. (1992) resulted in underestimation of the 1,3-butadiene uptake from chamber studies. In addition, they investigated whether the model was sensitive to the different values obtained for the muscle:air partition coefficients for the mouse and rat and determined that the species-specific coefficients provided the best fits to their 1,3-butadiene uptake results for the two species. Medinsky et al. also determined that the inclusion of lung metabolism improves the model fit for the mouse, especially at lower exposure concentrations, but has little effect for the rat.

Based on their model simulations, Medinsky et al. (1994) suggested that lung metabolism may play an important role in 1,3-butadiene uptake and carcinogenesis. Their model predicts locally generated concentrations of EB that are 15 times greater in the mouse lung than in the rat lung, for a 6-h exposure to 10 ppm. Medinsky et al. recommended that more research be done to characterize 1,3-butadiene metabolism and target cells in the mouse lung and to understand the pharmacokinetics of DEB in different species. They further claimed that "quantitation of the concentrations of [1,3-butadiene], [EB], and [DEB] in target and non-target tissues of rats and mice after exposure to [1,3-butadiene] is essential for validation of existing models before these models can be applied to predict behavior in humans."

One of the major strengths of the Medinsky et al. (1994) model is that these investigators experimentally measured partition coefficients and confirmed the results of Johanson and Filser (1993), suggesting that the empirical values for the partition coefficients for 1,3-butadiene differ significantly from the theoretical values used in previous models. Medinsky et al. also conducted closed-chamber experiments to obtain validation data for their model and investigated the role of lung metabolism in 1,3-butadiene uptake. Some limitations of the model include the fact that metabolism was restricted to the liver and lung, although other tissues are known to metabolize 1,3-butadiene as well (Chapter 3). In addition, the alveolar ventilation rates were determined by fitting experimental closed-chamber data, and there are uncertainties about the actual values for organ microsomal contents. Finally, only 1,3-butadiene oxidation was described in the lung, although rate constants for further metabolism of EB are also available from Csanády et al. (1992). Further developments of this model are discussed in Sections 9.2.7.4 and 9.2.9.

9.2.7. Blaine Presentations

The results of four PBPK modeling exercises for 1,3-butadiene were presented at the International Symposium on the Evaluation of Butadiene and Isoprene Health Risks held in Blaine, Washington, in June 1995. The proceedings of the symposium were published in a special issue of the scientific journal *Toxicology* (vol. 113) in 1996. None of the models was described in detail. The published results of these modeling efforts are summarized briefly below.

9.2.7.1. *Kohn and Melnick (1996)*

Kohn and Melnick modified their 1993 model for mice and rats by (a) distributing blood among arterial and venous spaces and tissue capillary beds, (b) separating the GI tract from the viscera compartment in order to more realistically model liver perfusion, (c) adding a compartment for the alveolar space in the lung, and (d) including GSH depletion and resynthesis.

Parameter values that were not available in the literature were obtained by optimization. These investigators found that the inclusion of the alveolar space eliminated the need to decrease alveolar ventilation rates by 40% as is done in some of the other models. They further noted that subcompartmentalization of the blood necessitated considerable extrahepatic metabolism in order to reproduce observed uptakes of 1,3-butadiene and EB. While generally reproducing uptake rates, the model overestimated blood EB concentrations. In addition, these investigators observed that the model was sensitive to the range of values that have been reported for some of the parameters, in particular the blood:air partition coefficient and certain enzymatic activities. They conclude that "[a] valid and useful toxicokinetic model must have reliable physiological and enzymological data for [1,3-butadiene] biotransformation before it can be credibly used for human risk assessment." Kohn's further refinements of this model are discussed in Section 9.2.10.

9.2.7.2. *Johanson and Filser (1996)*

This presentation basically summarized Johanson and Filser's 1993 model, which was discussed above. Some results of extending the model to humans were also presented, although the parameter values used for humans were not reported and it is not clear how some of them were obtained. The extended model predicts relative internal doses of EB (based on steady-state concentrations or AUCs for mixed venous blood) for mice, rats, and humans in the ratio 1.6:1.0:0.3.

9.2.7.3. *Csanády et al. (1996)*

Csanády and Filser and colleagues presented a modified version of Johanson and Filser's model. The modifications are (a) V_{max} for 1,3-butadiene was based on in vivo rather than in vitro data, (b) disposition of DEB for the mouse only was included using the Csanády et al. (1992) in vitro data for EB oxidation and DEB hydrolysis and GSH conjugation, and (c) production of hemoglobin-EB adducts was incorporated. The modified model predicts relative internal doses of EB (AUC for blood) for mice, rats, and humans in the ratio 1.3:1.0:0.3. Predicted Hb-EB adduct levels for mice and rats were in good agreement with experimental measurements. Filser and colleagues maintain that inclusion of an intrahepatic first-pass effect is necessary to resolve the problem occurring in the Medinsky et al. and Kohn and Melnick models whereby the models can simulate 1,3-butadiene uptake data, but then blood levels of EB are overpredicted. The investigators are currently refining this model further.

9.2.7.4. *Sweeney et al. (1996)*

Sweeney et al. presented a modified version of the 1994 Medinsky et al. model. The reported modifications were (1) addition of a GSH submodel that included diurnal variations, (2) addition of a DEB submodel, and (3) incorporation of nonenzymatic elimination of EB. However, it was not clear how parameter estimates for modifications 2 and 3 were obtained and whether or not the DEB submodel included further metabolism of DEB or merely its production and distribution. Sweeney et al. also investigated the possibility that EB transfer from blood to liver enzymes is diffusion-limited rather than flow-limited, and examined the effects of using optimized parameters for the phase I oxidation of 1,3-butadiene to EB and for phase II reactions involving EB. (The phase I parameters were rejected because they yielded results inconsistent with observations.) These investigators found that while their base modified model overestimates blood EB concentrations by about an order of magnitude, the model variations with diffusion limitation and optimized parameters both adequately simulate observed concentrations. In addition, the base and diffusion-limited models were able to reasonably simulate measured liver EB concentrations taking into account the 3-5 minute lag time between cessation of exposure and tissue removal, while the model variation with optimized parameters apparently overestimated phase II metabolism. Thus, the authors concluded that the parameter values derived from in vitro rates were more appropriate than the optimized values. They further concluded that, overall, the diffusion-limited model yielded results most consistent with experimental data. A more recent refinement of this model was published in 1997 and is discussed in Section 9.2.9.

9.2.8. Reitz et al. (1996)

In 1996 Reitz et al. prepared an unpublished PBPK model for the Chemical Manufacturers Association, which presented the model to EPA for consideration. The Reitz et al. model was based on the Medinsky et al. (1994) model, with modifications as well as inclusion of DEB elimination and extension of the model to humans. A major difference between the two models is that because the Medinsky et al. model overestimates EB blood levels compared to measurements by Himmelstein et al. (1994), and overestimates EB exhalation compared to data obtained by Medinsky and colleagues from their chamber experiments, Reitz et al. chose not to use the in vitro constants for EB metabolism measured by Csanády et al. (1992) but rather to estimate the EB metabolic parameters from in vivo data. Reitz et al. state that they have “found it necessary to ‘calibrate’ in vitro measurements with selected in vivo studies in order to obtain accurate simulations of in vivo data” for PBPK models of other chemicals as well. A further difference between the two models is that the Reitz et al. model does not include lung metabolism but rather assumes that all metabolism occurs only in the liver. These investigators

provide a number of unconvincing reasons for removing lung metabolism from the model. More to the point, the alternative approach they have taken to estimating the EB metabolism parameters using in vivo data really provides estimates only of whole-body metabolism, and it would be difficult to credibly partition the whole-body metabolism to specific tissue compartments.

Reitz et al. derived new parameter estimates for EB metabolism in the mouse as follows. First, they assumed that oxidation of EB to DEB is insignificant compared to EB hydrolysis or conjugation with GSH, so the oxidation pathway was ignored during the derivation of rate constants for the other pathways. Second, they modeled GSH conjugation using Michaelis-Menten kinetics. V_{max} was set at 350 μmol/h/kg based on the in vivo clearance results of Kreiling et al. (1987). A K_m value of 4.2 μmol/L was derived by optimization, fitting the data of Himmelstein et al. (1994). Note that these values differ substantially from those used by Medinsky et al. (1994) for the liver (V_{max}=154,000 μmol/h/kg and K_m=35,300 μmol/L, yielding a first-order clearance rate [i.e., V_{max}/K_m] of 4.36). Reitz et al. claim that the first-order rate constant used by Medinsky et al. was left unchanged during the optimization for K_m and that once V_{max} and K_m were obtained, the first-order rate constant was adjusted. This is unclear, however, because the first-order clearance rate used by Medinsky et al. was for GSH conjugation, while the first-order clearance rate used in the Reitz et al. model is for hydrolysis. Reitz et al. assume first-order hydrolysis for all species based on the results of Kreiling et al. for rats, a species in which hydrolysis plays a key role in EB elimination, suggesting that EB metabolism was not saturated at exposure levels up to 5000 ppm. Reitz et al. then adjusted the "first-order rate constant" so that 20% of EB metabolism from a 6-h exposure to 8000 ppm occurs via the hydrolysis pathway to fit the urinary metabolite data of Sabourin et al. (1992). The first-order clearance rate was eventually set to 6.3 L/kg/h, again significantly different from the value of 0.47 derived from the V_{max} and K_m values used by Medinsky et al.

For EB oxidation, Reitz et al. rely on V_{max} and K_m values based on in vitro data provided by the Chemical Industry Institute of Toxicology (CIIT; i.e., Medinsky and colleagues) for rats, mice, and humans—presumably from liver preparations, although this is not stated. It is unclear why the in vitro values presented for the mouse differ from those reported by Csanády et al. (1992) (V_{max} of 1.3 nmol/mg protein/min vs. 0.20 and K_m of 140 μmol/L vs.15.6, respectively). The in vitro V_{max} values were then normalized for protein content, apparently using the same protein concentration values used for the Medinsky et al. model. Because of the differences in the in vitro measurements, the parameter values for EB oxidation used in the Reitz et al. mouse model differ from those used by Medinsky et al. (V_{max} of 170 μmol/h/kg vs. 26 and K_m of 140 μmol/L vs. 15.6, respectively). These CIIT data for EB oxidation appear to be the same data published by Seaton et al. in 1995 and used in the Sweeney et al. (1997) model; although

Sweeney et al. corrected the V_{max}s to account for hydrolyzed DEB and obtained values of 176.6 and 57.1 μmol/h/kg for the mouse and rat, respectively, vs. 170 and 42 used by Reitz et al.

Then, Reitz et al. used a parallelogram approach for interspecies extrapolation to derive new parameter values for EB hydrolysis and conjugation with GSH in the rat. Under this approach, in vivo metabolic activities for the rat are estimated from in vitro activities using the same relationship between in vitro and in vivo activities observed for the mouse. (Recall that Reitz et al. assumed that all metabolism occurring in vivo is from liver activity.) Reitz et al. assumed that the apparent first-order rate constants (i.e., Vmax/Km) derived from the Csanády et al. (1992) in vitro data reflected enzyme content in each species. They then estimated in vivo rate constants for the rat by multiplying the in vivo rate constants for the mouse by the ratio of the in vitro first-order rate constants for the liver and by the ratio of liver weights (as a fraction of body weight). Thus, an in vivo first-order clearance rate for EB hydrolysis of 13.2 L/kg/h was derived for the rat by multiplying 6.3 by ([2.48/0.26]/[5.79/1.59]) (from Csanády et al., 1992, Table II) and by (0.05/0.0624). Similarly, an in vivo V_{max} of 345 μmol/h/kg was estimated for GSH conjugation in the rat by multiplying 350 by ([241/13.8]/[500/35.3]) (from Csanády et al., 1992, Table III) and by (0.05/0.0624). The K_m value for GSH conjugation was assumed to be the same across species, even though the K_m values used by Reitz et al. for EB oxidation range from 140 in mice to 500 in humans. Parameter estimates for EB oxidation (V_{max} and K_m) for the rat were derived from in vitro rat data provided by CIIT, as discussed above for the mouse.

The basis for the human 1,3-butadiene oxidation parameter values is not revealed in Reitz et al.'s report; however, the values were apparently obtained in the same manner as those used by Medinsky et al. for the rat and mouse. For the rat and mouse, Medinsky et al. used the in vitro data of Csanády et al. (1992) but used different microsomal protein concentration values. For humans, Reitz et al. apparently also used the in vitro data of Csanády et al. as well as the liver protein concentrations used by Medinsky et al. for the rat and mouse.

Human metabolic parameter values for EB conjugation with GSH and hydrolysis were derived according to the parallelogram approach described above. For GSH conjugation, an in vivo V_{max} of 44.6 μmol/h/kg was estimated for humans by multiplying the mouse value of 350 by the ratio of the in vitro first-order rate constants for the liver from Csanády et al. (1992) ([45.1/10.4]/[500/35.3]) and by the ratio of liver weights (0.026/0.0624). For hydrolysis, Reitz et al. used the midpoint of the range of normalized first-order rate constants for the 12 human liver samples reported in Csanády et al. (1992) and compared that to a corresponding rate constant for mice, yielding a ratio of 12.1:1. Had they used the midpoint of the actual first-order rate constants reported for three human livers, which included those with the highest and lowest enzyme activities, the ratio would have been about 20% lower, 9.6:1. Reitz et al. then mistakenly multiplied the ratio of 12.1 for enzyme activity by the in vivo first-order rate constant

for hydrolysis of 13.2 L/kg/h derived *for the rat* rather than the value of 6.3 estimated for the mouse and by the ratio of liver weights. Had they used the correct in vivo estimate, they would have obtained a parameter value of 31.8 L/kg/h for the first-order rate constant for hydrolysis in humans rather than the value of 66.6 that they use in their model. Parameter estimates for EB oxidation (V_{max} and K_m) for humans were derived from in vitro human data provided by CIIT, as discussed above for the mouse.

To extend the model to include DEB elimination, Reitz et al. estimated DEB partition coefficients and metabolic parameters as follows. Partition coefficients were estimated from data provided to Reitz et al. by Thornton-Manning of the Inhalation Toxicology Research Institute (ITRI) on blood and tissue levels of DEB in female rats and mice exposed to 62.5 ppm 1,3-butadiene for 4-6 h. (These data were apparently later published by Thornton-Manning et al., [1997].) Reitz et al. report postexposure blood levels of 13.6 pmol DEB/g in rats and 345 pmol/g in mice. They also claim that within a given species, similar levels of DEB were detected in bone marrow, mammary tissue, ovary tissue, and fat (6.3-10.5 pmol/g in rats and 169-264 pmol/g in mice). On the basis of these data, Reitz et al. calculated an average tissue:blood concentration ratio of 0.6, which they used as the DEB tissue:blood partition coefficient for all tissues of mice, rats, and humans. However, Sweeney et al. (1997) later published experimentally derived tissue:blood partition coefficients for DEB in the mouse that ranged from 1.41 for the kidney (i.e., richly perfused tissue) to 2.20 for fat. Based on reports by Bond of CIIT that the vapor pressure of DEB was too low for calculation of the blood:air partition coefficient using vial equilibrium techniques, Reitz et al. arbitrarily set the blood:air partition coefficient to 1,000, a high value, for all species in their model.

Reitz et al. estimated the kinetic constants for hydrolysis and GSH conjugation of DEB using in vitro V_{max} and K_m values provided by CIIT for rats, mice, and humans. On the basis of the relatively high K_m values, they tentatively assume that both DEB hydrolysis and conjugation with GSH are first-order processes, "pending development of better in vivo data," noting that in vitro K_ms obtained for EB did not accurately predict in vivo behavior. Thus, DEB elimination was described using a single first-order clearance term reflecting the sum of the first-order rate constants for hydrolysis and conjugation, which were purportedly derived from the in vitro data. However, the first-order terms that Reitz et al. report could not be replicated. The CIIT data for EB hydrolysis and conjugation with GSH appear to be the same data published in 1996 by Boogaard and Bond and by Boogaard et al., respectively, except that Reitz et al. apparently misreported the rat K_m for GSH conjugation as 240 μM rather than 24,000 μM. Using the corrected K_m value, it appears that Reitz et al. mistakenly used the *microsomal* protein concentrations for normalizing the GSH conjugation results. Using the cytosolic protein concentrations for GSH conjugation yields corrected first-order clearance terms (summing

hydrolysis and conjugation) of 4.5 and 8.4 L/kg/h for the rat and mouse, respectively (vs. the 2.80 and 3.84 for rat and mouse, respectively, reported by Reitz et al.). It is unknown whether or not the value presented by Reitz et al. for humans is also in error.

Parameter values used in the Reitz et al. model are summarized in Table 9-6. Except for the EB and DEB metabolic parameters and DEB partition coefficients, which were estimated as described above, mouse and rat parameter values were identical to those used by Medinsky et al., with the exception of mouse lung and rapidly perfused tissues organ volumes, which differed slightly. For humans, blood flows and partition coefficients were assumed to be the same as for the mouse, with the exception of the muscle:blood partition coefficient for 1,3-butadiene, where the rat value was used. No reference was cited for human values for the physiological parameters and organ volumes.

For validation of the new metabolic constants for EB hydrolysis and conjugation with GSH (with EB oxidation set to 0) for the mouse, the modified model was used to predict the blood EB concentrations measured by Himmelstein et al. (1994), although these data had been used for parameter optimization. Predicted levels ranged from 20% too high to 80% too low. These predictions are better than those from the original Medinsky et al. model, which had overestimated blood EB concentrations by more than fourfold. Reitz et al. also evaluated the ability of the modified model to predict data from CIIT on EB exhalation during 1,3-butadiene exposure. The modified model performed better than the original Medinsky et al. model, which had overpredicted EB exhalation at all exposure levels; however, the modified model underestimated exhalation at higher exposures and overestimated exhalation at lower exposures. The modified model was also used to simulate the EB gas uptake data of Kreiling et al. (1987); it gave a reasonable fit to these data, as had the original Medinsky et al. model.

Similarly, the new metabolic constants for EB hydrolysis and conjugation with GSH (with EB oxidation set to 0) for the rat were used to predict the blood EB concentrations measured by Himmelstein et al. (1994), and the modified model gave results within a range of 0.5-1.4 times the observed levels, which were an improvement over the overpredictions of the Medinsky et al. model. The modified rat model also provided a reasonable fit to the EB gas uptake data of Filser and Bolt (1984), as had the original Medinsky et al. model.

For validation of the extended model including DEB metabolism, Reitz et al. simulated the blood concentration data of Himmelstein et al. (1994) for mice exposed to 71, 602, or 1282 ppm 1,3-butadiene for 6 h and blood concentration data from ITRI for mice exposed to 62.5 ppm 1,3-butadiene for 4 h. Predicted values of blood DEB ranged from 6.5 times lower at 71 ppm to 1.1 times higher at 1282 ppm compared to the Himmelstein et al. observations; the predicted value was 2.2 times lower than the ITRI value at 62.5 ppm. Together, these comparisons suggest that the model may be underestimating blood DEB for mice at lower exposure levels.

Table 9-6. Parameter values used in the Reitz et al. (1996) PBPK model.

Parameter	Mouse	Rat	Human
Physiological parameters[a]			
Alveolar ventilation (L/h/kg)	41	17	4.96
Cardiac output (L/h/kg)	41	17	4.96
Body weight (kg)	0.028	0.325	70.0
Blood flows (fraction of cardiac output)[a]			
Liver	0.25	0.25	0.25
Fat	0.09	0.09	0.09
Lung	1.0	1.0	1.0
Slowly perfused tissues	0.15	0.15	0.15
Rapidly perfused tissues	0.51	0.51	0.51
Organ volumes (fraction of body weight)[a]			
Liver	0.0624	0.050	0.026
Fat	0.1	0.09	0.21
Lung	0.0078	0.0053	0.0077
Slowly perfused tissues	0.71	0.72	0.60
Rapidly perfused tissues	0.0198	0.0347	0.0563
Partition coefficients for 1,3-butadiene[b]			
Blood:air	1.34	1.49	1.34
Liver:blood	1.01	0.80	1.01
Lung(& rapid):blood	1.10	0.62	1.10
Muscle(slow):blood	2.99	0.99	0.99
Fat:blood	14.3	14.9	14.3
Partition coefficients for EB[b]			
Blood:air	36.6	50.4	36.6
Liver:blood	1.15	1.43	1.15
Lung(& rapid):blood	1.54	1.09	1.54
Muscle(slow):blood	0.645	0.393	0.645
Fat:blood	2.49	2.74	2.49
Partition coefficients for DEB[c]			
Blood:air	1000	1000	1000
Liver:blood	0.6	0.6	0.6
Lung(& rapid):blood	0.6	0.6	0.6
Muscle(slow):blood	0.6	0.6	0.6
Fat:blood	0.6	0.6	0.6

Table 9-6. Parameter values used in the Reitz et al. (1996) PBPK model (continued).

Parameter	Mouse	Rat	Human
Rate constants for oxidative metabolism of 1,3-butadiene[d]			
V_{max} (μmol/kg/h)	338	62.0	64.4
K_M (μmol/L)	2.0	3.75	5.14
Rate constants for EB metabolism[c]			
Oxidation V_{max} (μmol/kg/h)	170.0	42.0	51.0
K_M (μmol/L)	140	150	500
GSH Conjugation V_{max} (μmol/kg/h)	350.0	345	44.6
K_M (μmol/L)	4.2	4.2	4.2
First order Hydrolysis K (L/kg/h)	6.3	13.2	66.6[e]
Rate constants for DEB metabolism[c,f]			
First order elimination K (L/kg/h)	3.84	2.80	2.17

[a]Mouse and rat values same as Medinsky et al. (1994) (see Table 9-5), except for mouse lung and rapidly perfused tissue organ volumes which differ slightly. Source of human values not reported.
[b]Mouse and rat values from Medinsky et al. (1994). Human values set equal to mouse values, except for muscle:blood partition coefficient for 1,3-butadiene where rat value was used.
[c]Derived as described in text.
[d]Mouse and rat values same as Medinsky et al. (1994) liver values. Human values derived as described in text.
[e]This value appears to be in error (see text). The corrected value is thought to be 31.8.
[f]The mouse and rat values appear to be in error (see text). The corrected values are thought to be 8.4 and 4.5, respectively. It is unclear whether or not the human value is also in error.

And those simulations were made using an apparently incorrect value for the first-order clearance term for DEB (see above). The corrected clearance term is over twice as large, suggesting that model predictions using the corrected term may underestimate DEB blood levels to an even greater extent. For validating the extended model in rats, the only data used were ITRI blood concentrations from a 4-h exposure to 1,3-butadiene, sampled at 2, 4, and 4.5 h. Predicted values of blood DEB ranged from 1.9 times higher at 2 h to 6.2 times higher at 4.5 h compared to the observed values.

There are no human data appropriate for validating the human model.

No comprehensive sensitivity analysis for the model parameters was reported. The only sensitivity analysis mentioned was for the blood:air partition coefficient for DEB, which was set to an arbitrary high value of 1000. Reitz et al. report that simulations with values ranging from 100 to 10,000 gave similar results.

A strength of the Reitz et al. model is that it was able to simulate observed levels of blood EB and DEB following 1,3-butadiene exposure fairly well, although the mouse blood EB results were used for parameter optimization, so a good fit to those data is to be expected, and the DEB

validation would need to be repeated using corrected clearance terms. A concern, however, is an apparent trend across the mouse blood DEB predictions, suggesting that the model results may deviate (underestimate actual levels) more at lower exposures, which are of greater environmental relevance. A trend also occurs with the rat blood EB predictions, although in this case the trend suggests the model overestimates blood EB levels at the lower exposures. The rat blood DEB data used for model validation were for one exposure level only; thus nothing about trends can be inferred.

Some limitations of the Reitz et al. model include the assumption that all metabolism occurs only in the liver, and the uncertainties in the metabolic constants derived from the unvalidated partitioning of EB clearance amongst the various metabolic pathways. In addition, values for some of the model parameters were obtained by optimization, and it is thus unclear what these parameters actually represent. Furthermore, the model raises serious issues about the use of in vitro versus in vivo data. In some cases the model uses metabolic constants derived from in vitro data, while in other cases, the in vitro data are deemed inadequate because the resulting model cannot replicate other experimental results. In the latter cases, metabolic constants were derived by fitting in vivo data, yielding parameter estimates that differ drastically from those based on the in vitro data and that suggest different structural forms for some metabolic pathways (e.g., first-order vs. saturable). For example, the K_m values for GSH conjugation of EB differed by almost four orders of magnitude. In sum, the Reitz et al. model is a dubious patchwork of metabolic rate constants, some fitted and some independently measured, some derived from in vitro data and some from in vivo data, some based on whole body clearance data and some based only on liver data.

An additional major limitation is the absence of validation for the human model. The parallelogram approach used for extrapolation to the rat can conceptually be used for extrapolation to humans, but given the uncertainties in the model, the unresolved discrepancies between the Reitz et al. model and other PBPK models for 1,3-butadiene, and the limited validation of both the mouse and rat versions, the human model has little credibility. Furthermore, while a model capable of predicting blood levels of EB and DEB may be a dosimetric improvement over default dosimetry assumptions, these blood levels alone do not explain species differences in target organ carcinogenic responses. Given the large number of questionable assumptions made by Reitz et al. in ascribing all metabolism to the liver and in partitioning whole-body EB clearance among various metabolic pathways, other tissue compartment metabolite concentrations obtained from their model would be highly unreliable.

9.2.9. Sweeney et al. (1997)

In 1997, Sweeney et al. (CIIT) published their own further revisions of the 1994 Medinsky et al. model for mice and rats. The revised model includes the disposition of DEB and some other metabolic reactions not depicted in the original model, such as nonenzymatic reactions of EB and DEB as well as GSH depletion and synthesis. The final model also includes an alternative pathway for 1,3-butadiene oxidation. The model contains the original six physiological compartments: liver, lung, fat, slowly perfused tissue, rapidly perfused tissue, and blood; however, the blood compartment was subdivided into arterial and venous compartments for modeling intravenous (iv) administration of EB and DEB.

The revised model uses the same physiological parameters, including blood flows and organ volumes, and the same partition coefficients for 1,3-butadiene and for EB as the original Medinsky et al. model (see Table 9-5). Arterial and venous blood subcompartments were assigned values of 2% and 4% of body weight, respectively. The same microsomal and cytosolic protein concentrations were also reportedly used; however, there appears to be a discrepancy with the mouse lung DEB hydrolysis V_{max} (see below). The source and value of lung cytosolic protein concentrations were not reported. A value of 61 mg protein/g lung, consistent with that cited in Boogaard et al. (1996), was apparently used for the rat; however, a value of about 50 mg protein/g lung seems to have been used for the mouse, rather than the 32 mg/g cited in Boogaard et al. Tissue:blood partition coefficients for DEB were experimentally determined using hexane as the partitioning medium because of the low vapor pressure of DEB. These coefficients and the metabolic constants used in the Sweeney et al. (1997) model are reported in Table 9-7.

The Sweeney et al. (1997) model describes the reactions depicted in Figure 9-1. Oxidative metabolism of 1,3-butadiene to EB, GSH conjugation of EB and DEB, hydrolysis of DEB, and depletion and synthesis of GSH are described in both the liver and the lung. Oxidation of EB to DEB and EB hydrolysis are described only in the liver, although Sweeney et al.'s Figure 2 depicts EB oxidation in the lung as well. Nonenzymatic first-order reactions of EB and DEB are modeled in all tissues except the slowly perfused compartment. In the final model, an alternative pathway for 1,3-butadiene oxidation in the liver and lung and a first-order elimination pathway in the slowly perfused compartment were also incorporated. All of these reactions are included in both the mouse and rat models.

The revised model uses the same rate constants for oxidative metabolism of 1,3-butadiene (to all products), based on the in vitro data of Csanády et al. (1992), as the original model. The Csanády et al. V_{max} and K_m values for EB hydrolysis and conjugation with GSH were also used; however, instead of using the latter values to derive a first-order rate constant as in the original model, GSH conjugation was described assuming a ping-pong mechanism and a K_m of 100

Table 9-7. Parameter values used in Sweeney et al. (1997) PBPK model[a].

Parameter	Mouse	Rat
Partition coefficients for DEB[b]		
Liver(& lung):blood	1.41	1.41
Kidney(& rapid):blood	1.53	1.53
Muscle(& slow):blood	1.82	1.82
Fat:blood	2.20	2.20
Rate constants for oxidative metabolism of 1,3-butadiene		
Liver (total) V_{max} (μmol/kg/h)[c]	338	62
K_m (μmol/L)[c]	2.0	3.75
Liver (to EB) V_{max} (μmol/kg/h)[d]	97	8.2
K_m (μmol/L)[d]	0.88	1.54
Liver (to other volatiles) V_{max} (μmol/kg/h)[e]	243	54
K_m (μmol/L)[e]	2.72	4.36
Lung (total) V_{max} (μmol/kg/h)[c]	21.6	1.01
K_m (μmol/L)[c]	5.01	7.75
Lung (to EB) V_{max} (μmol/kg/h)[d]	6.4	0.13
K_m (μmol/L)[d]	1.6	3.18
Lung (to other volatiles) V_{max} (μmol/kg/h)[e]	16.1	0.88
K_m (μmol/L)[e]	9.5	9.14
Fraction to EB (2-product model)[f]	0.192	0.241
Rate constants for metabolism of EB		
Liver oxidation		
One enzyme V_{max} (μmol/kg/hr)[g]	176.6	57.1
K_m (μmol/L)[g]	145	141
Two enzyme V_{max1} (μmol/kg/hr)[h]	32.5	10
K_{m1} (μmol/L)	15.6[c]	141[g]
V_{max2} (μmol/kg/hr)[I]	144.1	47.1
K_{m2} (μmol/L)[g]	145	141
Liver hydrolysis V_{max} (μmol/kg/hr)[c]	754	260
K_m (μmol/L)[c]	1590	260
Liver GSH conjugation V_{max} (μmol/kg/hr)[j]	154,000	78,100
K_m (μmol/L)[j]	35,300	13,800
K_m^{GSH} (μmol/L)[k]	100	100
Lung GSH conjugation V_{max} (μmol/kg/hr)[j]	4088	819
K_m (μmol/L)[j]	36,500	17,400
K_m^{GSH} (μmol/L)[k]	100	100

Table 9-7. Parameter values used in Sweeney et al. (1997) PBPK model[a] (continued).

Parameter	Mouse	Rat
Rate constants for metabolism of DEB		
Liver hydrolysis V_{max} (μmol/kg/h)[m]	4193	5555
K_m (μmol/L)[m]	8100	2700
Liver GSH conjugation V_{max} (μmol/kg/h)[n]	50,342	60,264
K_m (μmol/L)[n]	6400	24,000
Lung hydrolysis V_{max} (μmol/kg/h)[m]	466.1	122.7
K_m (μmol/L)[m]	7500	7100
Lung GSH conjugation V_{max} (μmol/kg/h)[n]	577	332
K_m (μmol/L)[n]	1700	4170
Nonenzymatic reaction rate constants[p] for EB (L/h)		
Blood	0.558	0.582
Liver	4.14	4.94
Lung	2.70	6.07
Fat	1.56	1.72
Muscle(& slow)	0	0
for DEB (L/h)[b]		
Blood	0.189	0.189
Liver	3.15	3.15
Kidney(& rapid)	4.1	4.1
Fat	2.8	2.8
Muscle(& slow)	0	0
First-order clearance rate for slowly perfused compartment[q]		
For EB (L/kg/h)	0.697	1.47
For DEB (L/kg/h)	0.762	3.07

[a] For physiological parameters and 1,3-butadiene and EB partition coefficients see Table 9-5. For parameters used in GSH submodel see Sweeney et al. (1997).
[b] Measured for mouse; assumed equivalent for rat.
[c] Same as Medinsky et al. (1994), based on Csanády et al. (1992) in vitro data.
[d] Obtained by optimization, based on mass balance data of Bond et al. (1986) (see Sweeney et al. (1997) for details).
[e] Obtained by optimization, using data reported by Himmelstein et al. (1997).
[f] Obtained by optimization, using Himmelstein et al. (1994) blood EB data.
[g] Based on Seaton et al. (1995) microsome data, with V_{max} corrected to account for hydrolyzed DEB. Rat and mouse K_ms are transposed compared to those reported by Seaton et al.; however, the values are similar so the effects are likely to be inconsequential.
[h] Based on Seaton et al. (1995) cDNA-expressed CYP 2E1 data.
[I] Difference between one-enzyme V_{max} and V_{max1}.
[j] From Csanády et al. (1992), although it's unclear where the lung cytosolic protein concentrations used for conversion are from.
[k] From Johanson and Filser (1993).
[m] Based on Boogaard and Bond (1996) microsome data, using Medinsky et al. (1994) microsomal protein concentration values, except for the mouse lung where a value of about 31 was apparently used rather than 20.
[n] Based on Boogaard et al. (1996) cytosol data; although a value of 50 was apparently used for the mouse lung cytosolic protein concentration rather than the value of 32 reported in Boogaard et al.
[p] Determined experimentally by Sweeney et al. (1997).
[q] Obtained by optimization for the rat using DEB blood clearance data of Valentine et al. (1997). Mouse values were estimated using the clearance rate ratios for the rat.

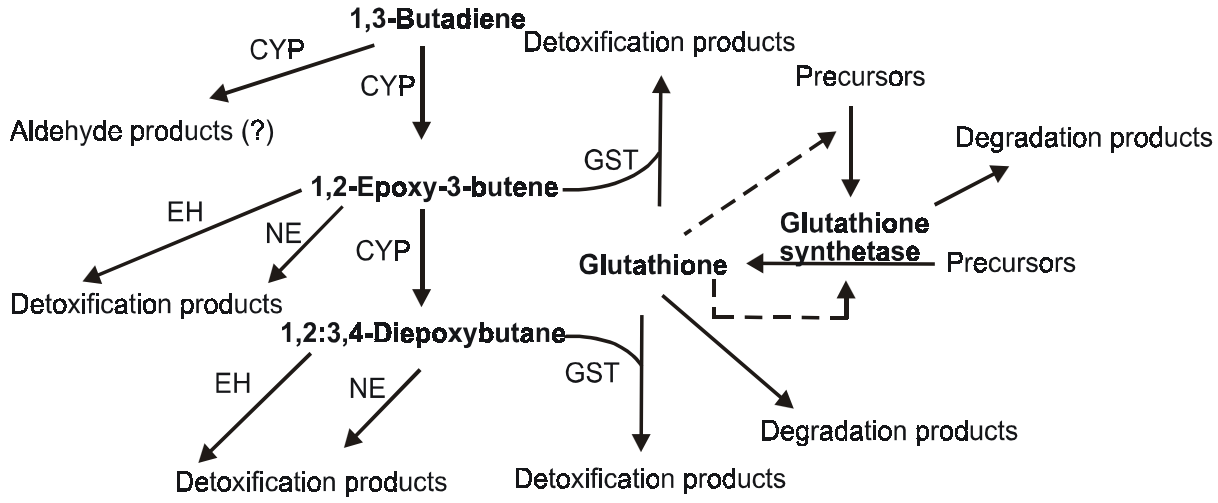

Figure 9-1. Reactions modeled in the PBPK model of Sweeney et al. (1997)[a].

[a]Reaction pathways are indicated by solid lines; inhibitory feedback is denoted by dashed lines. CYP = cytochrome P-450. EH = epoxide hydrolase. GST = glutathione S-transferase. NE = nonenzymatic reactions.

Source: Sweeney et al., 1997.

μmol/L for GSH as proposed in the Johanson and Filser (1993) model. Sweeney et al. explain that while a simpler description might be adequate in scenarios dealing with 1,3-butadiene exposure, they wanted a more general description that would also apply to EB exposures resulting in low levels of GSH.

To derive parameters for EB oxidation in the liver, Sweeney et al. used the kinetic data of Seaton et al. (1995) from mouse, rat, and human liver microsomes instead of the Csanády et al. data, for reasons that are not stated. First, using the kinetic data for DEB hydrolysis of Boogaard and Bond (1996), the EB oxidation rates were corrected to take into account any DEB that may have undergone hydrolysis before it could be measured as oxidation product. Then, to derive parameters for a "two-enzyme" EB oxidation scenario, the Michaelis-Menten rate equation was split into two terms to portray EB oxidation by both a high-affinity, low-capacity isozyme and a low-affinity, high-capacity isozyme. CYP 2E1 was considered to be the high-affinity enzyme (subscript "1" in Table 9-7) and was assigned a V_{max} based on the EB oxidation rate measured by Seaton et al. for cDNA-expressed CYP 2E1 and, for the mouse, the smaller K_m for liver microsomes observed by Csanády et al. The low-affinity enzyme (subscript "2") was assigned the larger K_m observed by Seaton et al. for liver microsomes and its V_{max} was estimated by subtracting V_{max} for the high-affinity enzyme from the corrected V_{max} based on the Seaton et al.

microsome data. For the rat, the Seaton et al. K_m was used for both enzymes. The oxidation reactions for 1,3-butadiene and EB were modeled as noncompetitive.

Parameter estimates for DEB hydrolysis were derived from the in vitro work of Boogaard and Bond (1996) on human, rat, and mouse liver and lung microsomes. However, the V_{max} values were converted using the protein concentration values used by Medinsky et al. (1994) rather than the values reported by Boogaard and Bond, which were identical to those used by Csanády et al. (1992). Furthermore, the mouse lung hydrolysis V_{max} value reported by Sweeney et al. appears to be either incorrect or the result of using a different value for lung microsomal protein concentrations than that used in the original model. The reported V_{max} is 50% higher than that which one would obtain using the original protein concentrations. Parameter estimates for conjugation of DEB with GSH were taken from the in vitro work of Boogaard et al. (1996) on rat and mouse liver and lung cytosolic fractions. The cytosolic protein concentrations used are consistent with those cited by Csanády et al. and Boogaard et al. except for the mouse lung (see above). DEB hydrolysis was modeled using simple Michaelis-Menten kinetics, while DEB conjugation with GSH was described assuming a ping-pong mechanism, as was done for EB conjugation.

Nonenzymatic reactions of EB and DEB were suggested by the results of the vial equilibration techniques used to determine the partition coefficients. EB and DEB losses were observed in all tissues except muscle even after GSH depletion, epoxide hydrolase inhibition, and heat inactivation of other enzymes. First-order rate constants for these nonenzymatic processes were estimated from the time course data for EB and DEB disappearance.

The submodel for GSH synthesis and degradation in the liver and lung is apparently the same submodel discussed in Sweeney et al. (1996), which was based on the model of D'Souza et al. (1988) with some modifications to account for diurnal variation. Some of the parameter estimates come from D'Souza et al. and others are estimated by optimization. It is not clear which datasets were used for optimization. According to Sweeney et al. (1996), declines in GSH observed by Himmelstein et al. (1995) in control and exposed animals were simulated well.

Sweeney et al. (1997) attempted to validate the DEB disposition part of their model against DEB blood clearance data from rats after intravenous administration of DEB (Valentine et al., 1997); however, they found that the DEB reactions already described in the model were insufficient to account for the rate of disappearance of DEB from the blood. To remedy this situation, Sweeney et al. determined that adding a first-order clearance term for DEB to the slowly perfused compartment, but not to any other compartments, substantially improved the fit of the model to the DEB clearance data. The value of the first-order clearance term was obtained by optimization. The biological relevance of this additional term is unclear, although Sweeney et al. note that epoxide hydrolases have been found in many tissues, including the skin, which is a

component of the slowly perfused compartment, although a minor component. These investigators further found that the same first-order clearance term for the slowly perfused compartment was needed to fit the rat blood clearance data of Valentine et al. following iv administration of EB, and an estimate of this clearance rate was also obtained by optimization. Sweeney et al. observed that the value of the first-order clearance term for rats was 1.47 times the ratio of V_{max} to K_m for liver hydrolysis for both EB and DEB, and thus used this formulation to estimate clearance rates for the mouse.

Model predictions of blood DEB peak concentrations and AUCs were then computed for comparison with unpublished data of Himmelstein obtained after tail vein administration of EB in mice. Simulations were conducted using both the one-enzyme and two-enzyme models for EB oxidation (see above). Predictions from the two-enzyme model were closer to experimental observations.

This revised model still substantially overpredicted blood EB levels for both rat and mouse following exposure to 1,3-butadiene. The incorporation of diffusional limitation in the liver that was investigated in the Sweeney et al. 1996 version of the model was rejected because it resulted in underpredictions of the mouse DEB blood levels observed after iv administration of EB and underpredictions of liver GSH levels. Thus, Sweeney et al. explored the possibility that there is an alternative oxidative pathway for 1,3-butadiene that does not yield EB. These investigators suggest that this possibility is consistent with data of Bond et al. (1986) showing that following rodent exposure to [^{14}C]1,3-butadiene, a large proportion of radiolabeled ^{14}C is exhaled as CO_2, by a biotransformation mechanism that has not yet been explained. In addition, Sweeney et al. cite the urinary metabolite results of Nauhaus et al. (1996) as indicating that there could be a substantial flux of 1,3-butadiene through a non-EB-producing pathway that Nauhaus et al. propose involves metabolism to acrolein and acrylic acid. (See Figure 3-1 for some possible pathways.)

Sweeney et al. experimented with two different methods for determining the proportion of 1,3-butadiene metabolism going to each pathway. For both approaches, total metabolism of 1,3-butadiene was assumed to be described by the rates used in the original Medinsky et al. (1994) model, as those rates were able to simulate the 1,3-butadiene uptake experiments. The first method used the single Michaelis-Menten rate equation for total metabolism and assumed that two products were produced. This two-product model assumed that the fraction metabolized to EB was independent of 1,3-butadiene concentration and the same for both the liver and lung. Parameter estimates for the value of the fraction were obtained by optimization using the blood EB concentration data of Himmelstein et al. (1994) for mice and rats exposed to 1,3-butadiene. The optimal value for the fraction of 1,3-butadiene metabolized to EB was 0.241 for the rat and 0.192 for the mouse, suggesting that the majority of 1,3-butadiene metabolism might be through

the hypothesized alternate pathway. Using this optimal fraction for the mouse, the model also provided a good fit to blood DEB and liver GSH data.

The second method assumed two separate pathways for 1,3-butadiene oxidation and involved partitioning total metabolism into separate rate equations for each pathway using blood 1,3-butadiene data reported by Himmelstein et al. (1997) and the mass balance data of Bond et al. (1986) to fit the parameters, although the Bond et al. data do not include any metabolites exhaled during the 6-h exposure period. Simulations using this approach suggest that the fraction of 1,3-butadiene metabolized to EB increases with decreasing 1,3-butadiene exposure.

The models using both approaches were also validated against additional data on 1,3-butadiene, EB, and DEB blood concentrations from Bechtold et al. (1995) and Thornton-Manning et al. (1995a). The degree of comparability of predictions with observations varies across species, experiment, metabolite, time, and exposure level; however, in general, it appears that at the lowest 1,3-butadiene exposure level evaluated (62.5 ppm), the two-product model provided better estimates for mouse blood levels than the two-pathway model, which tended to overestimate levels, especially for EB. Both methods overestimated rat blood levels of EB and DEB from exposure to 62.5 ppm 1,3-butadiene by about 2-6 times. Differences between the two approaches are likely to be more dramatic at lower exposure levels, as the fraction of 1,3-butadiene metabolized to EB increases in the two-pathway model; however, there are no blood data for validation at lower exposures.

While different approaches to modeling some of the mechanistic pathways were explored, no comprehensive sensitivity analysis for any of the model parameters was reported.

On the basis of the model simulations of blood levels following iv administration of EB and DEB, Sweeney et al. conclude that extrahepatic and extrapulmonary metabolism is required to describe the clearance of these compounds. They suggest that this conclusion is also consistent with the discrepancy between tissue:blood partition coefficients and the ratio of tissue and blood concentrations measured by Thornton-Manning et al. following in vivo exposure to 1,3-butadiene. The lower concentration ratios for tissues such as heart and spleen, which are not considered major organs for xenobiotic metabolism, suggest that EB and DEB are being further metabolized throughout the body. Sweeney et al. note that "[u]ncertainty as to the nature of these reactions makes extrapolation to other species tenuous."

Another major conclusion of Sweeney et al. is that observations of blood EB and DEB concentrations in mice and rats are best explained by the existence of an alternative oxidative pathway for 1,3-butadiene metabolism that does not generate EB. These investigators suggest that such a pathway might also explain the urinary metabolite data of Nauhaus et al. (1996) and the exhaled $^{14}CO_2$ data of Bond et al. (1986). Sweeney et al. further suggest that some support for the two-pathway model for 1,3-butadiene oxidation can be found in experiments in which

mice were exposed to both 1,3-butadiene and styrene (Leavens et al., 1996). Styrene coexposure was found to result in a decreased rate of 1,3-butadiene metabolism, while EB blood concentrations were increased. Sweeney et al. propose that styrene might be competing with 1,3-butadiene for a non-EB-producing pathway, forcing more 1,3-butadiene metabolism to EB, even while the total rate of 1,3-butadiene metabolism decreases. They suggest that if this is the case, an individual's specific complement of P-450 isozymes may be an important determinant of that individual's risk from 1,3-butadiene exposure.

One of the strengths of the work of Sweeney et al. is that these investigators conducted actual experiments to obtain measurements for some of the parameters (e.g., partition coefficients for DEB) as well as to generate additional data for model validation. They also evaluated different hypotheses for resolving blood EB overpredictions in the original model. Their final model, using either approach for describing the proposed alternative pathway for 1,3-butadiene oxidation, generally yields fairly good predictions of blood EB and DEB concentrations in mice and rats exposed to 1,3-butadiene. Model validation was conducted using multiple independent data sets.

The main limitations of the Sweeney et al. model are that several of the reactions described are hypothetical and a substantial number of the parameters have been estimated by optimization. The work of these investigators provides a good example of how PBPK modeling is a valuable tool for generating hypotheses that can be tested, and that result in a better understanding of underlying mechanisms that can in turn lead to the development of more definitive models. At this point, however, the model is inadequate for use in risk assessment. While the model generally predicts blood EB and DEB concentrations in mice and rats exposed to 1,3-butadiene fairly well, there are serious uncertainties regarding some of the processes being modeled and some of the parameter values used that make extrapolation of the model to humans and to lower exposure levels of 1,3-butadiene highly uncertain, especially given the absence of human data needed to validate any such extrapolations.

9.2.10. Kohn (1997)

Kohn's 1997 paper does not present a comprehensive new 1,3-butadiene PBPK model, but rather summarizes his comparisons of different assumptions for modeling 1,3-butadiene uptake data. Kohn observes that the consequences of the mathematical simplifications used to model complex biological systems are rarely explored, and in this paper he investigates the assumption of flow limitation commonly used in gas inhalation PBPK models.

The assumption of flow limitation presupposes that the permeability of a tissue to a chemical is sufficiently high that the levels of the chemical in the tissue itself and in the blood in the tissue capillary space are nearly at equilibrium and the tissue and its capillary space can be treated as

one compartment. As long as the permeability is high with respect to the blood flow rate to the tissue, the uptake of the chemical from the blood to the tissue is limited by the blood flow rate. A single-pass extraction ratio, ϵ, is defined as the rate of uptake of a chemical by the tissue divided by the sum of the rates of transport from capillary blood to tissue and venous spaces and from the tissue into capillary blood.

Kohn compares three models of 1,3-butadiene uptake and disposition by the mouse in closed inhalation chambers. The first model ("consolidated blood" model) is a classical PBPK model in which blood is consolidated into explicit arterial and venous blood compartments. The model also includes compartments for lung, liver, kidney, fat, rapidly perfused tissues ("viscera"), and slowly perfused tissues ("muscle"). 1,3-Butadiene in the chamber air was treated as partitioning into the arterial blood, and the distribution of 1,3-butadiene between compartments was assumed to be flow-limited.

The second model is based on the work of Anderson et al. (1987). It infers a gas exchange compartment, representing the lung capillary bed, which equilibrates with air inhaled from the chamber. This implicit compartment, along with implicit venous and arterial blood compartments, is treated as having no volume and is not represented in the mass balance equations. No storage of 1,3-butadiene occurs in these blood compartments, and blood concentrations of 1,3-butadiene are assumed to be in equilibrium with the tissues. With the exception of the blood compartments, the tissue compartments are the same as in the first model. 1,3-Butadiene distribution is again assumed to be flow-limited.

The third model ("distributed blood" model) distributes the blood among explicit compartments for arterial and venous blood and for the capillary beds for each individual tissue represented in the model (lung, liver, kidney, fat, viscera, muscle, and gastrointestinal tract). The GI tract was separated from the viscera compartment to better depict hepatic perfusion. The model also includes an explicit alveolar space.

The values of the physiological parameters, partition coefficients, and metabolic constants are identical for all three models, as applicable, and are summarized in Table 9-8. Tissue volumes, blood flow rates, alveolar ventilation, and cardiac output are reportedly consensus values taken from the literature. Capillary bed volumes were taken from Altman and Dittmer (1971). Mouse tissue:blood partition coefficients were taken from Medinsky et al. (1994) with the exception of the muscle value, which was deemed unreliable because of concerns that the mouse muscle tissue may have been contaminated by some attached fat; instead Medinsky et al.'s rat muscle:air value was divided by their mouse blood:air value to get a muscle:blood coefficient. Kohn reports that the blood:air partition coefficient of Medinsky et al. could not reproduce the equilibrium chamber concentration data of Kreiling et al. (1986b) for mice treated with dithiocarb to inhibit P450 activity; thus an average of the Medinsky et al. (mouse and rat) and the Johanson and Filser

(1993) (rat) values was used. The kidney partition coefficient was apparently derived by dividing the rat kidney:air partition coefficient of Johanson and Filser by the mouse blood:air coefficient of Medinsky et al., and values for the viscera and GI tract were similarly derived from Johanson and Filser's rat spleen:air coefficient. The extraction ratio, ϵ, occurring in the distributed blood model, was allowed to vary but was assumed to have the same value for all tissues.

Metabolic constants for oxidation of 1,3-butadiene in the liver and lung were derived from the in vitro data of Csanády et al. (1992). A V_{max} value for oxidation in the kidney was derived from data of Sharer et al. (1992). No K_m value was available for the kidney, so the lung value was used. A first-order rate constant for absorption onto the chamber walls and animal fur was taken from Medinsky et al. (1994).

The models were first used to simulate the 1,3-butadiene uptake data from the chamber experiments of Kreiling et al. (1986b). All three models fit the data for dithiocarb-treated mice, with ϵ values between 0.5 and 0.99 providing adequate fit for the distributed blood model. Then, Kohn investigated the effect of varying ϵ on the fit of the distributed blood model to the Kreiling et al. data for uninhibited mice exposed to 100 ppm 1,3-butadiene. An extraction ratio of 0.5 gave the best fit; however, even with a value of 0.99, the distributed blood model did not overpredict uptake as much as did the flow-limited models. The flow-limited models overpredicted uptake for each 1,3-butadiene exposure level, whereas the distributed blood model with ϵ=0.5 fit all the data fairly well, although it did start to overpredict uptake for the 5000 ppm exposure group after 6 h of exposure. Kohn also explored and rejected the hypothesis that overpredictions of 1,3-butadiene uptake by the flow-limited models might be remedied if 1,3-butadiene were sequestered in red blood cells.

The models were also used to simulate the mouse blood 1,3-butadiene levels following 6 h of exposure observed by Himmelstein et al. (1994). Each model reproduced the data within 20% error, although the distributed blood model provided a better fit at the highest exposure. Kohn notes that the consistency of results across the three models for the blood data is not unexpected because steady-state levels are largely determined by the partition coefficients.

A sensitivity analysis was conducted to ascertain which parameters most influence the predicted chamber concentrations of 1,3-butadiene following 1 h of exposure. Cardiac output and ventilation rate were among the most influential parameters. In the distributed blood model the extraction ratio was also an important parameter. Of the partition coefficients, the blood:air coefficient was determined to have the most influence. Sensitivities to V_{max} for the metabolizing enzymes increased with increasing exposure level and at the lowest exposure of 100 ppm were much less influential than the extraction ratio.

Table 9-8. Parameter values used in Kohn (1997) PBPK models.

Parameter	Model		
	Consolidated blood	**Anderson et al. (1987)**	**Distributed blood**
Alveolar ventilation ($L/h/kg^{0.7}$)[a,b]	20.2	20.2	20.2
Cardiac output ($L/h/kg^{0.7}$)[a]	15.3	15.3	15.3
Body weight (kg)[c]	0.028	0.028	0.028
Tissue compartment volumes (% body weight)[a]			
Arterial blood[d]	1.25		0.661
Venous blood[d]	3.75		1.983
Lung	0.6	0.6	0.6
Alveoli			5
GI tract			7.5
Liver	5.5	5.5	5.5
Kidney	1.67	1.67	1.67
Fat	6	6	6
Viscera	11.43	11.43	3.93
Muscle	64.5	64.5	64.5
Capillary bed volumes (% tissue volume)[e]			
Lung blood			11
GI tract blood			2.9
Liver blood			11
Kidney blood			10.2
Fat blood			3
Viscera blood			7.1
Muscle blood			1.3
Blood flow rates (% cardiac output)[a]			
GI tract			18.1
Liver	22.5	22.5	4.4[f]
Kidney	16.3	16.3	16.3
Fat	5	5	5
Viscera	22.4	22.4	22.4
Muscle	33.8	33.8	33.8

Table 9-8. Parameter values used in Kohn (1997) PBPK models (continued).

Parameter	Model		
	Consolidated blood	Anderson et al. (1987)	Distributed blood
Partition coefficients for 1,3-butadiene			
Blood:air[g]	1.95	1.95	1.95
Lung:blood[h]	1.10	1.10	1.10
Liver:blood[h]	1.01	1.01	1.01
Kidney:blood[i]	0.687	0.687	0.687
Fat:blood[h]	14.3	14.3	14.3
Viscera:blood[i]	0.649	0.649	0.649
Muscle:blood[i]	1.10	1.10	1.10
Rate constants for oxidative metabolism of 1,3-butadiene			
Liver V_{max} (mmol/L/h)[j]	4.66	4.66	4.66
K_m (μM)[j]	2	2	2
Lung V_{max} (mmol/L/h)[j]	1.33	1.33	1.33
K_m (μM)[j]	5.01	5.01	5.01
Kidney V_{max} (mmol/L/h)[k]	12.9	12.9	12.9
K_m (μM)[m]	5.01	5.01	5.01
Microsomal protein concentrations (mg protein/g tissue)			
Liver[n]	30	30	30
Lung[p]	9	9	9
Kidney[q]	9	9	9

[a] Consensus values from literature sources.
[b] 70% of minute volume per Arms and Travis (1988).
[c] Average from experiments of Kreiling et al. (1986b).
[d] Blood outside of capillary spaces was distributed between arteries and veins in a 1:3 ratio (Menzel et al., 1987).
[e] From Altman and Dittmer (1971).
[f] Hepatic artery only.
[g] Average of Medinsky et al. (1994) rat and mouse values and Johanson and Filser (1993) rat value.
[h] From Medinsky et al. (1994).
[I] See text.
[j] From Csanády et al. (1992).
[k] From Sharer et al. (1992).
[m] Set equal to lung value.
[n] From Alberts et al. (1983).
[p] From Smith and Bend (1980).
[q] From Coughtrie et al. (1987).

Kohn concludes that improper assumptions and optimized parameters can hinder identification of the correct model. He recommends verification of the accuracy of the mathematical representations of inhalation, distribution, and metabolism to reduce uncertainties about the PBPK models.

Although limited to 1,3-butadiene disposition, Kohn's 1997 modeling experiments provide some interesting observations pertaining to the 1,3-butadiene PBPK model assumptions and parameter estimates. A strength of this work is that Kohn attempted to estimate all parameter values from the literature rather than by optimization; although specific values were not always available and sometimes values for parameters assumed to be similar (e.g., from other tissues or species) had to be substituted. Then he explored the implications of the basic assumption of flow limitation using various model representations.

9.3. SUMMARY

1,3-Butadiene is metabolized to three epoxide intermediates and potentially to other reactive metabolites as well. Comparisons of tissue levels of EB and DEB between mice and rats suggest that DEB may be an important determinant of the differences in cancer potency between mice and rats (Thornton-Manning et al., 1995a). However, EB is also a reactive metabolite, and it has been suggested that EB may be important, for example, in the rat mammary gland tumorigenicity of 1,3-butadiene (Thornton-Manning et al., 1998). In addition, Perez et al. (1997) have suggested that the epoxydiol metabolite, 3,4-epoxy-1,2-butanediol, forms more hemoglobin adducts than EB in both rats and humans exposed to 1,3-butadiene, yet this important metabolite has been neglected in the current PBPK models. While much progress has been made in recent years towards elucidating the differences in cancer potency between mice and rats, a complete picture of the relative potencies of the various reactive metabolites at different tissue sites is still far from clear.

Furthermore, uncertainties in the existing PBPK models and data make them unreliable for use in human risk assessment. Serious uncertainties exist pertaining to the model structures and assumptions, parameter values, and validation. There is a clear progression in the models over time, as more experimental data have become available for both parameter estimation and model validation and as different hypotheses have been tested. However, even among recent models, there are important discrepancies that need to be resolved. In fact, as the models have gotten more complex, models from different teams of investigators have become more divergent in certain respects.

The earliest models were limited by the available experimental data, and some parameters were estimated theoretically. Later models were able to incorporate more experimentally derived parameters; however, several nonetheless rely on optimization to estimate critical parameters.

Furthermore, as blood EB data became available for validation, it became apparent that models that adequately simulated 1,3-butadiene and EB uptake experiments nevertheless overestimated blood EB levels. The next generation of models proposed a variety of different hypotheses for dealing with this problem, and none of these divergent hypotheses has been adequately tested. For example, Johanson and Filser suggest that intrahepatic metabolism resolves the issue, while Reitz et al. claim the problem lies in extrapolation from in vitro measures of metabolic constants and Sweeney et al. have proposed that there is an alternative pathway responsible for a substantial proportion of the oxidative metabolism of 1,3-butadiene.

Other discrepancies among the models arise in the treatment of extrahepatic and extrapulmonary metabolism and the structural forms of the various phase I and phase II reactions. The recent work of Kohn (1997) also raises questions about the basic assumption of flow limitation. In addition, only the most recent models include disposition of the diepoxide metabolite DEB, and none of the existing models include the disposition of the epoxydiol or potentially reactive nonepoxide metabolites (see below).

With respect to the parameter values, there are disagreements about the ventilation rate, which is a key parameter for determining 1,3-butadiene delivery, and about metabolic parameters. For example, the V_{max} and K_m values used by Sweeney et al. for conjugation of EB with GSH in the liver differ by orders of magnitude from those used by Reitz et al. for both the mouse and the rat. Even the V_{max}/K_m rates differ by more than 14-fold. Similarly, the V_{max}/K_m rates for EB hydrolysis differ by more than 13-fold. Recall that the Sweeney et al. rates are based on in vitro data, while Reitz et al. rejected the in vitro data and estimated their parameter values from in vivo data. The validity of the in vitro metabolic data is a critical issue for any PBPK-based extrapolation of carcinogenic risk from rodents to humans because there are no appropriate human in vivo PBPK data for 1,3-butadiene and thus interspecies extrapolation must rely on in vitro data or allometric scaling.

Furthermore, the human in vitro data available for extension of the rodent PBPK models to humans are limited. The measurements that have been made suggest there is a high degree of inter-individual variability in metabolic enzyme activities. For example, Seaton et al. (1995) reported a 60-fold variation in the initial rate of EB oxidation to DEB among 10 human liver samples. (See also Table 3-4.) If the human in vitro data used as a basis for interspecies extrapolation in PBPK modeling do not represent the variability in the human population, the final results could be seriously biased. In addition to standard interindividual variability in the activities of phase I and phase II enzymes, humans are known to have important polymorphisms in the types of isoforms of glutathione-S-transferase and epoxide hydrolase that they express, and these polymorphisms may have substantial effects on 1,3-butadiene metabolism. For example,

sensitivity to DEB-induced genotoxicity in human lymphocyte cultures has been shown to be highly associated with lack of the GSTT1 gene (e.g., Norppa et al., 1995; see also Chapter 4).

Another area of uncertainty is model validation. The existing models have been subjected to limited validation. Virtually all of the model reports claim that the existing models adequately fit the validation data, despite important differences among the models. In some cases, this is not surprising because some of the model parameters have been determined by optimization against data similar to those being used for validation. In other cases, it appears that gas uptake data are relatively insensitive to various features of the models and might be of limited use in and of themselves for model validation. Ideally, for the PBPK models to be more reliable, they should be validated against tissue concentration data for various metabolites in various tissues over time and for a variety of exposure concentrations, especially at the low- exposure end. More recently, some of these data have become available (Chapter 3), although they must be interpreted with caution because it appears that metabolites in some of the tissues are subject to further metabolism during the lag time between the termination of exposure and the measurement of tissue concentrations. Blood metabolite measurements have thus far provided the most useful data for model validation. Because there are no human in vivo PBPK data for validating any 1,3-butadiene PBPK model extended to humans, it is especially critical that the rodent models be correct in their descriptions of the underlying biology and be well validated.

Recent studies suggest some additional considerations that might affect the accuracy and utility of 1,3-butadiene PBPK models. For example, the work of Perez et al. (1997) suggests that it might be important to include the epoxydiol metabolite, 3,4-epoxy-1,2-butanediol, in future models. These investigators determined that epoxydiol-hemoglobin adduct levels were substantially higher than EB-hemoglobin adduct levels in butadiene-exposed rats and humans. Furthermore, Kemper et al. (1998) suggest that the 3-butene-1,2-diol metabolite, which is the hydrolysis product of EB and an important metabolite in humans, can be metabolized to other reactive intermediates in addition to the epoxydiol. Moreover, Richardson et al. (1998) conclude from their studies of urinary metabolites following EB administration that the biotransformation of 1,3-butadiene to DEB or the epoxydiol metabolite should be considered an integrated step. Richardson et al. suggest that this might explain the inability of the PBPK models, which generally model the reactions separately, to predict blood EB and DEB levels.

Recent studies also demonstrate that there is stereoselectivity in the metabolism of 1,3-butadiene and its metabolites, and there may be differences in the mutagenic and carcinogenic potency of the metabolite stereoisomers as well (e.g., Krause and Elfarra, 1997; Richardson et al., 1998; Oe et al., 1999). If stereoselectivity in 1,3-butadiene metabolism is important, then parameter estimates derived from in vitro and in vivo studies of commercial 1,3-butadiene metabolites might not reflect what happens in vivo following 1,3-butadiene exposure.

Another issue that has not yet been addressed in any of the 1,3-butadiene PBPK models is metabolism in the bone marrow, which is a target organ in both mice and humans. Maniglier-Poulet et al. (1995) have measured 1,3-butadiene metabolism to EB in vitro by mouse and human bone marrow cells. EB formation was stimulated by hydrogen peroxide but not by NADPH, suggesting peroxidase-mediated metabolism. These investigators suggest that "[a]lthough the extent of conversion is markedly lower than in either rat or mouse hepatic microsomes, in-situ conversion of [1,3-butadiene] to [EB] in bone marrow should be considered in future models of [1,3-butadiene] metabolism and pharmacokinetics."

9.4. CONCLUSIONS

As discussed above, the existing PBPK models and data cannot yet adequately explain the interspecies differences in 1,3-butadiene carcinogenicity. Uncertainties in the model structures and parameter values also prohibit their use in refining risk assessment dosimetry at this time. Some areas in which more research is needed include (a) investigation of the alternative oxidation pathway for 1,3-butadiene hypothesized by Sweeney et al. (1997), (b) resolution of the issue of the validity of the in vitro metabolic data for extrapolating to in vivo exposure, (c) evaluation of the necessity for including intrahepatic first-pass hydrolysis of EB and possibly other integrated reactions, (d) assessment of the impacts of stereoselective metabolism on the PBPK models, (e) investigation of the validity of the flow limitation assumption, (f) evaluation of the kinetics of the epoxydiol metabolite and possibly other metabolites in rodents and humans, (g) clarification of the values of various physiological parameters, in particular the ventilation rate, (h) more measurements of extrahepatic and extrapulmonary metabolism, (i) better characterization of the distribution of values for the human metabolic rates, and (j) more time-course measurements of blood and tissue concentrations of metabolites for model validation.

There is strong evidence that ovarian atrophy in the mouse is attributable specifically to DEB, the diepoxide metabolite of 1,3-butadiene (Chapter 5). Therefore, a PBPK model describing DEB disposition following 1,3-butadiene exposure could conceivably be used to extrapolate the risk of ovarian atrophy from mice to humans; however, the existing PBPK models are inadequate for this purpose given the substantial uncertainties and unresolved discrepancies described above. With respect to carcinogenicity, the state of the science is much less certain. More information on the specific roles of the various metabolites in the various target tissues would be necessary before any dosimetric predictions from PBPK modeling of 1,3-butadiene could be translated into human cancer risk estimates.

In conclusion, the existing PBPK models and data are inadequate for developing a reliable alternative to the default methodology of using exposure to the parent compound as a dose surrogate for extrapolation of the carcinogenic or reproductive risks from animals to humans.

And, given the dramatic interspecies differences in cancer response between the mouse and rat, any attempt to extrapolate the cancer risk in rodents to humans using the default methodology would involve far greater uncertainties than basing a cancer risk assessment on the occupational data of Delzell et al. (Chapter 7). Ideally, a reliable, well-validated PBPK model with parameter values for humans could also be applied to analyzing different human exposure scenarios (e.g., extrapolating from occupational to environmental exposures). However, there are too many uncertainties in the PBPK modeling for that to be practicable at this time.

10. QUANTITATIVE RISK ASSESSMENT FOR 1,3-BUTADIENE

This chapter presents the quantitative risk analyses performed for the critical health effects reviewed in this document. Section 10.1 provides the quantitative cancer risk assessment based on the leukemia results from the Delzell et al. occupational epidemiology study reviewed in Chapter 7. Section 10.2 presents quantitative analyses based on the cancer results from the 1993 NTP mouse bioassay described in Chapter 6. Section 10.3 presents the results of benchmark dose modeling of several of the reproductive and developmental endpoints observed in experimental animals, reviewed in Chapters 5 and 4 (dominant lethal effects). Section 10.4 develops Reference Concentrations (RfCs) for certain reproductive and developmental effects, which are the most sensitive noncancer endpoints that have been observed in available toxicity studies. Finally, Section 10.5 summarizes the key quantitative risk estimates.

10.1. EPIDEMIOLOGICALLY BASED CANCER RISK ASSESSMENT

10.1.1. Exposure-Response Modeling

For quantitative risk assessment, it is generally preferable to use high-quality epidemiologic data, when available, over toxicologic data. In the past, available epidemiologic data on 1,3-butadiene have been inadequate for quantitative cancer risk assessment because of the lack of historical exposure information, and all but the most recent previous risk assessments relied primarily on models based on the NTP mouse bioassay studies.

The retrospective mortality study by Delzell et al. (1995) of a cohort of synthetic rubber production workers exposed to 1,3-butadiene (reviewed in Chapter 7) presents an opportunity to perform a quantitative cancer risk assessment based on human data. The investigators developed a job exposure matrix (JEM) for 1,3-butadiene, styrene, and benzene based on industrial hygiene data, which contained estimates of the average daily exposure (in ppm based on the 8-h TWA) and the number of annual peaks (defined as $\geq$ 100 ppm for 1,3-butadiene and 50 ppm for styrene) for each area and job code for each study year. The investigators were then able to estimate cumulative exposures (ppm*years) and number of peak exposures (peak years) for each individual worker by linking the JEM with the study subjects' work histories.

Delzell et al. (1995) investigated the relationship between cumulative exposure to 1,3-butadiene and leukemia mortality using Poisson regression analysis (Frome and Checkoway, 1985). The models controlled for the potentially confounding effects of age (40-49, 50-59, 60-69, 70-79, 80+), years since hire (10-19, 20-29, 30+), calendar period (1950-59, 1960-69, 1970-79, 1980-89, 1990-91), and race (black, white, other). Plant was considered as a possible confounder but was dropped from the final models because it did not affect the estimated parameters for 1,3-butadiene or styrene. Few subjects were exposed to benzene, and benzene did

not appear to confound the relationship between 1,3-butadiene or styrene exposure and leukemia mortality. Hence, the model results presented in the report did not control for benzene exposure.

Different functional forms of the relationship between the relative rate (RR) and measures of exposure were evaluated by Delzell et al. (1995), including the following:

(1) Log-linear: $RR = e^{\beta X}$
(2) Power: $RR = e^{\beta[\ln(1+X)]}$
(3) Linear excess: $RR = 1 + \beta X$
(4) Polynomial excess: $RR = 1 + \beta_1 X^p + \beta_2 X^q + ...$

where X represents the 1,3-butadiene or styrene exposure categories using the midpoints of the intervals, β represents the estimated model parameters, and the powers "p" and "q" are fixed real numbers. Although many polynomial functions (model 4) were considered, only the results from a square root model were presented because this was considered to provide the best fit. This model may be represented as:

(5) Square root: $RR = 1 + \beta_1 X^{½}$.

A positive exposure-response relationship between cumulative exposure to 1,3-butadiene or styrene and leukemia mortality was observed from the Poisson regression analyses. This relationship was evident both in models that represented these exposures as categorical variables (see Table 59 in Delzell et al., 1995) and in models where exposure was represented using continuous variables as described above. The regression parameter for 1,3-butadiene cumulative exposure (adjusted for styrene) was found to be statistically significantly greater than 0 ($p<0.05$) in all of the models evaluated, whereas nonsignificant relationships were observed for styrene (adjusted for 1,3-butadiene). The 1,3-butadiene and styrene exposures among exposed study subjects were found to be moderately correlated (Spearman's rank correlation, r=0.53). The relationship between 1,3-butadiene cumulative exposure and leukemia mortality appeared to be independent of the styrene exposure and was not appreciably altered by inclusion of styrene cumulative exposure in the model. On the other hand, the relationship between styrene cumulative exposure and leukemia mortality was weakened and irregular when 1,3-butadiene cumulative exposure was controlled for. These findings suggest that 1,3-butadiene cumulative exposure is a more likely explanation for the leukemia excess observed in this cohort than styrene cumulative exposure.

Analyses of peak years of 1,3-butadiene exposure indicated an association between this variable and leukemia mortality even after controlling for cumulative exposure, but this relationship was irregular in the categorical regression analyses. Excluding exposures that occurred within 5 or 10 years of death (i.e., lagging exposures) only slightly increased the exposure-response relationship for 1,3-butadiene cumulative exposure, whereas excluding exposures within 20 years of death weakened and almost eliminated the relationship (see Table 63 in Delzell et al., 1995).

Subsequently, Health Canada obtained the individual data on this cohort from Delzell et al. and performed additional analyses (Health Canada, 1998). Their approach was similar to that of Delzell et al. (1995), but some of the refinements made by Health Canada are noteworthy. These refinements include finer stratification of age, calendar year, and years since hire, as well as use of the actual mean cumulative exposure of 362.7 ppm-years for the highest exposure group of "200+ ppm-years" rather than the apparently arbitrary value of 250 ppm-years used by Delzell et al. The Health Canada report fit the same models as Delzell et al. with the exception that the model with the square root of cumulative exposure (model 5 above) was replaced by one that included cumulative exposure raised to the power α, which was estimated. This is a more general model, which contains the "square root" model of Delzell et al. (1995) as a special case [α=0.5], with α describing the *shape* of the relationship. This model is subsequently referred to as the "shape" model. These refinements provide a more attractive basis for quantitative risk assessment, and thus the models fitted by Health Canada were adopted for this analysis.

The results reported by Health Canada (1998) on the effect of cumulative 1,3-butadiene exposure on the leukemia mortality rate under several models of the relative rate are summarized together with results from Delzell et al. (1995) in Table 10-1. The smaller deviance values reported by Health Canada reflect the fact that they used more levels in their stratification. The differences in the regression coefficients from the two analyses were modest, and the Health Canada report indicates that these differences were not found to be statistically significant. The power and the shape models appear to provide the best fit (i.e., lowest deviance). However, the difference in goodness of fit between these models is slight and thus it is difficult to select one model as the "best" model on this basis. The Health Canada models are presented graphically in Figure 10-1.

10.1.2. Prediction of Lifetime Excess Risk of Leukemia Mortality

The relative rate models presented in the report by Health Canada (1998), summarized in Table 10-1, were used as a basis for predicting the excess risk of leukemia mortality for varying levels of continuous environmental exposures to 1,3-butadiene. These risk estimates were made using the relative rate estimates and an actuarial program that accounts for the effects of

Table 10-1. Results from exposure-response models of cumulative exposure to 1,3-butadiene using alternative structural forms reported by Health Canada (1998) and Delzell et al. (1995)[a].

Structural model form[b]	Health Canada (1998)		Delzell et al. (1995)	
	Model deviance	β estimate (S.E.)[c]	Model deviance	β estimate (S.E.)[c]
Log-linear: $RR = e^{\beta X}$	176.721	0.0029[d] (0.0014)	486.0	0.0041[e] (0.0019)
Linear: $RR = 1 + \beta X$	174.713	0.0099[e] (0.0065)	486.0	0.0068[e] (0.0050)
Power: $RR = e^{\beta[\ln(1 + X)]}$	171.547	0.2850[e] (0.0976)	485.6	0.2028[e] (0.0972)
Shape: $RR = 1 + \beta X^{\alpha}$	171.994	0.4548[f] (β) (0.8222) 0.3999[f] (α) (0.2733)	485.6	0.1293[e] (β) (0.1024) 0.5 (α) (0) [g]

[a] Adapted from Table 11-3 in Health Canada (1998) and Table 67 in Delzell et al. (1995). Additional information on the LRT *p*-values for the Health Canada parameter estimates was provided by Health Canada (personal communication from Michael Walker to Leslie Stayner, 16 June 1999). Results presented are adjusted for age, calendar year, years since hire, race, and cumulative styrene exposure.
[b] X denotes cumulative 1,3-butadiene exposure (ppm-yrs).
[c] S.E. is the standard error for the exposure parameter estimates.
[d] likelihood ratio test (LRT) *p*-value=0.0578.
[e] LRT *p*-value<0.05.
[f] LRT *p*-value<0.05 for model with both α and β versus null model.
[g] α = ½ was fixed by Delzell et al. (1995).

competing causes of death.[1] U.S. age-specific mortality rates for all race and gender groups combined (NCHS, 1993) were used to specify the leukemia and all-cause background rates in the actuarial program. The risks were computed up to age 85 for continuous exposures to 1,3-butadiene. The occupational 1,3-butadiene exposures in the epidemiologic study were converted to continuous environmental exposures by multiplying the occupational exposure estimates by a factor to account for differences in the number of days exposed per year (365/240 days) and

[1]This program is an adaptation of the approach previously used in BEIR IV. Health Risks of Radon and Other Internally Deposited Alpha Emitters. (1988) Washington, DC: National Academy Press; pp. 131-134.

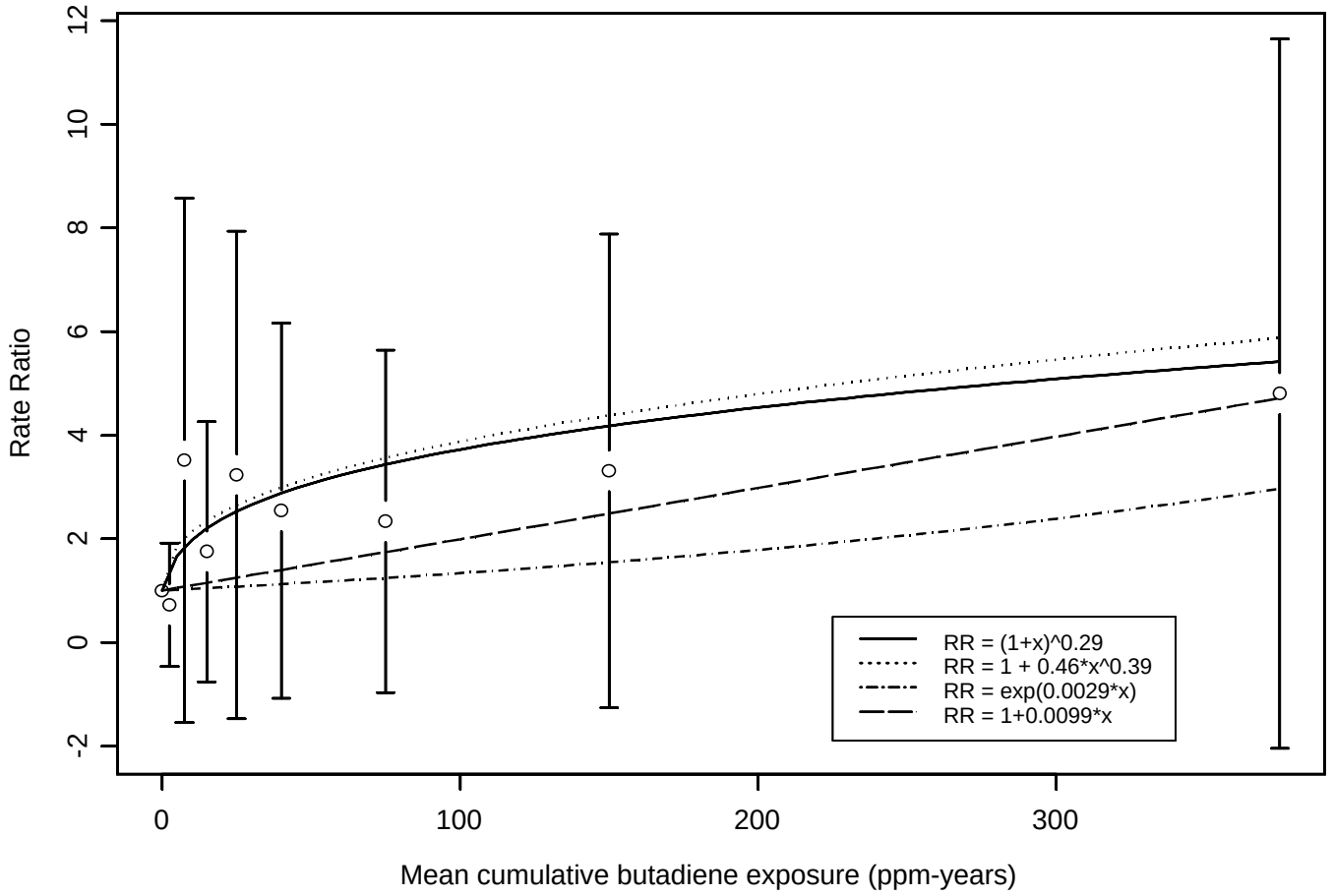

Figure 10-1. Predicted rate ratios from Health Canada (1998) Poisson regression models[a].

[a] Open circles with error bars represent rate ratios and 95% confidence intervals from regression analyses with 1,3-butadiene exposure expressed as a categorical variable. The four lines represent different exposure-response models, as defined in the legend, with 1,3-butadiene exposure as a continuous variable. Additional information was provided by Health Canada for the calculation of the confidence intervals (personal communication from Michael Walker to Leslie Stayner, 18 June 1999).

another factor to account for differences in the amount of air inhaled per day (20/10 m^3). The reported standard errors for the 1,3-butadiene regression coefficients were used to compute the upper 95% confidence limits for the relative rates based on a normal approximation.

Point estimates and one-sided upper 95% confidence limits for the excess risk of leukemia mortality associated with varying levels of environmental exposure to 1,3-butadiene based on the alternative model forms are illustrated in Figures 10-2 to 10-5. Estimates of leukemia mortality risks and exposure levels corresponding to levels of risk of potential regulatory interest are presented in Tables 10-2 and 10-3. These estimates vary by several orders of magnitude depending on the model used, particularly at low levels of risk. For example, at the 1-in-a-million risk level, the maximum likelihood estimates for continuous lifetime 1,3-butadiene exposure range from 0.26 parts per billion (ppb) (based on the loglinear model) to 1.1×10^{-8} ppb (based on the shape model).

Consistent with the proposed EPA cancer risk assessment guidelines (U.S. EPA, 1996, 1999), these results were also used to estimate the exposure levels (EC_p, "effective concentration") and their associated 95% lower confidence limits (LEC_p) corresponding to varying levels of excess risk (p) ranging from 0.1% to 10%. These estimates are summarized in Table 10-4. Although the proposed EPA guidelines emphasize the derivation of exposure levels associated with a 10% excess risk level, this does not seem reasonable in this instance. The 10% level of risk is associated with exposure levels that are higher than most of the exposures experienced by the workers in this epidemiologic study. Furthermore, based on the actuarial program described above, a relative rate of 19 would be required for adults over the age of 20 to achieve a lifetime excess risk of leukemia death of 10%, but the leukemia standardized mortality ratios (SMRs) reported by Delzell et al. (1995) were considerably lower.[2] Hence, these considerations suggest that using a 10% risk level would be an upward extrapolation in this case. A 1% or even lower (e.g., 0.1%) excess risk level would seem to be a more reasonable choice in this circumstance. Based on the actuarial program, the relative rates for excess risks of 1% or 0.1% are 2.7 and 1.2, respectively. These rates better correspond with the set of SMRs reported by Delzell et al. (1995). The exposure levels corresponding to a 1% excess risk level are illustrated for the four relative rate models in Figures 10-2 to 10-5. When a 1% excess risk level is used, the LEC_{01} from these analyses ranges from 0.037 to 0.87 ppm, depending on the model.

[2]The maximum reported SMR was 13.33. This SMR was based on two leukemia deaths among black men from plant #2 with at least 10 years of work (not all of which was salaried) and at least 20 years of elapsed time since hired. (See Table 29 of Delzell et al., 1995.)

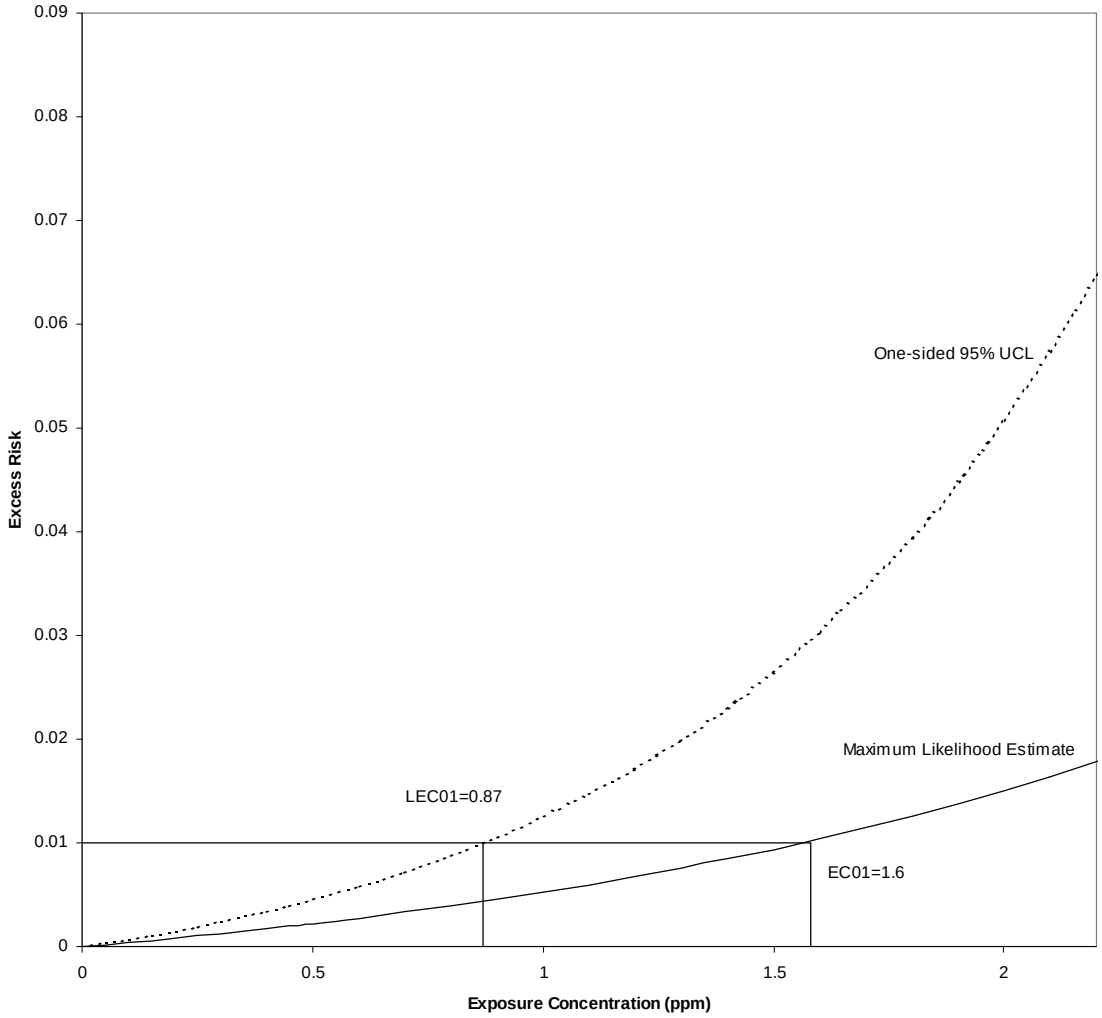

Figure 10-2. Excess risk and 95% upper confidence limit estimates for leukemia mortality based on the log-linear model reported by Health Canada (1998)[a].

[a]Log-linear model: $RR=e^{\beta X}$

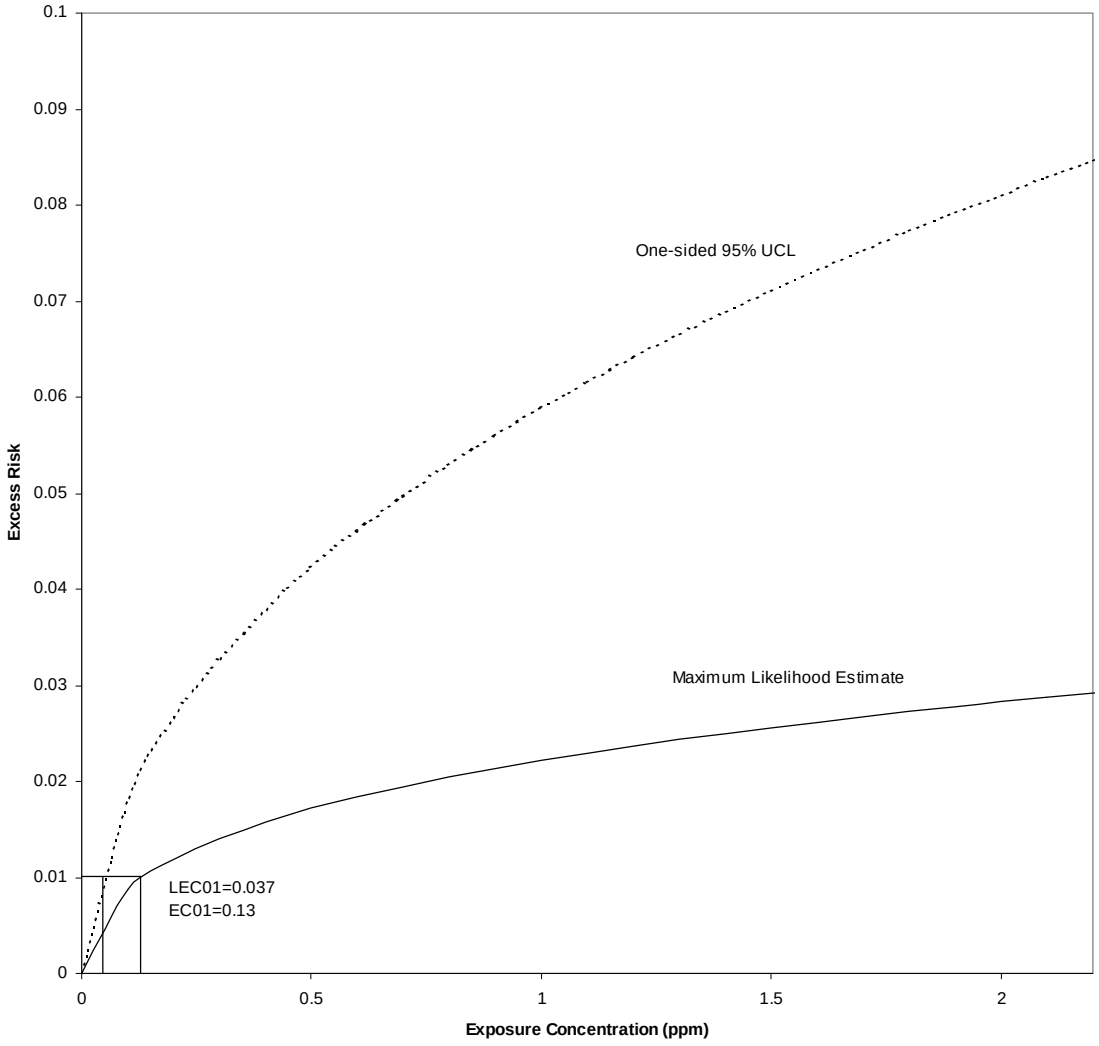

Figure 10-3. Excess risk and 95% upper confidence limit estimates for leukemia mortality based on the power model reported by Health Canada (1998)[a].

[a]Power model: $RR=e^{\beta[\ln(1+X)]}$

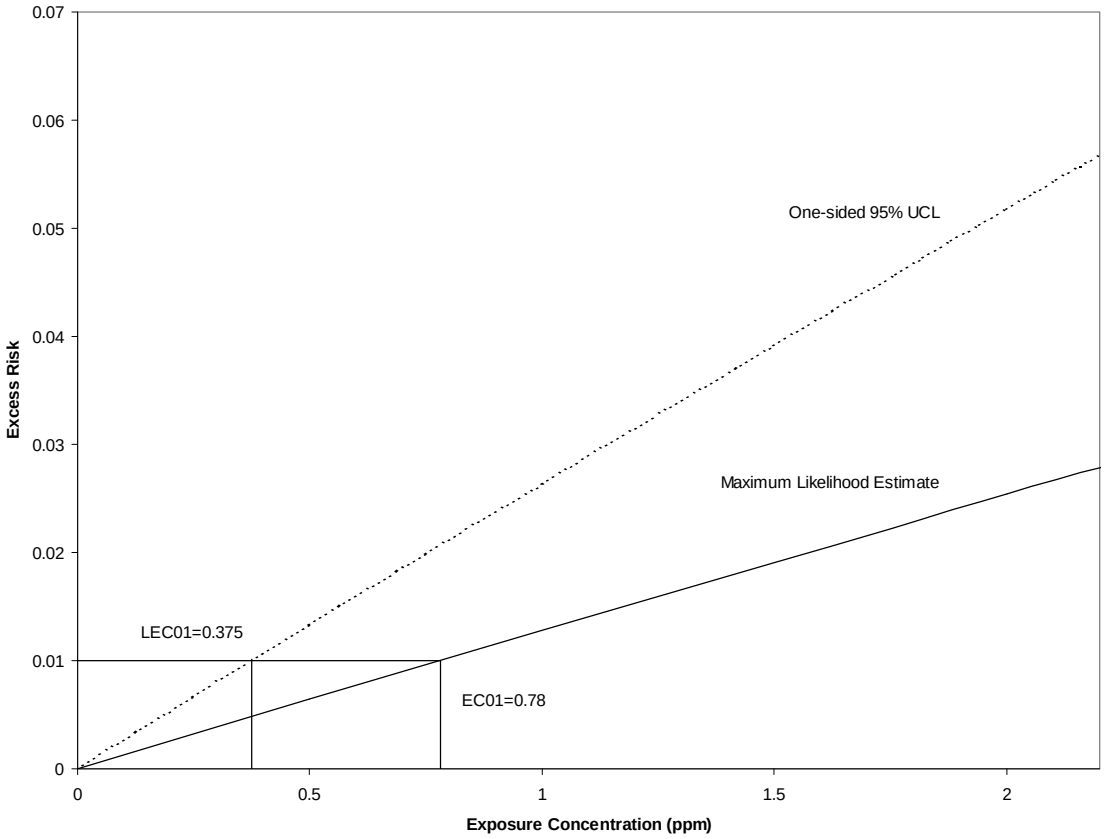

Figure 10-4. Excess risk and 95% upper confidence limit estimates for leukemia mortality based on the linear model reported by Health Canada (1998)[a].

[a]Linear model: $RR = 1 + \beta X$.

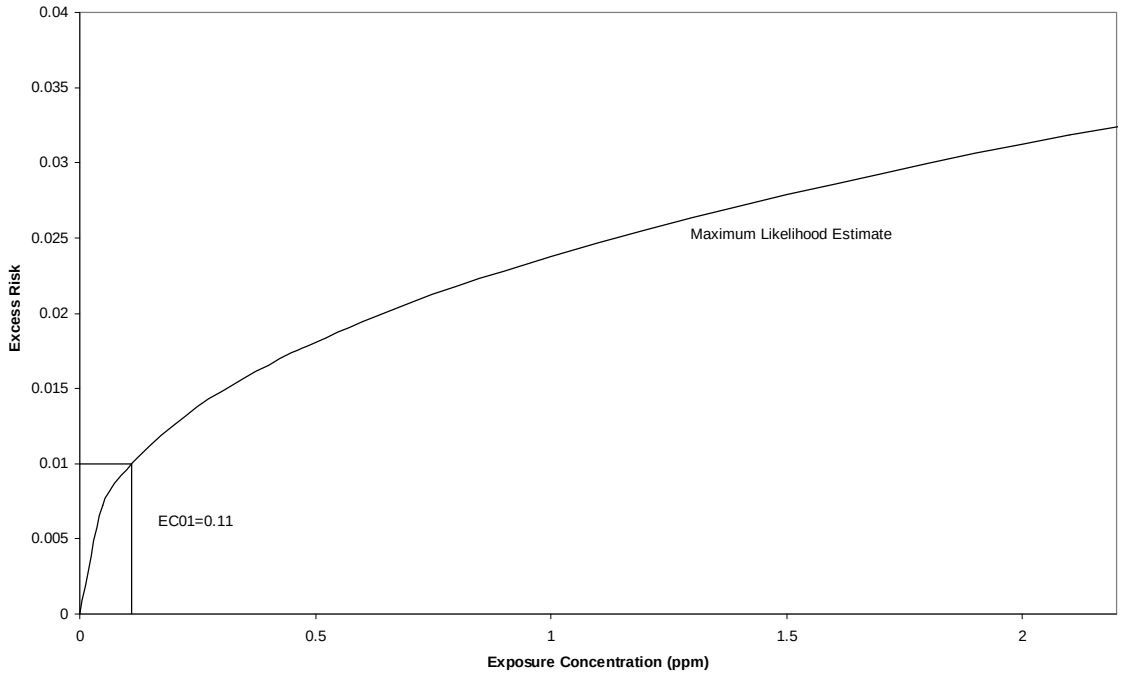

Figure 10-5. Excess risk estimates for leukemia mortality based on shape model reported by Health Canada (1998)[a].

[a]Shape model: $RR=1+\beta x^{\alpha}$; upper confidence limits could not be calculated.

Table 10-2. Maximum likelihood estimates (MLEs) of excess leukemia mortality risk with 95% upper confidence limits (95% UCL) for continuous lifetime exposures to varying concentrations of 1,3-butadiene based on several models reported by Health Canada (1998) and U.S. population rates.

Model	Concentration (ppm)	MLE excess risk	95% UCL excess risk
Log-linear: $RR = e^{\beta X}$	1.0×10^{-4}	3.8×10^{-7}	6.8×10^{-7}
	1.0×10^{-3}	3.8×10^{-6}	6.8×10^{-6}
	1.0×10^{-2}	3.8×10^{-5}	6.8×10^{-5}
Power: $RR = e^{\beta[\ln(1+\times)]}$	1.0×10^{-4}	3.7×10^{-5}	5.8×10^{-5}
	1.0×10^{-3}	3.5×10^{-4}	5.5×10^{-4}
	1.0×10^{-2}	2.4×10^{-3}	4.0×10^{-3}
Linear: $RR = 1 + \beta X$	1.0×10^{-4}	1.3×10^{-6}	2.7×10^{-6}
	1.0×10^{-3}	1.3×10^{-5}	2.7×10^{-5}
	1.0×10^{-2}	1.3×10^{-4}	2.7×10^{-4}
Shape: $RR = 1 + \beta X^{\alpha}$	1.0×10^{-4}	6.1×10^{-4}	NA[a]
	1.0×10^{-3}	1.5×10^{-3}	NA
	1.0×10^{-2}	3.8×10^{-3}	NA

[a] Confidence intervals were not calculated for the "Shape" model because the Health Canada report did not include the variances and covariances of its two exposure parameter estimates.

Table 10-3. Maximum likelihood estimates (MLEs) and 95% lower confidence limits (95% LCL) of continuous exposure concentrations associated with varying excess risk levels for leukemia mortality based on relative rate results of several models reported by Health Canada (1998) and U.S. population rates.

Model	Excess risk	MLE (ppm)	95% LCL (ppm)
Log-linear: $RR = e^{\beta X}$	1×10^{-6}	2.6×10^{-4}	1.5×10^{-4}
	1×10^{-5}	2.6×10^{-3}	1.5×10^{-3}
	1×10^{-4}	2.6×10^{-2}	1.5×10^{-2}
Power: $RR = e^{\beta[\ln(1+X)]}$	1×10^{-6}	2.7×10^{-6}	1.7×10^{-6}
	1×10^{-5}	2.7×10^{-5}	1.7×10^{-5}
	1×10^{-4}	2.7×10^{-4}	1.7×10^{-4}
Linear: $RR = 1 + \beta X$	1×10^{-6}	7.7×10^{-5}	3.7×10^{-5}
	1×10^{-5}	7.7×10^{-4}	3.7×10^{-4}
	1×10^{-4}	7.7×10^{-3}	3.7×10^{-3}
Shape: $RR = 1 + \beta X^{\alpha}$	1×10^{-6}	1.1×10^{-11}	NA[a]
	1×10^{-5}	3.5×10^{-9}	NA
	1×10^{-4}	1.1×10^{-6}	NA

[a] Confidence intervals were not calculated for the "Shape" model because the Health Canada report did not include the variances and covariances of its two exposure parameter estimates.

Table 10-4. Maximum likelihood (EC_p) and 95% lower-bound (LEC_p) estimates of the continuous exposure concentrations associated with varying levels of excess risk (p) of leukemia mortality.

Structural model form	Percentage excess risk (p)	1,3-Butadiene exposure levels (ppm)		Ratio[a]
		Maximum likelihood (EC_p)	Lower 95% bound (LEC_p)	
Log-linear model: $RR = e^{\beta X}$	10	4.6	2.6	3.8×10^{-2}
	1	1.6	0.87	1.1×10^{-2}
	0.1	0.24	0.14	7.1×10^{-3}
Power: $RR = e^{\beta[\ln(1+X)]}$	10	1.2×10^{2}	3.2	3.1×10^{-2}
	1	0.13	3.7×10^{-2}	2.7×10^{-1}
	0.1	3.3×10^{-3}	1.9×10^{-3}	5.3×10^{-1}
Linear model: $RR = 1 + \beta X$	10	8.34	4.01	2.5×10^{-2}
	1	0.780	0.375	2.7×10^{-2}
	0.1	0.0775	0.0373	2.7×10^{-2}
Shape: $RR = 1 + \beta X^{\alpha}$	10	42	NA	NA[b]
	1	0.11	NA	NA
	0.1	3.5×10^{-4}	NA	NA

[a]The ratio is the excess risk (p/100%) divided by the one-sided lower 95% confidence limit on the exposure estimate (LEC_p).
[b]Confidence intervals were not calculated for the "Shape" model because the Health Canada report did not include the variances and covariances of its two exposure parameter estimates.

10.1.3. Sources of Uncertainty

It is apparent from the results presented in Tables 10-2 through 10-4 that one major source of uncertainty is the choice of the model for the prediction of risk. The range of values of the LEC at the 10% excess risk level are within 2-fold, while the range at the 1% excess risk level spanned just over one order of magnitude, and the range for the 0.1% level spanned nearly two orders. In this instance, it seems most reasonable to utilize the results at the 1% risk level because this corresponds to exposures within the range of this epidemiologic study. However, it is not possible to clearly choose one of the relative rate models as the best for risk assessment purposes because none of the models has a biologic basis. Furthermore, all the models summarized in Table 10-1 fit the observed data nearly as well. Moreover, for a given linear extrapolation, the ratios in Table 10-4 show that the sensitivity of the result to the choice of excess risk level varies considerably for these models, with the linear model, as expected, being the least sensitive.

Another major source of uncertainty in this analysis is the potential misclassification of exposures in the study by Delzell et al. (1995). This is a frequent limitation of nearly all epidemiologic studies of this type for quantitative risk assessment purposes. The exposures of this study were based on modeling a relatively extensive set of data. However, questions have been raised concerning the accuracy of exposure estimates, particularly for some ill-defined tasks (letter from Elizabeth Moran, CMA, to Aparna Koppikar, March 25, 1996). For example, the work histories of maintenance laborers do not indicate whether they were vessel cleaners (a high-exposure category) or building cleaners (a low-exposure category). The full impact of this potential exposure misclassification is unknown, but preliminary re-analyses by Delzell et al. suggest that “the impact would have been to dampen and, possibly, to distort dose-response relationships” (letter from Delzell and Macaluso, University of Alabama, to Aparna Koppikar, April 2, 1996).

Another concern about the study has been expressed regarding the assignment of peak exposures in the analysis, which were defined as average exposures equal to or greater than 100 ppm over 15 minutes. It has been suggested that there were tasks with extremely high peak exposures (thousands of ppm) over very short time periods (seconds to a few minutes) (letter from Delzell and Macaluso, University of Alabama, to Aparna Koppikar, April 2, 1996). The models used in this risk assessment were based on cumulative exposure and did not consider the potential for the effects of peak exposures. The potential impact of work area assignments and butadiene peaks on leukemia mortality in this study population is an active area of research among the investigators at the University of Alabama at Birmingham (UAB), who conducted the study by Delzell et al. (1995).

In addition, there is the possibility that the excess of leukemia observed in the UAB study is related to confounding by another chemical also used at the plants. Initially, the analyses conducted by the UAB considered potential confounding by benzene and styrene. Benzene was only weakly associated with leukemia in this analysis, and its effect was eliminated when the analysis was controlled for 1,3-butadiene and styrene exposures (Macaluso et al., 1996). Leukemia mortality did appear to increase with styrene exposure, although not as strongly and as consistently as with exposure to 1,3-butadiene. Studies of workers exposed to styrene alone have not demonstrated an increased risk of leukemia (Bond et al., 1992; Okun et al., 1985; Wong, 1990; Frentzel-Beyme et al., 1978; Kogevinas et al., 1993). Thus, it appears highly unlikely that either benzene or styrene exposure was a significant confounder in this study. It was recently suggested that exposure to dithiocarbamates may potentially confound the observed association between 1,3-butadiene exposure and leukemia in the study by Delzell et al. (Irons and Pyatt, 1998). Dithiocarbamates, however, have not been associated with leukemia risk, and at this time the suggestion is little more than conjecture (see discussion in Section 7.3.3).

Other uncertainties are not so much inherent in the dose-response modeling or in the epidemiologic data themselves, but rather occur in the process of obtaining more general Agency risk estimates from these specific results. EPA cancer risk estimates are typically derived to represent an upper bound on increased risk of cancer incidence for all sites affected by an agent for the general population. For experimental animal studies, this is accomplished by using tumor incidence data and summing across all the tumor sites demonstrating significantly increased incidences. Furthermore, the most sensitive sex and species is customarily used in order to be protective of the general human population. However, in estimating comparable risks from the Delzell et al. epidemiologic data, certain limitations are encountered. First, the Delzell et al. study is a retrospective mortality study, and cancer incidence data are not available for the entire cohort. Second, the epidemiologic data are for a healthy male worker cohort and may not be representative of the general population. Finally, 1,3-butadiene is a multi-site carcinogen in rodents (see Chapter 6), and, while increased risk of leukemia was observed in the epidemiologic study, the study may not have had sufficient statistical power to detect tumor increases at all sites. The issue of study power is discussed further below, and how these issues were dealt with quantitatively in deriving the final cancer risk estimates is addressed in the next section.

As discussed above, the rodent bioassay data clearly show that 1,3-butadiene is a multisite carcinogen. This raises a concern that there may not have been enough statistical power in the epidemiology study to detect increases for all the possible tumor sites. On the other hand, this was a large cohort study (over 15,000 subjects), with a long follow-up time (up to 49 years; 25% of the subjects had died by the end of the follow-up), so for most tumor sites, this should not be a problem. The main tumor site that might be at issue is the lung, which was the most

sensitive site in both male and female mice. Lung cancer is fairly common in humans; this reduces the power of an epidemiology study to detect an increase in lung cancer. A crude "power" calculation is presented below to estimate the lung cancer results one might expect to have observed in the epidemiology study if humans are as sensitive as female mice to the lung cancer effects of 1,3-butadiene. The calculation is based on the average employment and exposure characteristics of the Delzell et al. cohort, exposure estimates and number of subjects available for 6 of the 8 plants, and the MLE of lung cancer risk for the female mouse (i.e., 0.1/ppm). It is also assumed that there is no confounding by smoking and that equivalent excess risks occur from equivalent cumulative exposures, with no latency. The epidemiology data are for lung cancer mortality, whereas the mouse unit risk estimate is for excess lung cancer incidence. However, lung cancer has a low survival rate, so this discrepancy should not substantially impact the calculation.

10.1.3.1 *Power Calculation for Lung Cancer for the Delzell et al. Cohort*

SBR + related activities: 15649 subjects from 8 plants

median years worked = 7.8

average person-years/subject = 24.7

59% started working before age 30

1. Calculate average cumulative exposure (ppm*years) for the 14,295 subjects in the six plants with exposure estimates (Delzell et al., 1995, Table 58).

Median cumulative exposure for 12,412 subjects with nonzero exposure = 11.3 ppm*years.

$$\frac{12412}{14295} \times (11.3) = (0.87) \times 11.3 = 9.8\, ppm * years$$

2. Calculate continuous partial lifetime exposure for period of follow-up.

$$\frac{9.8\, ppm * years}{7.8\, years} \times \frac{7.8\, years}{24.7\, years} \times \frac{240\, days}{365\, days} \times \frac{10 m^3 / day}{20 m^3 / day} = 0.130\, ppm$$

3. Calculate MLE of risk for partial human lifetime based on female mouse lung cancer data.

from TOXRISK: EC_{10} = 7.1918 ppm

for human lifespan = 70 years

exposure starting at age 30 years

exposure ending at age 55 years (i.e., 30 + 24.7)

risk at age 55

$$\frac{0.1}{7.1918\,ppm} = 0.014\,ppm^{-1}$$

4. Calculate partial lifetime individual excess risk per person.

$$0.130\,ppm \times 0.014\,ppm^{-1} = 0.00181$$

5. Calculate expected number of excess lung cancer cases.

$$14295 \times 0.00181 = 26$$

Background expected lung cancer cases from the six plants = 312 (314 observed)

6. The statistical power to detect a predicted RR of 338/312 = 1.083 is given by Beaumont and Breslow (1981) as:

$$Z_{1-\beta} = Z_{\alpha} - 2\left(\sqrt{RR} - 1\right)\sqrt{Expected}$$

$$Z_{1-\beta} = 1.645 - 2\left(\sqrt{1.083} - 1\right)\sqrt{312} = 0.208\,.$$

From cumulative normal frequency distribution tables, Z = 0.208 corresponds with A = 0.08.

From Snedecor and Cochrane (1989), probability of a value > Z (Z negative) = 0.5 + A

(Z positive) = 0.5 − A

A = 0.08 and Z positive. Therefore, power = 0.5 - 0.08 = 0.42 = 42%

i.e., the power to detect a statistically significant increase in lung cancer if true SMR = 108 is 42%.

Thus, according to the above calculation, one might have expected to see 26 *excess* lung cancer cases in the epidemiology study. In fact, only 2 excess lung cancer cases were observed in the workers from the 6 plants over a background of 312 expected cases. However, as calculated above, the study has low statistical power to detect such a small proportional excess (power to detect a statistically significant increase in risk if the true SMR = 338/312 = 108 is estimated to be 42%).

10.1.4. Summary and Conclusions

Risk estimates for environmental exposures to 1,3-butadiene are derived from an analysis by Health Canada (1998) of the Delzell et al. (1995) occupational retrospective cohort mortality study of approximately 16,000 workers in 6 North American styrene-butadiene rubber manufacturing plants. The study results reflect follow-up from 1943 through 1991, with an average follow-up of 25 years, and with about 25% of the cohort deceased. Although overall mortality and all cancer mortality were below expected values based on general population regional rates, the increase in leukemias was statistically significant (SMR = 1.43, 95% CI = 1.04-1.91) for all ever-hourly men (Delzell et al., 1996). The consistency of this leukemia result across plants and with findings from previous epidemiology studies of 1,3-butadiene plus other data led to the conclusion that this observed increase was caused by 1,3-butadiene. The availability of high-quality epidemiologic data based on individual worker exposure estimates and the significant exposure-response results for 1,3-butadiene observed by Delzell et al. motivated the decision to base a quantitative cancer risk assessment on this database.

Although this cohort had been previously studied (Matanoski et al., 1989, 1990; Matanoski and Schwartz, 1987; Matanoski and Santos-Burgoa, 1994), the Delzell et al. update and analyses are noteworthy for their extensive work on exposure estimation based on detailed reviews of individual job histories and a job exposure matrix (Delzell et al., 1995; Macaluso et al., 1996). The careful work on exposure allowed better estimates of risk and exposure response. Exposure metrics included cumulative ppm-years and years multiplied by annual number of peak exposures of at least 100 ppm for at least 15 minutes. Additional individual worker exposure information on both styrene and benzene allowed analyses to be adjusted for these potential confounding exposures. The Delzell et al. (1995) report includes these analyses.

The Delzell et al. study used Poisson regression analysis with nine categories of cumulative exposure for 1,3-butadiene and nine categories of exposure for styrene. The analysis also included, as covariates, adjustments for age, race, calendar year, and years since hire. Benzene was ruled out as a potential confounder and was not included in the relative rate models. Relative rate models within the Poisson regression analyses included the (a) log-linear, (b) power, (c) linear, and (d) square root models. The parameter representing cumulative 1,3-

butadiene exposure was found to be statistically significant in all the models evaluated, and all models fit the data adequately in the observable range. The cumulative styrene exposure parameter was positive for all the models, but not statistically significant. Results with the peak exposures metric were irregular and are not given further consideration here.

Subsequently, Health Canada obtained the data on this cohort from Delzell and performed additional analyses of cumulative 1,3-butadiene exposure (Health Canada, 1998). Their approach was similar to that of Delzell et al. (1995), but several refinements made by Health Canada are noteworthy. These include finer stratification of age, calendar year, and years since hire, as well as use of the actual mean cumulative exposure for the highest exposure group. The Health Canada report fit the same models as Delzell et al., with the exception that the model with the square root of cumulative exposure was replaced with one that included cumulative exposure raised to the power α, which was estimated.

The quantitative risk analysis presented here uses the results of the Health Canada analyses to extrapolate risk from occupational work-time exposure to environmental continuous exposure. This was simply done by calculating the cumulative exposure to 1,3-butadiene for continuous instead of intermittent occupational conditions to obtain the relative rate of leukemia mortality predicted for each year up to age 85. These predictions, together with U.S. population rates for leukemia and all-causes mortality, were used to estimate excess risk up to age 85 using an actuarial method that adjusted for competing risks. This does not mean that an average lifetime of 85 years was assumed, rather the methodology uses actual age-specific survival rate data, up to age 85. These techniques have been used before by EPA as well as other government agencies.

After calculation of the exposure-response relationship, the low-exposure extrapolation is done in two ways reflecting the different approaches used in EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986) and in the proposed guidelines (U.S. EPA, 1996, 1999), which, as of this writing, are undergoing revision based on public comments and comments from review by an EPA Science Advisory Board panel. Although neither version of the guidelines provides specific guidance for modeling human data, the guidelines differ in their general approach to low-dose extrapolation. EPA has generally used a linear model to fit human data, and, under the 1986 Guidelines, the risk estimates are calculated as a potency or slope factor derived from applying the same linear model to lower exposure concentrations. Under the proposed guidelines, the risk estimates are obtained by first calculating a "point of departure" at the low end of the range of observation, using any of the appropriate models, and then extrapolating to 0 by means of a straight line (linear extrapolation default). The LED_{10} (i.e., 95% lower confidence limit on a dose associated with 10% extra risk) is proposed as the standard

point of departure. However, the LEC_{01} is used here because 1% is within the observable range of increased leukemia deaths for the different 1,3-butadiene exposure groups in the Delzell et al. study, and, in fact, a 10% extra risk level would require *upward* extrapolation. Furthermore, exposure levels are expressed as exposure concentrations rather than doses; thus an LEC is used rather than an LED.

The results of the extrapolations using the four relative rate models were listed in Tables 10-2 through 10-4 and presented in Figures 10-2 to 10-5. The results show that in the observable excess risk range of 1%, the MLEs of continuous exposure (EC_{01}) vary about 15-fold from 0.11 to 1.6 ppm, and the LEC_{01} estimates range from 0.037 to 0.87, or about 24-fold. As the extrapolation extends 10-fold to a 0.001 excess risk level, the MLEs of exposure from the various models diverge much more, almost a 700-fold range from 0.00035 ppm to 0.24 ppm. At the 10^{-6} risk level, the MLEs of exposure diverge by more than seven orders of magnitude. Clearly, the final risk estimates based on the 1986 guidelines' extrapolation procedures are highly dependent on the choice of model.

As mentioned above, under the 1986 guidelines approach, the default model of choice for human data has been the linear relative rate model. This choice is based more on historical precedence and biological plausibility arguments than on statistical fit or conservatism. In fact, for a 10^{-6} risk level, using the MLE from the linear model for 1,3-butadiene would be much less protective of public health than would be the MLE from either the shape or power models. In addition, EPA has historically used MLEs for cancer risk estimates from human data rather than upper bounds as used with animal data. Using this approach with the Delzell et al. leukemia mortality data, the linear model yields a maximum likelihood potency (slope) estimate of 1.3×10^{-2} /ppm.

Using the linear extrapolation default approach under the 1996 and 1999 proposed guidelines, the 1% excess risk level is chosen for determining the point of departure because it is within the observable response range of leukemia deaths, and 10% is actually above the observable range. At the 1% level, the different models provide MLE exposure estimates (i.e., EC_{01}) ranging from 0.11 to 1.6 ppm, and the 95% LCLs (i.e., LEC_{01}) on exposure range from 0.037 to 0.87 ppm. Without specific guidance for choice of model from the proposed guidelines, potency estimates based on linear extrapolation from points of departure from each of the models examined by Health Canada (1998) are presented in Table 10-5. The linear extrapolation default is used based on the known genotoxicity of 1,3-butadiene, via its metabolites.

The cancer potency estimates, using EC_{01}s as the point of departure, range from 6.3×10^{-3}/ppm (log-linear model) to 9.1×10^{-2}/ppm (shape model). The cancer potency estimates, based on the available LEC_{01}s from three models, range from 0.011/ppm (log-linear) to 0.27/ppm (power), with the linear model yielding 0.027/ppm.

Of the various estimates presented in Table 10-5, the potency estimate of 0.027/ppm was selected to represent the excess leukemia mortality risk from the occupational data. Use of the 95% lower bound reflects some of the uncertainties inherent in the epidemiologic data, as well as the policy position expressed in the 1996 and 1999 proposed guidelines. In addition, although various models adequately fit the observed data, there is no compelling reason to deviate from the linear model historically used for human data.

However, as discussed in Section 10.1.3 above, we actually wish to estimate excess risk of cancer incidence, not mortality. Therefore, another calculation was done using the same linear relative rate model and age-specific leukemia incidence rates for 1994-1998 (NCI, 2001; Table XIII-2: all leukemias, all races, males & females) from SEER (Surveillance, Epidemiology and End Results program of the National Cancer Institute) in place of the leukemia mortality rates in the actuarial program. This calculation assumes that leukemia incidence and mortality have the same exposure-response relationship for 1,3-butadiene exposure and that the incidence data are for first occurences of leukemia or that relapses provide a negligible contribution. The calculation also relies upon the fact that the leukemia incidence rates are small compared to the all-cause mortality rates. The resulting 1,3-butadiene exposure concentration estimates for an excess risk of 1% are an EC_{01} of 0.528 ppm and an LEC_{01} of 0.254 ppm. Linear extrapolation from the LEC_{01} (i.e., $0.01/LEC_{01}$) yields a unit risk estimate of 0.04/ppm for leukemia incidence.

An adjustment factor of 2 was then applied to this unit risk estimate to reflect evidence from rodent bioassays suggesting that extrapolating the excess risk of leukemia in a male-only occupational cohort may underestimate the total cancer risk from 1,3-butadiene exposure in the general population. First, studies in both rats and mice indicate that 1,3-butadiene is a multisite carcinogen. It is possible that humans exposed to 1,3-butadiene may also be at risk of cancers other than leukemia and that the epidemiologic study had insufficient power to detect excess risks at other sites (see below). Second, both the rat and mouse studies suggest that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males, and the female mammary gland was the only 1,3-butadiene-related tumor site common to both species.

The mammary tumor unit risk estimated from the female mouse (most sensitive species) data is just slightly lower (maximum likelihood estimate [MLE] = 0.02/ppm, 95% upper confidence limit [UCL] = 0.03/ppm) than the human (male) leukemia risk (0.04/ppm based on linear extrapolation from the LEC_{01} for leukemia incidence). Thus, EPA decided to apply an adjustment factor of 2 to the leukemia risk estimate, resulting in a unit risk estimate of 0.08/ppm intended to cover the combined risks for leukemia and mammary cancer and to provide additional protection to account for the fact that small increases in risk at other sites, particularly the lung, cannot be ruled out.

Table 10-5. Cancer potency (unit risk) estimates for leukemia mortality based on linear extrapolation from the LEC_{01} or EC_{01} calculated from the models presented by Health Canada (1998).

Model	EC_{01} (ppm)	Potency estimate (ppm^{-1}) ($0.01/EC_{01}$)	LEC_{01} (ppm)	Potency estimate (ppm^{-1}) ($0.01/LEC_{01}$)
Log-linear	1.6	6.3×10^{-3}	0.87	1.1×10^{-2}
Power	0.13	7.7×10^{-2}	.037	2.7×10^{-1}
Linear	0.78	1.3×10^{-2}	0.375	2.7×10^{-2}
Shape	0.11	9.1×10^{-2}	NA	NA

The Delzell et al. study was a large cohort study (over 15,000 subjects) with a long follow-up time (up to 49 years; 25% of the subjects had died by the end of the follow-up), so for most tumor sites there should be sufficient power to detect an increased risk. The main tumor site that might be at issue is the lung, which was the most sensitive site in both male and female mice. Lung cancer is fairly common in humans; therefore, the epidemiology study may have lacked the statistical power to detect an increase in lung cancer. The crude "power" calculation presented in Section 10.1.3.1 suggested that if humans were as sensitive as female mice to the lung cancer effects of 1,3-butadiene, one might have expected to see 26 *excess* lung cancer cases in the epidemiology study. In fact, only 2 excess lung cancer cases were observed in the workers from the 6 plants over a background of 312 expected cases. However, the study has low statistical power to detect such a small proportional excess (power to detect a statistically significant increase in risk if the true SMR = 338/312 = 108 is estimated to be 42% according to the method of Beaumont and Breslow [1981] as presented in Section 10.1.3.1), and a lung cancer SMR of 107 (319 observed/297 expected) was observed for the ever hourly workers for the 8 plants (although there was no increased lung cancer risk in the overall cohort [SMR=101] or in the subgroup of ever hourly workers with $\geq$10 years worked and $\geq$20 years since hire [SMR=100]).

The only process group associated with an increased lung cancer SMR was maintenance (SMR = 141 observed/114 expected = 124). However, 7 mesotheliomas were also observed in maintenance workers (9 among ever hourly workers in the total cohort), suggesting that these workers may have been exposed to asbestos, a known lung carcinogen. Furthermore, the evidence for the association between the increased lung cancers in the maintenance workers and 1,3-butadiene exposure is weakened by the fact that lung cancers are not increased in other

process groups which exhibited increases in leukemia cases (e.g., 1,3-butadiene production), the absence of a positive relationship with number of years worked, the absence of a trend with increasing years since hire, and the fact that the increase was attenuated when state, rather than national, lung cancer rates were used for comparison (Sathiakumar et al., 1998). Thus, the overall evidence of an association between lung cancer and 1,3-butadiene exposure is tenuous.

On the other hand, because the background rate of lung cancer is high, the power of the study to detect small increases in lung cancer risk is low, and, without adjusting for amount of smoking, it is difficult to make firm conclusions. Workers are not allowed to smoke in the plants because of the explosive potential of 1,3-butadiene; therefore, the workers may have had lower cigarette consumption, and this could easily mask a small increase in lung cancer risk. Thus, while the study does not provide good evidence of an association between lung cancer and 1,3-butadiene exposure, one cannot rule out a small increase in risk.

Some increases were also observed for laryngeal cancer in the Delzell et al. study, however, these are based on small numbers (for the overall cohort: 17 observed, 15 expected). On the other hand, the increases are associated with process groups in which excess leukemias are observed. No data are provided for duration of exposure or other exposure characteristics. Thus, while the evidence for an association between laryngeal cancer and 1,3-butadiene exposure is meager, a small increase in laryngeal cancer cannot be ruled out.

In conclusion, a lifetime excess cancer unit risk estimate of 0.08/ppm for the general population was obtained by applying an adjustment factor of 2 to the unit risk estimate of 0.04/ppm for leukemia incidence which was derived from the Delzell et al. male worker cohort data. The adjustment factor of 2 is intended to cover the potential risk of female mammary cancer, which was observed in both mice and rats, and to provide further protection to account for the fact that some increases in risk at other sites–particularly the lung, which was the most sensitive site in female and male mice–cannot be ruled out.

10.2. CANCER RISK ESTIMATES BASED ON RODENT BIOASSAYS

10.2.1. Rat-Based Estimates

Cancer risk estimates based on the 1981 Hazleton rat inhalation study of 1,3-butadiene were presented in EPA's 1985 1,3-butadiene risk assessment (U.S. EPA, 1985). 95% upper-limit incremental lifetime unit cancer risk estimates for humans were calculated using the linearized multistage (LMS) model, after estimating the equivalent human dose assuming 1,3-butadiene retention based on results of a 1985 NTP absorption study (NTP, 1985; see EPA's 1985 report for further details). The upper limit based on the male rat tumor incidence data for Leydig cell tumors, pancreatic exocrine tumors, and/or Zymbal gland carcinomas was 4.2×10^{-3} per ppm 1,3-butadiene exposure. The upper limit based on the female rat tumor incidence data for mammary

gland carcinomas, thyroid follicular tumors, and/or Zymbal gland carcinomas was 5.6×10^{-2} per ppm 1,3-butadiene exposure.

These rat-based estimates are not considered the most appropriate estimates of human risk; they are merely presented for comparison purposes. EPA believes that the mouse is likely to represent a better rodent model for human cancer risk from 1,3-butadiene (see below) and that the cancer risk estimates derived from the epidemiologic data are the best available estimates for human risk.

10.2.2. Mouse-Based Estimates

Cancer risk estimates based on the 1984 NTP mouse inhalation study were presented in EPA's 1985 1,3-butadiene risk assessment; however, revisions to these estimates are warranted because of the new data provided by the 1993 NTP mouse inhalation bioassay, which examined cancer response from exposure to lower 1,3-butadiene concentrations than those used in the 1984 study (NTP 1984, 1993; see Chapter 6). Groups of male and female $B6C3F_1$ mice were exposed to 1,3-butadiene concentrations of 0, 6.25, 20, 62.5, 200, or 625 ppm 1,3-butadiene for 6 h/day, 5 days/week, for up to 104 weeks. Significant increases in tumor incidence were observed at multiple sites: the hematopoietic system (lymphomas, histiocytic sarcomas), heart (hemangiosarcomas), lung, forestomach, Harderian gland, liver, preputial gland (males), ovary (females), and mammary gland (females), when adjusted for intercurrent mortality. Significant increases in lung cancer incidence were observed in female mice at 1,3-butadiene exposure levels down to 6.25 ppm, the lowest level tested.

10.2.2.1. *Quantal*

When EPA estimates cancer risks for humans from rodent bioassay data, the risk estimates are generally calculated from the incidence of rodents of the most sensitive species, strain, and sex bearing tumors at any of the sites displaying treatment-attributable increases. In the case of 1,3-butadiene, so many sites demonstrated significant tumor increases attributable to 1,3-butadiene that background levels of tumor-bearing animals obfuscate the effects of 1,3-butadiene when all these tumor sites are combined. Therefore, risk estimates were derived from the incidence of female (most sensitive sex) mice with malignant lymphomas, heart hemangiosarcomas, lung tumors (alveolar/bronchiolar adenomas or carcinomas), mammary gland tumors (carcinomas, adenocanthomas, or malignant mixed tumors), or benign or malignant ovary granulosa cell tumors (Table 10-6). These sites were considered the most responsive sites with low background tumor incidence. Most of the impact on the low-dose linear extrapolation is from the lung tumor response, because the lung tumor incidences show the largest increases at the lowest exposures. The 625 ppm exposure group was not included in the dose-response

analysis because all of the mice were dead by week 65, and the tumor response was already virtually saturated in the 200 ppm exposure group. Note also that mice that died before the time of observation of the first tumor were considered to be not at risk and were excluded from the incidence denominators.

Table 10-6. Dose-response data for linearized multistage model.

Administered exposure (ppm)	**Control**	**6.25**	**20**	**62.5**	**200**
Human equivalent exposure (ppm)	0	1.1	3.6	11	36
Number of mice with tumors[a] /Number of mice at risk[b]	6/50	19/49	26/50	31/50	46/49

[a]Lymphocytic lymphomas, heart hemangiosarcomas, alveolar/bronchiolar adenomas or carcinomas, mammary gland tumors (carcinomas, adenocanthomas, malignant mixed tumors), or benign or malignant ovary granulosa cell tumors.
[b]Female mice surviving to the time of the first significant tumor, which was a lymphocytic lymphoma at day 203.

Human equivalent exposures were based on ppm 1,3-butadiene exposure, adjusted for continuous daily exposure (e.g., 6.25 ppm × 6/24 × 5/7 = 1.12 ppm). No attempt was made to adjust for internal doses of reactive 1,3-butadiene metabolites because the PBPK data were inadequate to develop reliable PBPK models (Chapter 9). No adjustments were made for 1,3-butadiene absorption because there are no adequate human data. Furthermore, there is no reason to expect nonlinearities in absorption at the lowest exposures (at least < 625 ppm). A 95% upper-limit incremental lifetime unit cancer risk (extra risk) for humans was calculated from the incidence data in Table 10-6 using the LMS model. The multistage model has the following form:

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)],$$

where P(d) represents the lifetime risk (probability) of cancer at dose d (human equivalent exposure concentration, in this case), and parameters $q_i \geq 0$, for I = 0,1,...,k.

Extra risk over the background tumor rate is defined as

$$[P(d) - P(0)] / [1 - P(0)].$$

Point estimates of the dose coefficients (q_is), and consequently the extra risk function, at any dose d, are calculated by maximizing the likelihood function with respect to tumor incidence data. The incremental lifetime unit cancer risk for humans (q_1^*) is defined as the 95% UCL on the parameter q_1, which is the linear dose coefficient, for extra risk. This 95% UCL represents a plausible upper bound for the true risk. The 95% UCL was calculated using the computer program GLOBAL86 (Howe and Van Landingham, 1986). Both the model and the curve-fitting methodology used are described in detail by Anderson et al. (1983).

The tumor incidence data in Table 10-6 generated the following results using the LMS model (GLOBAL86):

MLEs of dose coefficients:

$q_0 = 0.2629$

$q_1 = 0.07643$

$q_2 = 0.0$

$q_3 = 0.0$

$q_4 = 0.0$

p-value for chi-square goodness of fit > 0.01

$q_1^* = 0.10$

MLE of risk for an exposure concentration of 1 ppm = 7.4×10^{-2}

MLE of risk for an exposure concentration of 1 ppb = 7.6×10^{-5}

MLE of exposure concentration for a risk of 0.10 (EC_{10}) = 1.4 ppm

95% UCL on exposure concentration for a risk of 0.10 (LEC_{10}) = 1.0 ppm

Thus, the incremental unit cancer risk estimate (95% UCL) for humans calculated from the mouse 1993 NTP inhalation bioassay results is 0.10 per ppm for continuous lifetime inhalation exposure to 1,3-butadiene. The MLE of risk appears to be nearly linear between 1 ppm and 1 ppb and is about 0.075 per ppm 1,3-butadiene exposure.

Under EPA's proposed new cancer risk assessment guidelines (U.S. EPA, 1996, 1999), unit cancer risk estimates for genotoxic chemicals, such as 1,3-butadiene, would be derived by straight linear extrapolation to 0 from the LED_{10} (estimated 95% UCL on the dose corresponding to a 10% extra cancer risk). Using the LEC_{10} generated for the LMS model by GLOBAL86 yields a unit cancer risk of 0.10/1.0 ppm = 0.10 per ppm, the same as the q_1^*. Using the EC_{10} yields $0.10/1.4 = 7.1 \times 10^{-2}$ per ppm.

The unit cancer risk estimate (95% UCL) derived above is intended to depict a plausible upper limit on the risk of developing any 1,3-butadiene-attributable tumor over a full (70-year) lifetime. However, using the quantal incidence data for total tumor-bearing mice in each exposure group does not fully characterize the cancer potency reflected by the mouse bioassay results. First, the methodology does not account for the fact that many of the mice in the higher exposure groups had tumors at multiple significant sites. Second, the methodology ignores the fact that survival was significantly decreased in female mice exposed to 20 ppm or more 1,3-butadiene as a result of fatal 1,3-butadiene-attributable tumors. Time-to-tumor analyses conducted for specific tumor sites are presented below and can be used to evaluate the time component of the cancer risk.

10.2.2.2. *Time-to-Tumor*

The mouse inhalation bioassay results demonstrate different dose-response relationships for different tumor sites. To assess the characteristics of the dose-response relationships for different tumor sites, time-to-tumor analyses were performed to adjust for competing mortality from cancer at other sites.

Time-to-tumor analyses were conducted from the individual mice data, including the 9-month and 15-month interim sacrifice data, for sites demonstrating increased cancer incidence. Benign and malignant tumors were combined for sites where appropriate. Thus time-to-tumor analyses were performed for lung alveolar/bronchiolar adenomas or carcinomas; lymphocytic lymphomas; histiocytic sarcomas; heart hemangiosarcomas; hepatocellular adenomas or carcinomas; Harderian gland adenomas or carcinomas; forestomach squamous cell papillomas or carcinomas; malignant or benign ovary granulosa cell tumors (female); and mammary gland adenocanthomas, carcinomas, or malignant mixed tumors (female). Preputial gland carcinomas in male mice were not analyzed because not all the tissues were examined microscopically.

Data from the 625 ppm exposure groups were excluded from analysis because of excessive early mortality, as in the quantal analysis discussed above. In addition, data from interim sacrifices for specific sites were excluded for exposure groups for which it appeared that complete histopathologic examination for that site was not performed on the entire interim sacrifice group.

Human equivalent exposures were based on ppm 1,3-butadiene exposure, adjusted for continuous daily exposure, as described above.

The general model used for the time-to-tumor (or time-to-response) analyses was the multistage Weibull model, which has the form

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)*(t - t_0)^z],$$

where P(d,t) represents the probability of a tumor (or other response) by age t (in bioassay weeks) for dose d (i.e., human equivalent exposure), and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for I = 0,1,...,k, where k = the number of dose groups − 1. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death (see below). The analyses were conducted using the computer software TOX_RISK version 3.5 (Crump et al., ICF Kaiser International, Ruston, LA), which is based on Weibull models taken from Krewski et al. (1983). Parameters are estimated using the maximum likelihood method.

Specific n–stage Weibull models were selected for the individual tumor types for each sex based on the values of the log likelihoods according to the strategy used by NIOSH (1991a). If twice the difference in log likelihoods was less than a chi-square with degrees of freedom equal to the difference in the number of stages included in the models being compared, then the models were considered comparable and the most parsimonious model (i.e., the lowest-stage model) was selected.

Tumor types were categorized by tumor context as either fatal or incidental tumors. Incidental tumors are those tumors thought not to have caused the death of an animal, and fatal tumors are thought to have resulted in animal death. Lymphocytic lymphomas, histiocytic sarcomas, and heart hemangiosarcomas were treated as fatal tumors, unless observed at an interim or terminal sacrifice, in which case they were considered incidental. Furthermore, these fatal tumors were deemed rapidly fatal, and t_0 was set equal to 0 (the data were believed insufficient to reliably estimate t_0 in any event). Tumors at all other sites were treated as incidental. This is basically the same determination as that made by NIOSH (1991a), except the NIOSH report dealt with preliminary data that did not distinguish histiocytic sarcomas from lymphomas. NIOSH further cited the work of Portier et al. (1986) analyzing tumor types in NTP historical controls to lend support to these tumor context assumptions.

Parameter estimates for the time-to-tumor analyses for each tumor type are presented in Tables 10-7 (based on female mouse data) and 10-8 (male mouse data). For all tumor types except the heart hemangiosarcomas (both sexes) and the forestomach (male mouse), the one-stage Weibull model was preferred. For male mice, the heart hemangiosarcomas and forestomach tumors were best described by the two-stage model, and for female mouse heart hemangiosarcomas, a three-stage model was preferred.

Table 10-7. Parameter estimates for multistage Weibull time-to-tumor model based on female mouse tumor incidence, without 625 ppm group.

Tissue	Q0	Q1	Q2	Q3	Z
Lymphocytic lymphoma	6.23×10^{-10}	1.67×10^{-10}	—	—	3.92
Histiocytic sarcoma	3.68×10^{-14}	1.23×10^{-14}	—	—	6.03
Heart hemangiosarcoma	0	0	0	2.88×10^{-17}	6.10
Lung	5.83×10^{-9}	3.40×10^{-9}	—	—	3.69
Liver	2.12×10^{-8}	2.11×10^{-9}	—	—	3.58
Forestomach	0	1.29×10^{-9}	—	—	3.43
Harderian gland	1.50×10^{-5}	2.06×10^{-6}	—	—	2.03
Mammary	2.47×10^{-6}	5.42×10^{-5}	—	—	1.27
Ovary	7.83×10^{-9}	1.48×10^{-8}	—	—	3.05

Table 10-8. Parameter estimates for multistage Weibull time-to-tumor model based on male mouse tumor incidence, without 625 ppm group.

Tissue	Q0	Q1	Q2	Z
Lymphocytic lymphoma	1.84×10^{-8}	1.28×10^{-9}	—	3.08
Histiocytic sarcoma	0.0	1.04×10^{-13}	—	5.50
Heart hemangiosarcoma	0.0	0.0	1.14×10^{-23}	10.0
Lung	1.38×10^{-7}	9.53×10^{-8}	—	3.27
Liver	1.40×10^{-4}	5.57×10^{-6}	—	1.83
Forestomach	9.68×10^{-10}	0.0	3.83×10^{-11}	3.39
Harderian gland	1.65×10^{-7}	7.45×10^{-8}	—	2.90

Human unit cancer risk (or potency) estimate results (extra risk) are presented in Tables 10-9 (based on female mouse data) and 10-10 (male mouse data). Mouse lung tumors convey the greatest amount of extrapolated risk to humans from both the female mouse data (q_1* = 0.14/ppm 1,3-butadiene exposure) and the male mouse data (q_1* = 0.10/ppm). Note that the unit risk estimate of 0.14/ppm generated from the female mouse lung tumor data using a time-to-tumor model is greater than the unit risk estimate of 0.10/ppm generated above from female mice bearing tumors at any of multiple sites when only the quantal data were used and decreased survival time was not taken into account.

Although the time-to-tumor modeling does help account for decreased survival times in the mice, considering the tumor sites individually does not convey the total amount of risk potentially arising from the sensitivity of multiple sites. To get some indication of the total unit risk from multiple tumor sites, assuming the multiple sites are mechanistically independent, the MLEs of the unit potency from the Weibull time-to-tumor models were summed across tumor sites, and estimates of the 95% upper bound on the summed unit potency were calculated. The TOX_RISK software provides MLEs and 95% UCLs for human risk at various exposure levels, allowing for calculation of unit potency estimates at those exposure levels.

When the MLEs of unit potency calculated at 1 ppb from the female mouse data were summed across the female mouse tumor sites, the MLE of the total unit risk was 0.23/ppm continuous lifetime 1,3-butadiene exposure. A 95% upper bound for the total potency was calculated by assuming a normal distribution for the risk estimates, deriving the variance of the risk estimate for each tumor site from its 95% UCL according to the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645\sigma,$$

where the standard deviation (SD) σ is the square root of the variance, summing the variances across tumor sites to obtain the variance of the sum of the MLEs, and calculating the 95% UCL on the sum from the variance of the sum using the same formula. The resulting 95% UCL on the unit potency for the total unit risk was 0.38/ppm. In comparison, summing the q_1*s across the female mouse tumor sites yielded 0.50/ppm.

The unit potencies were also summed using a Monte Carlo analysis and the software Crystal Ball version 4.0 (Decisioneering, Denver, CO). Normal distributions were assumed for the unit potency for each tumor site, with the mean equal to the MLE and σ as calculated from the above formula. A distribution around the sum of the MLEs was then generated by simulating the sum of unit potencies picked from the distributions for each tumor site (according to probabilities determined by those distributions) 10,000 times. The mean for the sum and the

Table 10-9. Human unit cancer risk estimates (extra risk, computed for risks of 10^{-6}) based on female mouse tumor incidences, without 625 ppm group, using multistage Weibull time-to-tumor model.

Tissue	Q1* (ppm^{-1})	MLE (ppm^{-1})	EC_{10} (ppm)	LEC_{10} (ppm)	$0.1/LEC_{10}$ (ppm^{-1})
Lymphocytic lymphoma	0.0239	0.0128	8.08	4.33	0.0231
Histiocytic sarcoma	0.1283	3.36×10^{-3}	30.8	0.806	0.1241
Heart hemangiosarcoma	4.27×10^{-3}	3.99×10^{-6}	11.6	9.24	0.0108
Lung	0.1404	0.0980	1.06	0.737	0.1357
Liver	0.0631	0.0366	2.82	1.64	0.0610
Forestomach	0.0215	0.0112	9.22	4.80	0.0208
Harderian gland	0.0443	0.0258	4.00	2.33	0.0429
Mammary	0.0321	0.0203	5.09	3.23	0.0310
Ovary	0.0358	0.0218	4.74	2.89	0.0346

Table 10-10. Human unit cancer risk estimates (extra risk, computed for risks of 10^{-6}) based on male mouse tumor incidences, without 625 ppm group, using multistage Weibull time-to-tumor model.

Tissue	Q1* (ppm^{-1})	MLE (ppm^{-1})	EC_{10} (ppm)	LEC_{10} (ppm)	$0.1/LEC_{10}$ (ppm^{-1})
Lymphocytic lymphoma	6.437×10^{-3}	2.220×10^{-3}	46.6	16.1	6.224×10^{-3}
Histiocytic sarcoma	0.02162	0.01394	7.42	4.78	0.02090
Heart hemangiosarcoma	0.01266	4.040×10^{-3}	12.0	7.59	0.01318
Lung	0.1023	0.06998	1.48	1.01	0.09890
Liver	0.04447	0.02720	3.80	2.33	0.04300
Forestomach	4.258×10^{-3}	1.660×10^{-5}	19.2	13.3	7.517×10^{-3}
Harderian gland	0.07402	0.05398	1.92	1.40	0.07157

95th percentile on the distribution were the same as the sum of MLEs and 95% UCL calculated above, as they should be. However, a sensitivity analysis based on the contribution to variance revealed that variability associated with the unit potency estimate for the histiocytic sarcomas was contributing more than 83% of the variance on the sum, and some of the simulated sums were negative (the distributions for the unit potency estimates were not constrained for the summation analyses). Excluding the histiocytic sarcomas yielded the same MLE of total risk of 0.23/ppm; however, the 95% UCL decreased to 0.29/ppm. The lung, which then contributes the most to the sum, contributed about 55% of the variance, followed by the liver with 20%, and no simulated sums were negative.

The same analyses were performed summing the estimates of unit potency derived from the male mouse data for the different tumor sites. The resulting MLE for the total unit risk was 0.18/ppm lifetime 1,3-butadiene exposure with a 95% UCL of 0.22/ppm. The lung contributed about 56% to the variance, followed by the Harderian gland with about 20%. Histiocytic sarcomas contributed only 3% in this case, and all simulated sums were positive.

Finally, the summation analyses were repeated for unit potency estimates calculated at 1 ppm exposure for comparison with the estimates calculated at 1 ppb. For the female mouse-based risks (excluding histiocytic sarcomas), the sum of the MLEs was 0.22/ppm (2% lower than at 1 ppb) and the 95% UCL on the sum was 0.28/ppm (4% lower than at 1 ppb). Thus, the total unit potency estimates are reasonably linear up to 1 ppm continuous lifetime exposure. Recall from Table 10-7 that the selected model for the heart hemangiosarcomas in the female mouse was nonlinear; however, the unit risk estimates based on the heart hemangiosarcomas at these extrapolated doses are lower than for the other sites and do not affect the total risk summed across tumor sites. Similarly, the results based on the male mouse (both the sum of the MLEs and the 95% UCL on the sum) calculated at 1 ppm were 2% lower than those calculated at 1 ppb. For the male mouse, the selected models for both the heart hemangiosarcomas and the forestomach tumors were nonlinear (Table 10-8); however, as with the female heart hemangiosarcomas, the risks from these sites have little impact on the total risk.

The results of these summation analyses are summarized in Table 10-11.

10.2.3. Discussion

Based on the analyses discussed above, the best estimate for an upper bound on human extra cancer risk from continuous lifetime exposure to 1,3-butadiene derived from animal data is about 0.3/ppm. This estimate reflects the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites (excluding histiocytic sarcomas) in the most

Table 10-11. Unit potency estimates (extra risk) summed across tumor sites.

Tumor Sites	Sum of MLEs (ppm^{-1})	95% UCL on sum (ppm^{-1})	Sum of q_1*s (ppm^{-1})
Female mouse tumor sites (calculated at 1 ppb)	0.23	0.38	0.50
Female sites excluding histiocytic sarcomas (at 1 ppb)	0.23	0.29	0.37
Female sites excluding histiocytic sarcomas (at 1 ppm)	0.22	0.28	0.36
Male mouse tumor sites (at 1 ppb)	0.18	0.22	0.27
Male mouse tumor sites (at 1 ppm)	0.17	0.21	0.26

sensitive species and sex (the female mouse). Histiocytic sarcomas were excluded because they introduced excessive variance into the upper bound and contributed only negligibly to the MLE of total unit risk.

The greatest source of uncertainty in this estimate is from the interspecies extrapolation of risk from mouse to human. The two rodent species for which bioassay data were available—mouse and rat—vary significantly in their carcinogenic responses to 1,3-butadiene, in terms of both site specificity and magnitude of response (Chapter 6). The mouse and rat also exhibit substantial quantitative differences in their metabolism of 1,3-butadiene to potentially reactive metabolites (Chapter 3). Unfortunately, existing pharmacokinetic models cannot yet adequately explain the species differences in carcinogenic response (Chapter 9), and it is possible that there are pharmacodynamic as well as pharmacokinetic differences between the mouse and rat with respect to their sensitivities to 1,3-butadiene.

The mouse was the more sensitive species to the carcinogenic effects of 1,3-butadiene exposure and, hence, the more conservative (i.e., public health protective) for extrapolation of risk to humans. In addition, the mouse appears to be the more relevant species for extrapolation to humans in terms of site specificity, in that 1,3-butadiene induces tumors of the lymphohematopoietic system in both mice and humans. Melnick and Kohn (1995) further

suggest that the genetic mutations observed in 1,3-butadiene-induced mouse tumors are analogous to genetic alterations frequently observed in human tumors.

In addition to uncertainties pertaining to the relevance of the rodent models to human risk, there is uncertainty in quantitatively scaling the animal risks to humans. Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation; however, current PBPK models are inadequate for this purpose (Chapter 9). In vitro metabolism data for humans suggest that interhuman variability in the capacity to metabolically activate 1,3-butadiene nearly spans the range between rats and mice (Chapter 3). Overall, based on current PBPK and genotoxicity data, one would generally expect humans to have lower cancer risks than mice from 1,3-butadiene exposure, but further advancements are needed in the PBPK models before these differences can be reliably quantified.

Another major source of uncertainty in the unit potency estimate of 0.3/ppm is the extrapolation of high-dose risks observed in the mouse bioassay to lower doses that would be of concern from human environmental exposures. A multistage Weibull time-to-tumor model was the preferred model because it can account for differences in mortality between the exposure groups in the mouse bioassay; however, it is unknown how well this model predicts low-dose extrapolated risks for 1,3-butadiene.

Uncertainties also pertain to the specific assumptions used in conducting these multistage Weibull time-to-tumor analyses. Alternative analyses were performed to consider the sensitivity of the results to some of these assumptions. For example, for each of the tumor types assumed to be fatal, alternative analyses were conducted in which the modeling software estimated t_0. In each case, the resulting q_1*s, EC_{10}s, and LEC_{10}s were identical to those generated when t_0 was set equal to 0 a priori.

In addition, analyses were performed on the lymphocytic lymphoma data including the 625 ppm group, because this was the exposure group most affected by lymphocytic lymphomas and relatively few animals in this group survived to develop tumors at other sites. From the female mouse data, the resulting q_1* was 0.515/ppm, or roughly twice that calculated when the 625 ppm group was excluded. From male mice, the q_1* was 0.0215/ppm, or roughly three times higher than that obtained when the 625 ppm group was excluded.

NIOSH (1991a) examined the sensitivity of its results for each tumor type to (a) model selection (i.e., stage of Weibull model) from among models deemed to be comparable, (b) tumor context assumptions, and (c) exclusion/inclusion of the 625 ppm exposure group, and generally found only small discrepancies in the results. Moreover, uncertainties in some of the model assumptions are trivial compared with the major uncertainties introduced by the interspecies and high- to low-dose extrapolations.

In conclusion, because of the high uncertainty in extrapolating 1,3-butadiene cancer risks from rodents to humans and the existence of good-quality occupational epidemiology data with exposure measures, the epidemiology-based risk estimates presented at the beginning of this chapter are the preferred human risk estimates. The rodent-based estimates are presented primarily for comparison purposes. Given that different quantitative methodologies and assumptions were used to calculate the various risk estimates, the estimated upper bound (95% UCL) on human incremental lifetime unit cancer risk from continuous 1,3-butadiene exposure was 6×10^{-2}/ppm based on the female rat tumors, 3×10^{-1}/ppm based on the female mouse tumors, and 2×10^{-2}/ppm and 6×10^{-3}/ppm based on lymphocytic lymphomas in female and male mice, respectively (lymphocytic lymphomas being the tumor site that most closely resembles the lymphohematopoietic cancers observed in male workers exposed to 1,3-butadiene). The human incremental lifetime unit cancer (incidence) risk estimate based on extrapolation from the leukemias observed in occupational epidemiology studies was 4×10^{-2}/ppm. A twofold adjustment to the epidemiology-based estimate was then applied to reflect evidence from the rodent bioassays suggesting that the epidemiology-based estimate may underestimate total cancer risk from 1,3-butadiene exposure in the general population (see Section 10.1.4), resulting in a lifetime excess cancer unit risk estimate of 8×10^{-2}/ppm.

10.3. DOSE-RESPONSE MODELING OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

10.3.1. Introduction

The reproductive and developmental effects of 1,3-butadiene are among the health effects (both cancer and noncancer) observed at the lowest exposure levels following short-term or chronic inhalation exposure. Data on reproductive and developmental effects were available from three types of studies for modeling and calculation of a benchmark concentration (BMC) and the lower confidence limit on the BMC (BMCL). The three types of studies were (a) prenatal developmental toxicity studies, (b) male-mediated dominant lethal studies, and (c) chronic studies. Data on mice were used in these analyses because there were few if any effects in the rat studies done. The studies are briefly described here, and pertinent endpoints used in the BMC and BMCL calculations are shown in the tables. For more detailed evaluation of these and other studies on 1,3-butadiene toxicity, the reader is referred to Chapters 4 and 5.

10.3.2. Prenatal Developmental Toxicity Studies

In the first type of study, a prenatal developmental toxicity, the effects of 1,3-butadiene were evaluated in mice exposed 6 h/day for 10-days via inhalation at 0, 40, 200, and 1,000 ppm

on gestation days (gd) 6-15 (Hackett et al., 1987b). Reduced fetal weights were seen in mice at all exposure levels (Table 10-12). Thus, 40 ppm was considered a LOAEL for mice.

Fetal weight data were modeled using three approaches. In the first approach, data were fit using a log-logistic model for developmental toxicity, as described by Allen et al. (1994a). The TERALOG model software (ICF Kaiser International, KS Crump Group) was used for this purpose. This analysis considers the within-dam grouping of fetal observations (using a beta-binomial approach to account for intralitter correlation) as well as litter size. To apply this model, the individual fetal weights were converted to dichotomous data using two different values as the cutoff for defining an adverse level of response:

- A decrease below the 5th percentile of the control distribution, and derivation of the EC_{05}[3] and LEC_{05}[4] associated with a 5% additional risk of obtaining a fetal weight below the 5th percentile of the controls, or
- A decrease below the 10th percentile of the control distribution, and derivation of the EC_{10} and LEC_{10} associated with a 10% additional risk of obtaining a fetal weight below the 10th percentile of controls.

These cutoffs were based on Kavlock et al. (1995) who showed that, using this approach, the BMDL estimates for a large database of similarly designed studies were close (on average) to the corresponding NOAELs. Using the "percentiles" function within EXCEL, the 5th and 10th percentiles of the control fetal weights were determined to be 1.13 and 1.169, respectively. The model can be expressed as:

$$P(d, s) = \alpha + \theta_1 s + [1 - \alpha - \theta_1 s]/\{1 + \exp[\beta + \theta_2 s - \gamma \log (d - d_0)]\},$$

where $P(d, s)$ is the probability of a low-weight fetus at dose d and litter size s, and the parameters α, β, γ, θ_1, and θ_2 are estimated by maximum likelihood methods. The intercept parameter (d_0; sometimes referred to as a threshold parameter, i.e., the point at which the model can no longer distinguish from background) was set equal to 0 in the model. Other parameter constraints were: $\gamma \geq 1$; $0 \leq \alpha - \theta_1 s \leq 1$.

[3]The EC is the effective (exposure) concentration associated with a given level of risk, 5% in this case. The EC is also known as the benchmark concentration (BMC).

[4]The LEC is the 95% lower confidence limit on the effective concentration associated with a given level of risk. The LEC is also known as the lower confidence limit on the benchmark concentration (BMCL).

Table 10-12. Fetal weight data from prenatal (developmental) toxicity study[a].

Exposure level	No. litters	Mean fetal weight/litter ± SD
0	18	1.341 ± .1068
40 ppm	19	1.282 ± .05806
200 ppm	21	1.126[b] ± .09849
1,000 ppm	20	1.038[b] ± .1118

[a]Species/strain: Pregnant CD-1 mice. Exposure time: Gestational day (GD) 6-15. Exposure regimen: 6 h/day. Exposure levels: 0, 40, 200, or 1,000 ppm.
[b]$p<0.05$.

Source: Hackett et al., 1987b.

In the second approach, fetal weight also was modeled as the average of mean fetal weights per litter using the continuous power model (Allen et al., 1994b). The THWC model software (ICF Kaiser International, KS Crump Group) was used for this purpose. Several cutoff values were used, again based on those suggested by Kavlock et al. (1995):

- A 5% reduction in mean fetus weight/litter from the control mean,
- A reduction in mean fetus weight/litter to the 25th percentile of the control distribution, and
- A reduction in mean fetus weight/litter to 0.5 SD of the control mean.

Again using the "percentiles" function within EXCEL, the 25th percentile of the mean fetal weights among the control fetuses was estimated to be 1.295.

The continuous power model can be expressed as

$$m(d) = \alpha + \beta d^{\gamma},$$

where $m(d)$ is the mean of the mean fetus weight/litter for dose d, and α, β, and γ are parameters estimated by maximum likelihood methods. The parameter constraints were: $\alpha \geq 0$; $\gamma \geq 1$.

Goodness of fit was determined by a χ^2 test for the log-logistic model, and by an F test for the continuous power model (U.S. EPA, 1995, Appendix A). The model was considered to provide an acceptable fit if the p value was greater than 0.05 and a graphical display of the data showed a good fit of the model.

The third approach used to model fetal weight data was the hybrid approach proposed by Gaylor and Slikker (1990) and further developed by Crump (1995). The BENCH_C model software (ICF Kaiser International, KS Crump Group) was used for this purpose. This approach uses all of the information contained in the original observations by modeling changes in mean response as a function of exposure concentration, but defines ECs and LECs in terms of probability of response. In order to derive probabilities of response from changes in average values, one must assume some distribution of mean fetal weights around the averages (an assumption of normality, with a constant SD for all concentrations, in this case). The continuous data are fit using a model that incorporates parameters from the quantal model. Several models are possible within the software for both continuous data and quantal risk estimates.
For this study, the log-logistic model (not including litter size) was used for the quantal risk estimates and the following model for the continuous portion of the hybrid model:

$$m(d) = m(0) + \sigma[N^{-1}(1-P_0) - N^{-1}\{(1 - P_0)[-1/[1+(\beta d^k)]]\}],$$

where N is the standard normal distribution function, m(d) is the mean response at dose d, σ is the SD of the response fixed for all dose groups, and β and k are the log-logistic model parameters estimated by the maximum likelihood method. The parameter constraints were $k \geq 1$; $\beta \geq 0$.

Crump (1995) indicated that a background rate (P_0) of 5% and an EC for 10% additional risk corresponds to a change from the control mean of 0.61 SD. Because a change in mean fetal weight/litter of 0.5 SD corresponded on average to a NOAEL in studies by Kavlock et al. (1995), a P_0 of 0.05 and an EC_{10} (10% additional risk) were used here.

Results of the modeling for fetal weight are shown in Table 10-13. The values were converted from a 6 h/day exposure to continuous exposure (6/24). Although a dose-duration adjustment is not typically applied for developmental toxicity endpoints, it was judged more appropriate, especially in the case of fetal weight, because of the many possible times during gestation when weight may be affected. In fact, exposure after the period of major organogenesis (gestation days 6-15 in the mouse) may result in further decrements in fetal body weight (RTI, 1999). Because the animals in this study were killed on gestation day 18, an additional adjustment from 10 days to 13 days could be considered. However, the adjustment for 6 h/day to 24 h/day is likely to be conservative, and any further adjustment for additional days of exposure may be overly conservative. The application of dose-duration adjustments to developmental toxicity data was explored and reported by Weller et al. (1999).

The log-logistic model resulted in an adequate fit of the data. Because the log-logistic model requires converting continuous data to quantal responses, the continuous power model also was applied but did not give an adequate fit with all four exposure levels. When it was fit to

Table 10-13. Fetal weight modeling.

Model	Response	Cutoff	EC[b] (ppm)	LEC[b] (ppm)
Log-logistic (1-4)[a]	Individual fetal weight	5th percentile	EC_{05} = 6.9	LEC_{05} =2.9
		10th percentile	EC_{10} = 10	LEC_{10} = 4.7
Continuous power (1-3)[a]	Mean fetal weight/litter	5% relative reduction	16.275	13.38
		25th percentile	11.28	9.17
		0.5 SD absolute reduction	13.09	10.64
Hybrid model[a] (1-4)	Mean fetal weight/litter	P_0 = 0.05	EC_{10} = 7.07	LEC_{10} = 3.325

[a]Exposure levels modeled in each case are shown in parentheses.
[b]Values were adjusted to 24-h exposures, e.g., (40) (6/24) = 10 ppm.

the first three exposure levels, an adequate fit was obtained. The continuous power model gave similar ECs and LECs but these were somewhat larger than those obtained with the log-logistic model except for the one based on a cutoff using the 25th percentile. The hybrid approach resulted in a quantal estimate of dose at the LEC_{10} that was lower than that for either the log-logistic or continuous power model.

All three models have strengths and limitations that must be considered. For example, neither the continuous power model nor the hybrid model are currently structured to account for intralitter correlation or litter size, whereas the log-logistic model accounts for both. The hybrid and the continuous power modeling approaches are based on mean fetal weight responses. With the hybrid approach, the EC_{10} and LEC_{10} estimates derived correspond to the concentration (and its lower bound) estimated to give a 10% risk of having a *mean* fetal weight below the cutoff. This is not the same as having a 10% risk of an individual fetus with a weight less than the cutoff. It is important to note that the SDs associated with average responses are smaller than those corresponding to individual observations, and so the cutoffs defined by the spread around the background mean (e.g., defined by assuming a 5% background response) tend to be closer to the background means than would be the case for individual responses. As a consequence, the models predict lower concentrations associated with smaller changes and the ECs therefore tend to be smaller than would be the case when the cutoff is based on individual responses. This may in part account for the fact that the hybrid approach gives a smaller EC_{10} than the log-

logistic model. In addition, the version of the hybrid model used here does not allow use of the SD (σ) for individual dose groups, so the σ at dose d_0 was used for all dose groups. The log-logistic model, on the other hand, makes use of the individual fetal weight data, although it does require conversion of continuous data to quantal responses.

Unlike the log-logistic approach or the continuous modeling approach, the hybrid approach has not been "calibrated" to find the optimal choices of P_0 and risk level to result in LECs (BMCLs) that on average are close to the NOAEL. Even though the LECs derived from the continuous power approach do not correspond directly to the probability of individual response, the options presented (e.g., a 5% relative reduction) have been shown to correspond on average to NOAELs (Kavlock et al., 1995) and hence there is some basis for selecting those options. In this case, it was not possible using the continuous power approach to obtain an adequate fit of the data except when the highest concentration was ignored. Because the EC and LEC values estimated from the continuous model were within the range of the remaining concentrations (0, 40, and 200 ppm), the modeling approach was acceptable, but it would be preferable to include the information contained in the 1,000 ppm data, if possible.

Based on the above considerations, the log-logistic model was considered the preferred approach for modeling fetal weight data from this study, i.e., the log-logistic model fit all four exposure levels adequately. Figure 10-6 shows the log-logistic model fit of the fetal weight data. The combinations of the 5^{th} percentile cutoff for a 5% additional risk or 10^{th} percentile cutoff for a 10% additional risk were shown by Kavlock et al. (1995) to result in BMDLs similar to the NOAEL. The LEC (BMCL) value for the 5^{th} percentile cutoff for a 5% additional risk of 2.9 ppm is the more health protective choice to use for the point of departure for the RfC.

10.3.3. Dominant Lethal Studies

In the second type of study used for BMCL calculations, the male-mediated effects of 1,3-butadiene were evaluated in three dominant lethal studies in which male CD-1 mice were exposed (Anderson et al., 1993, 1995, 1996, 1998; Brinkworth et al., 1998)[5] and subsequently mated with unexposed females. All three studies were conducted in the same laboratories under essentially the same conditions and with the same personnel.

10.3.3.1. *Anderson et al. (1993, 1995, 1996)*

In the first Anderson et al. study (reported in 1993,1995, 1996), males were exposed to 0, 12.5, or 1,250 ppm for 6 h/day, 5 days/week, for 10 weeks, then mated immediately after exposure ended. Two females were mated with each male. One group of females at each

[5]Drs. Anderson and Brinkworth very kindly supplied the individual animal data from all three of these studies after obtaining agreement from the sponsors for use in the modeling of data for this effort.

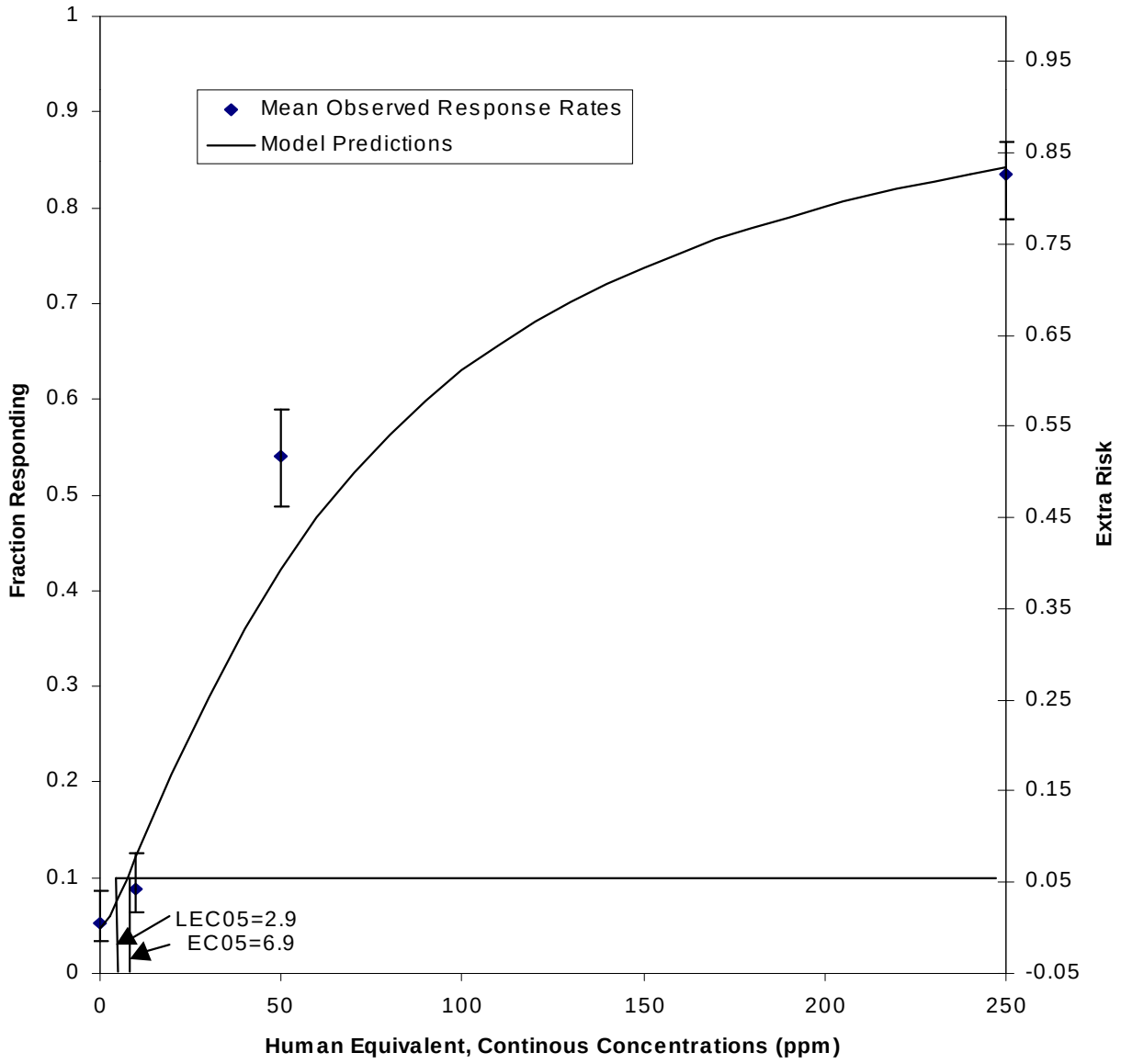

Figure 10-6. Log-logistics model for fetal weight data, with data points and standard error bars.

exposure level was killed on gestation day 17 (reported in Anderson et al., 1993), while another was allowed to litter (reported in Anderson et al., 1996). Data from the final study report (Anderson et al., 1995) were used to obtain individual animal data for modeling. The data used for modeling are summarized in Tables 10-14a and 10-14b. In the prenatal component of the study, there was a statistically significant decrease in the mean number of implantations, and a significant increase in incidence and percentage of early deaths, late deaths, and abnormal fetuses at 1,250 ppm. At 12.5 ppm, a statistically significant increase in incidence of late deaths (with and without dead fetuses) and abnormal fetuses (including runts) was noted. However, incidence of late deaths and abnormal fetuses was lower at 1,250 ppm than at 12.5 ppm, perhaps because of the large increase in early deaths at the higher exposure level. For the purposes of modeling the data, early and late deaths (including dead fetuses) are combined into "all deaths," resulting in a consistently increasing response with increasing exposure level. In addition, the number of abnormal fetuses was changed from that reported by Anderson et al. (1993) to include only those with actual malformations, whereas the runts (those with <75% the body weight of the rest of the litter) were considered in an analysis of fetal body weights. Skeletal evaluations were conducted on a small subset of normal and abnormal fetuses (each abnormal fetus and a normal littermate as well as one control corresponding to each abnormal fetus were examined). There was no clear indication of any increase in skeletal defects among the animals examined. The mean fetal body weights from the analysis of individual data are shown in Table 10-14a and indicate a slight but insignificant decrease at 1,250 ppm.

In the postnatal component of the study, there was a significant decrease in the number of implantations and litter size at birth, as well as an increase in postimplantation loss at 1,250 ppm. Litter size at weaning was similar to that at birth, because very few pups died between birth and weaning. No changes were detected at 12.5 ppm. Total litter weight at birth was significantly decreased at 1,250 ppm, but when mean pup weight per litter was calculated, there was no difference between the offspring of control males and offspring of treated males; in fact, there was a slight increase in the 1,250 ppm pup weights (data not shown). This is consistent with data from Anderson et al. (1995) indicating that male and female body weights of F1 offspring from treated males were increased above controls at 8 through 71 weeks of age. No further analysis of these data was conducted.

Table 10-15 shows the EC_{05}, LEC_{05}, EC_{10}, and LEC_{10} values for various endpoints from the Anderson et al. study (1993, 1995, 1996). Implant data from both the prenatal and postnatal

Table 10-14A. Male-mediated developmental toxicity, prenatal component[a].

Exposure level	No. males exposed (no. mated)	No. preg. females[b]	Total no. implants (mean ± SD)	No. early deaths (mean[c] ± SD)	No. late deaths (mean[c] ± SD)	No. late deaths incl. dead fetuses (mean[c] ± SD)	No. abnormal fetuses (no. litters)[d]	Fetal weight mean[e] ± SD (no. litters)
0	25 (23)	23	278 (12.09 ± 1.276)	13 (0.050 ± .0597)	0	2 (0.007 ± .022)	0	1.142 ± .1055 (23)
12.5 ppm	25 (25)	24	306 (12.75 ± 2.507)	16 (0.053 ± .0581)	7 (0.023[f] ± .038)	8 (0.026[g] ± .0424)	4(2)	1.116 ± .1030 (24)
1250 ppm	48 (48)	38	406 (10.68[f] ± 3.103)	87 (0.204[h] ± .1611)	6 (0.014[h] ± .0324)	7 (0.016 ± .0339)	1 (1)	1.109 ± .0935 (37)

[a]Species/strain: CD-1 mice, adult males. Exposure regimen: 6 h/day, 5 days/week. Exposure time: 10 weeks. Exposure levels: 0, 12.5 ppm, 1250 ppm.
[b]Used in prenatal component.
[c]Based on number of implants/litter.
[d]Not including runts.
[e]Based on number of live fetuses/litter.
[f]Statistically significantly different from controls, $p \leq .01$.
[g]Statistically significantly different from controls, $p \leq .05$.
[h]Statistically significantly different from controls, $p \leq .001$.

Source: Anderson et al., 1993, 1995, 1996.

Table 10-14B. Male-mediated developmental toxicity, postnatal component[a].

Exposure level	Litter size at birth mean ± SD (no. litters)	No. implants mean ± SD (no. litters)	No. postimplantation losses (mean[b] ± SD)	Litter size at weaning mean ± SD (no. litters)
0	12.22 ± 2.16 (18)	205 12.81±2.07 (16)	10 (0.625±.500)	12.17±2.07 (18)
12.5 ppm	11.14 ± 1.71 (21)	210 12.35±1.69 (17)	19 (0.790±.676)	10.95±1.99 (20)
1,250 ppm	8.31[c]+3.48 (36)[d]	335 10.47[c]±2.90 (32)	80 (2.50[c]±1.78)	9.03[c]±2.53 (33)

[a]Species/strain: CD-1 mice, adult males. Exposure regimen: 6 h/day, 5 days/week. Exposure time: 10 weeks. Exposure levels: 0, 12.5 ppm, 1250 ppm.
[b]Per pregnant female.
[c]Statistically significantly different from controls, $p \leq .001$.
[d]Three litters completely resorbed.

Source: Anderson et al., 1993, 1995, 1996.

components of the study were fit using a linear model because no corpora lutea data were available for normalizing the implant data. The model used was

$$y(d) = \alpha + \beta d,$$

where y(d) is the mean response for the endpoint of interest at dose (concentration) d.

For all other endpoints, the log-logistic model (TERALOG software), as discussed above for fetal weight modeling, was used because the individual animal data for the dominant lethal studies were available. Adjustment for duration of exposure was applied after the modeling (i.e., the model outputs for EC and LEC were multiplied by the factor (6/24)(5/7)). The endpoints included in the modeling were those that appeared significantly related to 1,3-butadiene exposure, and included early prenatal deaths, late prenatal deaths, all prenatal deaths, abnormal fetuses in the prenatal component of the study, and postimplantation loss in the postnatal component. Dead fetuses were not included in late prenatal deaths but were included in all prenatal deaths. Abnormalities included only external effects detected in fetuses and did not include skeletal or visceral effects (only a small subset of fetuses was examined for skeletal effects), and also did not include runts or one fetus with blood in the amniotic sac. The litter size used in the model was the number of live fetuses for each litter because only live fetuses were

examined for abnormalities. Fetal weights were analyzed separately and did not appear to be changed significantly with treatment. Fertility, numbers of dead fetuses, and postnatal deaths did not appear to be significantly related to treatment and were not included in the analyses. Litter size at weaning was not included because of the relatively small number of postnatal deaths, and litter size at birth was captured by an analysis of all prenatal deaths.

As shown in Table 10-15, the EC and LEC estimates for early deaths and all deaths were similar because early deaths dominated the "all deaths" values. The values for postimplantation loss from the postnatal portion of the study also were similar even though this value may include some deaths that occurred after prenatal day 17 (when fetuses in the prenatal component were killed) up to postnatal day 0 when pups were delivered and examined.

Table 10-15. ECs and LECs for male-mediated developmental toxicity[a].

Prenatal component						Postnatal component	
Estimate (ppm)	**No. implants**	**No. early deaths**	**No. late deaths[b]**	**No. all deaths**	**No. abnormal fetuses[c]**	**No. implants**	**No. post-implantation losses**
EC_{05}	89	55	∞	53	∞	67	98
LEC_{05}	56	40	∞	39	∞	46	54
EC_{10}	178	116	∞	112	∞	135	206
LEC_{10}	112	85	∞	83	∞	93	114

[a]Exposures were adjusted to 24-h daily exposures, e.g., (12.5) (6/24) (5/7) = 2.2 ppm. Entries with the "∞" symbol indicate essentially flat dose-response curves.
[b]Not including dead fetuses.
[c]Not including runts.

Source: Anderson et al., 1993, 1995, 1996.

10.3.3.2. *Anderson et al. (1998)*

The second dominant lethal study, by Anderson et al. (1998), involved exposure of male CD-1 mice to 0, 12.5, 65, or 130 ppm for 6 hr/day, 5 days/week, for 4 weeks. Mating of males began after 1 day of rest at the end of exposure with two females per male. Both females, if pregnant, were included in the examination of effects reported in the paper. Data are shown in Table 10-16. The ECs and LECs are shown in Table 10-17. Implant data were fit using a linear

Table 10-16. Male-mediated developmental toxicity[a].

Exposure level	No. fertile males	No. implants Mean ± SD[b] (no. preg. females)	No. early deaths (mean ± SD)[c]	No. late deaths (mean ± SD)[c]	No. late deaths incl. dead fetuses (mean ± SD)[c]	No. abnormal fetuses[d]	Fetal weight mean ± SD (no. litters)
0	25	572 12.43 ± 2.60 (46)	17 (0.039 ± 0.10)	7 (0.011 ± 0.04)	13 (0.023 ± 0.05)	0	1.178 ± .1962 (11)
12.5 ppm	24	528 12.00 ± 2.37 (44)	12 (0.022 ± 0.05)	3 (0.005 ± 0.02)	12 (0.022 ± 0.05)	0	1.107 ± .1941 (14)
65 ppm	23	559 12.42 ± 2.58 (45)	44 (0.083[e] ± 0.11)	10 (0.017 ± 0.04)	14 (0.024 ± 0.05)	0	1.049 ± .0830 (13)
130 ppm	24	562 11.96 ± 2.52 (47)	49 (0.082[e] ± 0.10)	7 (0.013 ± 0.03)	16 (0.030 ± 0.05)	0	1.161 ± .1801 (16)

[a]Species/strain: CD-1 mice, adult males. Exposure regimen: 6 h/day, 5 days/week. Exposure time: 4 weeks. Exposure levels: 0, 12.5, 65, 130 ppm.
[b]Mean per litter.
[c]Mean per implant per litter.
[d]Excludes runts.
[e]Statistically significantly different from controls, $p<.01$.

Source: Anderson et al., 1998.

Table 10-17. ECs and LECs for male-mediated developmental toxicity[a].

Estimate (ppm)	No. implants	No. early deaths	No. late deaths[b]	No. all deaths
EC_{05}	∞ [12]	14 [23]	∞ [86]	17 [18]
LEC_{05}	∞ [7.3]	8.9 [11]	∞ [29]	9.5 [10]
EC_{10}	∞ [24]	30 [49]	∞ [CF]	37 [38]
LEC_{10}	∞ [15]	19 [23]	∞ [CF]	20 [21]

[a]Exposures were adjusted to 24-h daily exposures, e.g., (12.5) (6/24) (5/7) = 2.2 ppm. Entries with the "∞" symbol indicate essentially flat dose-response curves, i.e., no estimates for the EC or LEC values could be derived. Values in brackets are the estimates from the alternative analyses checking sensitivity of results. All fits described the data satisfactorily (P-values all greater than 0.10). CF indicates that convergence failed, likely due to the flat dose-response curve.
[b]Not including dead fetuses.

Source: Anderson et al., 1998.

model, as for the Anderson et al. (1993, 1995, 1996) study. In the 1998 study, there were often, but not always, two pregnant females per male. Because, to our knowledge, no dose-response models exist that incorporate the added layer of nesting (litter within male) present with these data sets, we have adopted the following procedure. When there were two litters for any experimental unit (male mouse), one of the two was randomly chosen for inclusion in the dose-response analysis. When there was only one litter for a particular male, that one litter was included in the analysis.

In order to assess the sensitivity of the results to that random assignment of litters to the dose-response analysis, the same analysis was conducted using the "leftover" litters (the litters not randomly chosen for inclusion in the original analysis). When there was only one litter, it was used in this alternative analysis as well as in the primary analysis. The results presented in Table 10-17 include both sets of EC and LEC estimates, one for the primary analysis (using the chosen litters) and one for the alternative analysis (using the leftover litters). However, when combinations of studies were considered, only the chosen litters were used (see below).

As in the first Anderson et al. study, this study reported significant effects of treatment with 1,3-butadiene on incidence of early deaths (Anderson et al., 1998). In this study, exposure levels of 65 and 130 ppm resulted in increased incidence of early deaths, but 12.5 ppm did not show a statistically significant increase in early deaths as in the first Anderson et al. (1993) study. There were no effects at any exposure level on late deaths, nor on incidence of abnormal fetuses

including runts. All the abnormal fetuses reported by Anderson et al. (1998) were runts (<75% of the weight of littermates), and no fetuses had structural abnormalities. Analysis of individual fetal weight data also showed no effect of exposure to 1,3-butadiene; however, data were available from only about one-half the litters because there apparently were problems in detecting pregnancies in a number of animals. In this analysis, fetal weight data were not included from "unplugged" females (those in which a vaginal plug was not detected) that eventually were found to be pregnant, nor from the "plugged" females that were considered to have been necropsied too early, i.e., those litters in which the fetuses were found to be underdeveloped for the presumed gestational age, because gestational age could not be accurately determined. Data from these litters were not combined with those from pregnancies of known gestational age, because fetuses grow rapidly during late gestation and as little as 1 day can make a significant difference in fetal weight. The lower sample size and other experimental considerations may explain the somewhat larger standard deviations for data from this study compared with those from the Anderson et al. (1993) study.

In Table 10-17, ECs and LECs for implants and late deaths could not be estimated in the primary analysis because they did not show a dose-response relationship, although there were cases in the alternative analyses where these values could be estimated. The ECs and LECs for early deaths and all deaths from the primary analysis were very similar to those from the alternative analysis. The values for early deaths and all deaths were similar to each other as well, again reflecting the fact that early deaths made up the majority of all deaths. The EC_{05} for both endpoints was between the two higher exposure levels tested, and the EC_{10} was above the highest exposure level. Given that the data are nested (i.e., fetuses within litters; two litters/male), and thus are similar to the developmental toxicity data evaluated by Allen et al. (1994b), the EC_{05} and LEC_{05} could easily be estimated. The LEC_{05} for both endpoints (early deaths and all deaths) was below the highest exposure level tested and near the 65 ppm exposure level (11.6 ppm adjusted value).

10.3.3.3. *Brinkworth et al. (1998)*

The third dominant lethal study, by Brinkworth et al. (1998), involved exposure of male CD-1 mice to 0, 12.5 ppm, and 125 ppm for 6 h/day, 5 days/week for 10 weeks. Mating of males began 4 days after the end of exposure with two females/male. Data are shown in Table 10-18. The ECs and LECs are shown in Table 10-19. The analysis was identical to that described above for the Anderson et al. (1998) study, in that a primary and an alternative analysis were done for the endpoints modeled. In this study, early deaths were significantly increased after exposure of males to 125 ppm, but not after 12.5 ppm. Incidence of late deaths, dead fetuses, and abnormal fetuses was not affected by treatment. Fetal weight also was not affected by exposure, but data

Table 10-18. Male-mediated developmental toxicity[a].

Exposure level	No. of fertile males	No. implants Mean ± SD[b] (no. preg. females)	No. early deaths (mean ± SD)[c]	No. late deaths (mean ± SD)[c]	No. dead fetuses (mean ± SD)[c]	Abnormal fetuses[d] (no. litters)	Fetal weight[e] Mean ± SD (no. litters)
0	23	576 13.09±2.604 (44)	32 (0.055±0.0691)	9 (0.014±0.0365)	1 (0.002±0.0099)	2 (2)	1.084±.1380 (9)
12.5 ppm	22	502 13.21±1.862 (38)	28 (0.055±0.0694)	13 (0.028±0.0647)	3 (0.006±0.0202)	1 (1)	1.004±.0719 (4)
125 ppm	25	602 13.09±1.532 (46)	59 (0.097[f]±0.0835)	14 (0.024 ± 0.0514)	4 (0.008±0.0235)	0	1.068±.1338 (9)

[a]Species/strain: CD-1 mice, adult males. Exposure regimen: 6 h/day, 5 days/week. Exposure time: 10 weeks. Exposure levels: 0, 12.5, 125 ppm.
[b]Mean per litter.
[c]Mean per implant per litter.
[d]Excludes runts.
[e]Only runts and a single littermate as well as one control were weighed.
[f]Statistically significantly different from controls, $p \leq .01$

Source: Brinkworth et al., 1998.

Table 10-19. ECs and LECs for male-mediated developmental toxicity[a].

Estimate (ppm)	No. implants	Early deaths	Late deaths[b]	All deaths
EC_{05}	∞ [50]	26 [20]	42[c] [∞]	19 [22]
LEC_{05}	∞ [13]	18 [11]	13[c] [∞]	9.9 [11]
EC_{10}	∞ [100]	31 [41]	89[c] [∞]	41 [25]
LEC_{10}	∞ [26]	23 [24]	28[c] [∞]	21 [23]

[a]Exposures were adjusted to 24-h daily exposures, e.g., (12.5) (6/24) (5/7) = 2.2 ppm. Entries with the "∞" symbol indicate essentially flat dose-response curves, i.e. no estimates for the EC or LEC values could be derived. Values in brackets are the estimates from the alternative analyses checking sensitivity of results.
[b]Not including dead fetuses.
[c]The fit of the log-logistic model to these data was relatively poor (p=0.04). All other fits described the data satisfactorily (p-values all greater than 0.10).

Source: Brinkworth et al., 1998.

are sparse and are likely biased in this case, because only those litters with fetuses considered to be runts (<75% or <80% the weight of the rest of the litter) were weighed, along with "matching" control litters. This likely explains the lower mean fetal weights in this study versus those seen in either the Anderson et al. (1993) or the Anderson et al. (1998) study. In addition, the SD was somewhat larger for these data than those from the Anderson et al. (1993) study, probably a reflection of the smaller sample size and other experimental considerations.

The ECs and LECs (Table 10-20) could not be calculated in the primary analysis for the number of implants, nor in the alternative analysis for late deaths. It was possible to calculate values for implants in the alternative analyses, but the fit of the model for late deaths was poor, so the estimates are not used. Values in brackets are the estimates from the alternative analyses checking sensitivity of results. All fits described the data satisfactorily (p-values all greater than 0.10). CF indicates that convergence failed, likely due to the flat dose-response curve; i.e., no estimates for the EC or LEC values could be derived. The ECs and LECs for early deaths were similar to those for all deaths, again reflecting the fact that early deaths make up the largest component of all deaths. The EC_{05} for both was similar to the highest exposure level tested (i.e., 22.3 ppm adjusted for continuous exposure), and, along with the LEC_{05}, could be estimated easily.

Table 10-20. ECs and LECs for male-mediated developmental toxicity from the combined studies[a].

Endpoint	Estimate (ppm)	Anderson et al. (1993)	Anderson et al. (1998)	Brinkworth et al. (1998)	Combined Anderson et al. (1993) and Brinkworth et al. (1998)[d]	Combined 3 studies[d]	Combined 3 studies except 1,250 ppm group[d]
Implants	EC_{05}	89	∞ [12]	∞ [50]	72	79	—
	LEC_{05}	56	∞ [7.3]	∞ [13]	50	53	—
	EC_{10}	178	∞ [24]	∞ [100]	144	158	—
	LEC_{10}	112	∞ [15]	∞ [26]	100	106	—
Early deaths	EC_{05}	55	14 [23]	26 [20]	50	44	24
	LEC_{05}	40	8.9 [11]	18 [11]	37	32	15
	EC_{10}	116	30 [49]	31 [41]	106	92	50
	LEC_{10}	85	19 [23]	23 [24]	77	67	28
Late deaths[b]	EC_{05}	∞	∞ [86]	42[e] [∞]	ND	ND	—
	LEC_{05}	∞	∞ [29]	13[e] [∞]	ND	ND	—
	EC_{10}	∞	∞ [CF]	89[e] [∞]	ND	ND	—
	LEC_{10}	∞	∞ [CF]	28[e] [∞]	ND	ND	—
All deaths[c]	EC_{05}	53	17 [18]	19 [22]	52	47	15
	LEC_{05}	39	9.5 [10]	9.9 [11]	38	34	10
	EC_{10}	112	37 [38]	41 [25]	109	99	31
	LEC_{10}	83	20 [21]	21 [23]	80	72	21

[a] Exposures were adjusted to 24-h daily exposures, e.g., (12.5) (6/24) (5/7) = 2.2 ppm. Values in brackets are the estimates from the alternative analyses checking sensitivity of results. Entries with the "∞" symbol indicate essentially flat dose-response curves, i.e., no estimates for the EC or LEC values could be derived. CF indicates that convergence failed, likely because of the flat dose-response curve. ND indicates "not done" — the failure of individual studies to estimate EC and LEC values suggested that analysis of combined data would be futile. "—" indicates that the combined analysis of data excluding the 1,250 ppm group was not done; only the analysis for early deaths and all deaths were done, since those endpoints showed the clearest dose-response relationship.
[b] Not including dead fetuses.
[c] Including dead fetuses.
[d] Only data on litters used in the primary analysis were used in combining studies for modeling.
[e] The fit of the log-logistic model to these data was relatively poor (p=0.04). All other fits described the data satisfactorily (p-values all greater than 0.10).

10.3.3.4. ***Analysis of Combined Dominant Lethal Studies***

As stated earlier, the three dominant lethal studies were conducted in the same laboratory, under essentially the same conditions, and by the same investigators. The only notable difference was the number of weeks of exposure (4 weeks in the Anderson et al. [1998] study versus 10 weeks in the other two studies). Because Adler and Anderson (1994) showed that the longer period of exposure did not increase the dominant lethal effects of 1,3-butadiene, the data were combined. Table 10-20 presents the results of the analyses for the combinations of studies. For completeness, results of analysis of the individual studies are also included.

Initially, only the data from Anderson et al. (1993) and Brinkworth et al. (1998) were combined because they were similar in terms of duration of exposure. Ultimately, all three studies were combined because the results indicated that the difference in duration of exposure was not crucial to estimation of the EC and LEC values.

Note that the random assignment of litters for inclusion in the dose-response analysis of the Anderson et al. (1998) study and the Brinkworth et al. (1998) study did not appear to affect the results to any great degree, with the exception of the estimates for implantations. Except for that case, the alternative estimates (in brackets in Table 10-20) that would have been obtained had the other litters been included in the analysis are very similar to those obtained from the primary analysis.

For implantations, the choice of primary or alternative data subsets did have a relatively large impact, because for the primary subset no downward trend in number of implants was detected, whereas for the alternative subset such a downward trend was in evidence. The apparent sensitivity of the implantation EC and LEC estimates to the choice of the data subsets suggests the need for additional investigation of this endpoint (including, perhaps, examination of whether 5% or 10% reductions in number of implantations are suitable reference points—response levels—for defining RfCs).

Both the Anderson et al. (1998) and the Brinkworth et al. (1998) studies, which had similar maximum exposure levels of 130 and 125 ppm, respectively, estimated very similar EC and LEC values, especially for all deaths. In fact, the EC and LEC estimates from both of those studies were less than the corresponding estimates from Anderson et al. (1993). The reason for this is as follows. In Anderson et al. (1998) and Brinkworth et al. (1998), the maximal observed increases in mean response, for all deaths as an example, were 0.058 and 0.066, respectively. Consistent with those observations, the models estimated EC_{05}s of slightly less than 22 or 23 ppm (the continuous exposure equivalents of 125 and 130 ppm, respectively). Given the rates of change in response observed in these two studies, the projections out to 1,250 ppm (the maximum experimental concentration in Anderson et al., 1993) would overestimate the response observed there. In fact, the observed average increase for the high-exposure group from the first

study was about 0.16; the projections from the two 1998 studies for the concentration corresponding to a 0.16 increase in response are about 350 ppm. When all the data were combined for modeling, the rate of increase of the dose-response curve was somewhat less and the fit at the high dose was better. However, the fit at the lower doses was not as good. Because we are most concerned with fitting the data in the range of the EC and LEC, it was decided to run the model for all three studies combined without the highest exposure level (1,250 ppm). When this was done, the ECs and LECs for early deaths and all deaths from the combined studies were very similar to those for the Anderson et al. (1998) and the Brinkworth et al. (1998) studies. Figure 10-7 shows the fit of the data for all prenatal deaths.

Combined analysis of the three studies provides many more data points suitable for dose-response analysis and enhances the credibility of the estimates precisely because of the general agreement observed among the results. We believe that the most suitable combination for estimating the EC_{05} and LEC_{05} is without the 1,250 ppm exposure level from the Anderson et al. (1993) study because this data point results in a higher estimate and a visual fit of the model to the data that is not as good at the EC_{05}. Therefore, a suitable basis for derivation of an RfC for 1,3-butadiene based on these dominant lethal results would be the value of 10 ppm (adjusted for continuous exposure) obtained when all of the studies are combined except for the 1,250 ppm exposure level and "all deaths" is the endpoint used (see Table 10-20). This value, while being the most health-protective, is consistent with values derived with other endpoints ("early deaths" with an LEC_{05} of 15 ppm), and with the estimates from either the Anderson et al. (1998) study or the Brinkworth et al. (1998) study estimates for early deaths or all deaths.

10.3.4. Chronic Studies

In the third type of study, reproductive effects of 1,3-butadiene were seen in lifetime studies in mice after chronic inhalation exposure to 6.25, 20, 62.5, 200, and 625 ppm for 6 h/day, 5 days/week (NTP, 1993). The lowest exposure level studied in mice (6.25 ppm) showed increased ovarian atrophy and was considered a LOAEL (Table 10-21). Minimal data from studies on rats suggested their lesser sensitivity to chronic exposure than for mice in that effects on fertility were noted only at high exposure levels (600 ppm and above).

In conclusion, each of these three types of studies indicated the potential of 1,3-butadiene to affect reproduction and development in mice at low levels of exposure.

The quantal Weibull model was used initially to model all data. In cases where this model did not provide a good fit of the data, a log-logistic model was used. The 15-month and chronic ovarian atrophy data could not be fit adequately using the quantal Weibull model. A log-logistic model similar to that used for fetal weight (setting θ_1s and θ_2s to zero) was found to fit the data well. The model was run to determine the probability of additional risk and extra risk.

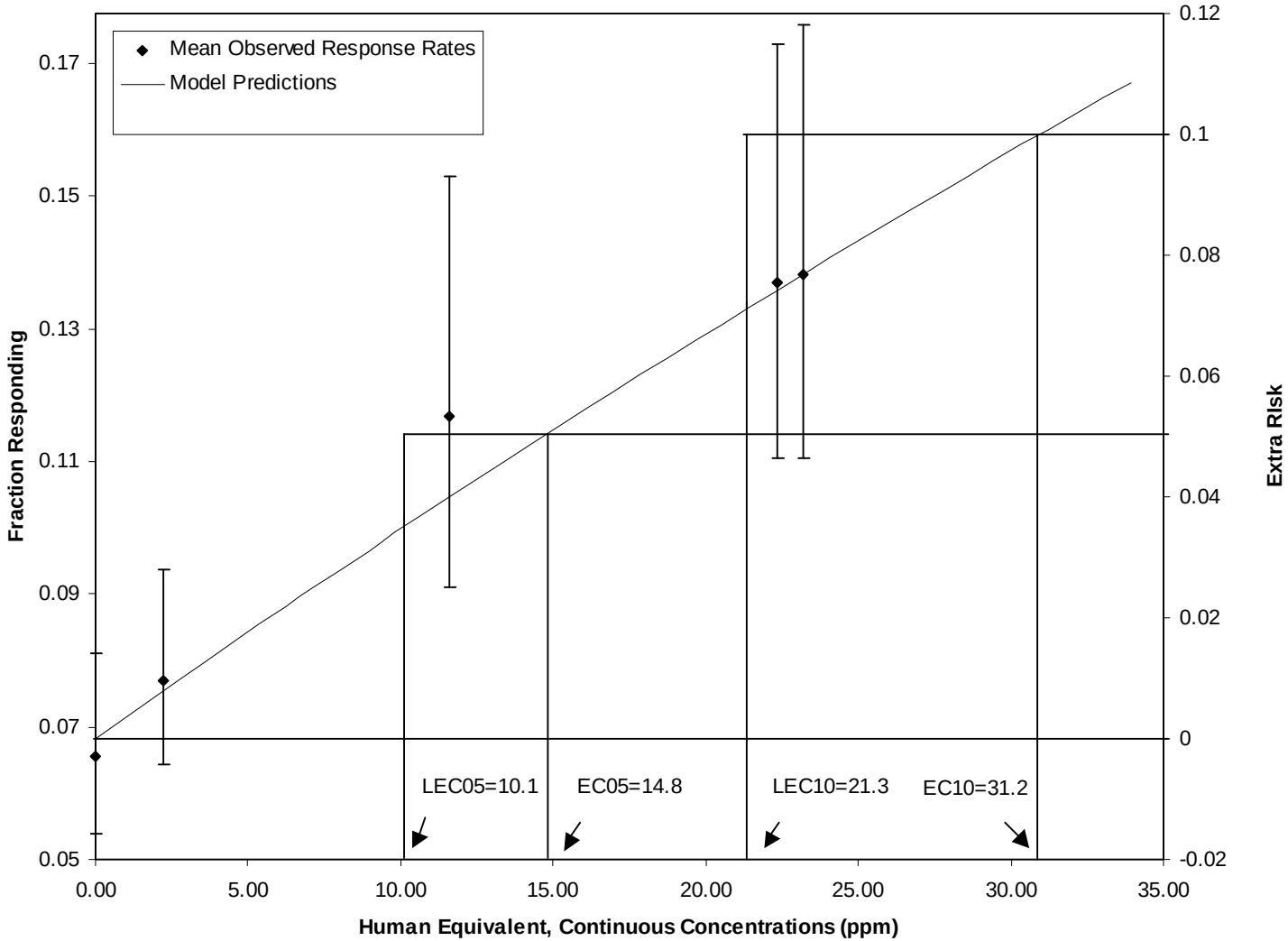

Figure 10-7. Log-logistic model for data on all prenatal deaths from the three dominant lethal studies, excluding the 1,250 ppm exposure group, with data points and standard error bars[a].

[a]The EC_{05}, LEC_{05}, EC_{10}, and LEC_{10} shown correspond to those in the last column of Table 10-20.

Table 10-21. NTP chronic study (1993)[a].

Incidence Data—Ovarian Atrophy

Exposure level	Ovarian atrophy—9 mo		Ovarian atrophy—15 mo		Ovarian atrophy—2 years	
	No. examined	% affected	No. examined	% affected	No. examined	% affected
0	10	0	10	0	49	8.16
6.25 ppm	—	—	10	0	49	38.78
20.00 ppm	—	—	10	10	48	66.67
62.50 ppm	10	0	10	90	50	84.00
200.00 ppm	10	90	10	70	50	86.00
625.00 ppm	8	100	2	100	79	87.34

Ovarian Atrophy — Lesion Distribution

Number (%)

Exposure level	Ovarian atrophy—9 mo			Ovarian atrophy—15 mo			Ovarian atrophy—2 years			
	Minimal	Mild	Moderate	Minimal	Mild	Moderate	Minimal	Mild	Moderate	Marked
0.00	0	0	0	0	0	0	1 (2)	2 (4)	1 (2)	0
6.25 ppm	—	—	—	0	0	0	0	15 (31)	4 (8)	0
20.00 ppm	—	—	—	1 (10)	0	0	1 (2)	23 (48)	8 (17)	0
62.50 ppm	0	0	0	1 (10)	7 (70)	1 (10)	3 (6)	18 (36)	21 (42)	0
200.00 ppm	0	0	9 (90)	0	1 (10)	6 (60)	0	9 (18)	34 (18)	0
625.00 ppm	0	0	8 (100)	0	0	2 (100)	0	19 (24)	47 (59)	3 (4)

Table 10-21. NTP chronic study (1993) (continued).

Incidence Data—Uterine Atrophy

Exposure level	Uterine atrophy—9 mo		Uterine atrophy—15 mo		Uterine atrophy—2 years	
	No. examined	No. (%) Affected	No. examined	No. (%) Affected	No. examined	No. (%) Affected
0	10	0	10	0	50	1 (2)
6.25 ppm	—	—	1	0	49	0
20 ppm	—	—	10	0	50	1 (2)
62.5 ppm	10	0	10	0	49	1 (2)
200 ppm	10	3 (30)	10	0	50	8 (16)
625 ppm	8	6 (75)	2	2 (100)	78	41 (53)

Incidence Data—Testicular Atrophy

Exposure level	Testicular atrophy—9 mo		Testicular atrophy—15 mo		Testicular atrophy—2 years	
	No. examined	No. (%) Affected	No. examined	No. (%) Affected	No. examined	No. (%) Affected
0	10	0	10	0	50	1 (2)
6.25 ppm	—	—	—	—	50	3 (6)
20 ppm	—	—	1	0	50	4(8)
62.5 ppm	—	—	—	—	48	2 (4)
200 ppm	10	0	10	0	49	6 (12)
625 ppm	10	6 (60)	7	4 (57)	72	53 (74)

[a]Species/strain: Male and female $B6C3F_1$ mice. Exposure regimen: 6 h/day, 5 days/week for 2 years. Exposure levels: 0, 6.25, 20, 62.5, 200, or 625 ppm.

Goodness of fit was determined by a χ^2 test. The model was considered to give a good fit if the *p* value was greater than 0.05 and a graphical display of the data showed a good fit of the model.

An attempt was made to model various levels of severity in the lesions seen, based on the data shown in Table 10-21. The data for moderate lesions were fit using the quantal Weibull model (Allen et al., 1994b) for dichotomous data. This model can be expressed as:

$$P(d) = 1 - \exp[-(\alpha + \beta d^{\gamma})],$$

where P(d) is the probability of response at exposure level d and α, β, and γ are parameters estimated from the observed dose-response data. Parameter constraints were $\alpha \geq 0$; $\beta \geq 0$; $\gamma > 0$. The model was run to determine the probability of additional risk. Goodness of fit was determined by a χ^2 test. The model was considered to provide an acceptable fit if the *p* value was greater than 0.05 and a graphical display of the data showed a good fit of the model.

Table 10-22 gives the results of fitting the log-logistic model to the 2-year ovarian atrophy data for exposure groups 1-5 and 1-4. The model gave a poor fit for all six exposure groups, because of leveling off of the response at exposures above 62.5 ppm (36 ppm adjusted for continuous exposure). The best fit of the model was for exposure groups 1-4, although the model also fit exposure groups 1-5 well (Figure 10-8; exposures adjusted for continuous exposure), and the EC_{10}s and LEC_{10}s obtained for groups 1-4 and 1-5 were similar. As expected, LEC_{10}s were lowest for ovarian atrophy at 2 years. Moderate ovarian atrophy at 2 years also was modeled using the quantal Weibull model with exposure groups 1-5 or 1-4. The EC_{10} and LEC_{10} were higher than those for all lesions. Ovarian atrophy data for all six exposure groups at 9 and 15 months were fit using the quantal Weibull or log-logistic model.

Uterine and testicular atrophy data also were modeled using the quantal Weibull model. The quantal Weibull model resulted in an acceptable fit of the 2-year uterine atrophy and testicular atrophy data (Table 10-22 and Figures 10-9 and 10-10; exposures adjusted for continuous exposure). However, the EC_{10}s and LEC_{10}s were much higher for these endpoints than for 9-month, 15-month, or 2-year ovarian atrophy data. LEC_{10}s were estimated because it has been shown that, for quantal responses, the LEC_{10} is near or below the range of detectable responses (Allen et al. 1994b). Also, the Proposed Guidelines for Carcinogen Risk Assessment propose use of an LEC_{10} as the default point of departure for low-dose extrapolation, and use of an LEC_{10} as a default for noncancer estimation of an RfC would be consistent with this approach (U.S. EPA, 1996, 1999).

Table 10-22. ECs and LECs for ovarian, uterine, and testicular atrophy using the quantal Weibull and log-logistic models[a].

Endpoint	Model	NOAEL/LOAEL	EC_{10} (ppm)	LEC_{10} (ppm)	*p*-Value
Ovarian atrophy — 2 years	Log-logistic (1-5)[b]	1.1 ppm (LOAEL)	0.32	0.22	0.11
			0.29[c]	0.21[c]	
	Log-logistic (1-4)		0.27	0.18	0.96
			0.24[c]	0.17[c]	
Ovarian atrophy — 2 year Moderate lesions only	Quantal Weibull (1-5)	1.1 ppm	3.02	2.35	0.55
	Quantal Weibull (1-4)		2.31	1.67	0.96
Ovarian atrophy — 15 mos	Log-logistic (1-6)	1.1 ppm	2.10	0.72	0.66
Ovarian atrophy — 9 mos	Quantal Weibull (1-6)	11 ppm	20.04	9.95	0.83
Uterine atrophy	Quantal Weibull (1-6)	11 ppm	29.37	18.43	0.66
Testicular atrophy	Quantal Weibull (1-6)	36 ppm	40.59	25.64	0.55

[a]Exposures were adjusted for continuous exposure, e.g., (6.25) (6/24) (5/7) = 1.1 ppm.
[b]Exposure levels included in the model.
[c]Extra risk. All other values are estimates of additional risk.

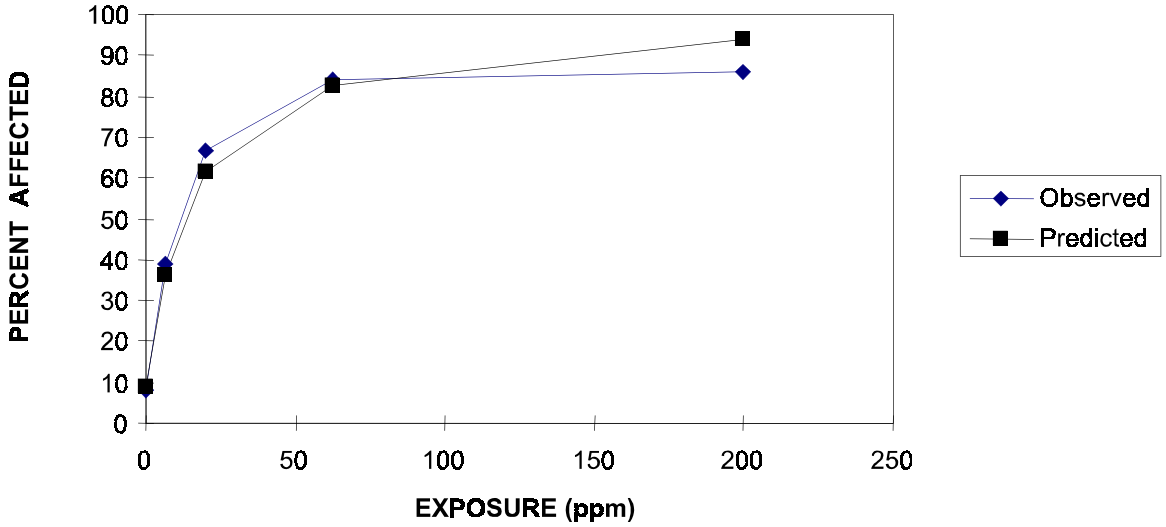

Figure 10-8. Ovarian atrophy (groups 1-5) using log-logistic model.

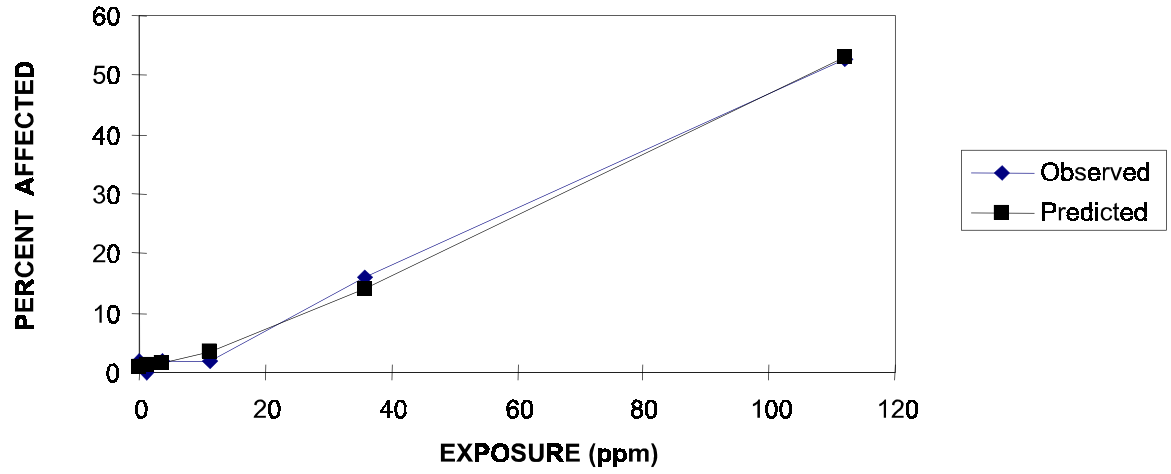

Figure 10-9. Uterine atrophy (groups 1-5) using quantal Weibull model.

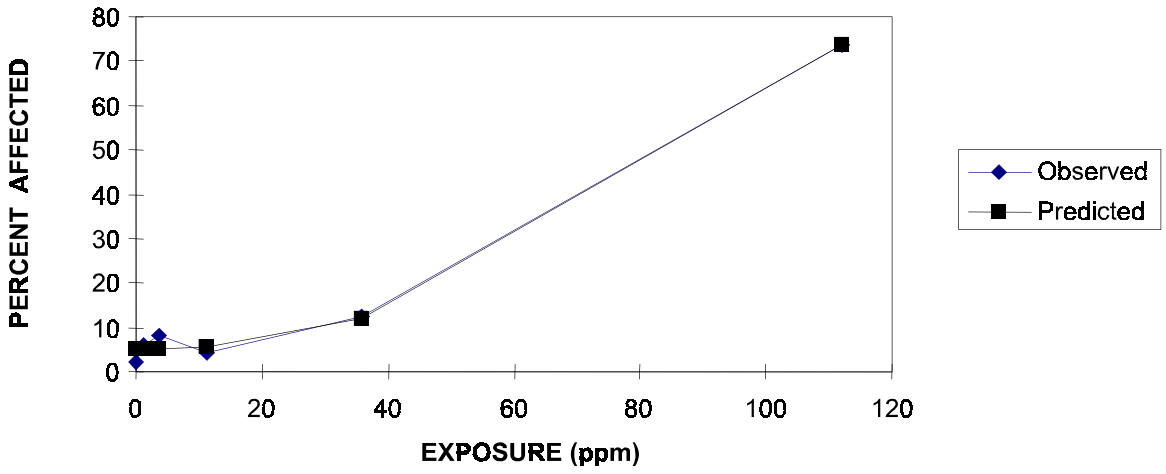

Figure 10-10. Testicular atrophy (groups 1-6) using quantal Weibull model.

Although some 9- and 12-month interim sacrifice data were available for ovarian, uterine, and testicular atrophy (Table 10-21), these were less than ideal for modeling because smaller numbers of animals were killed and not all dose groups were represented. In addition, some animals died or became moribund and were killed before the 2-year death time point (see Chapter 6). To include the interim data and to account for the variability in time of death, time-to-response analyses were done using the multistage Weibull model as discussed in Section 10.2.2.2. Exposures were adjusted to the equivalent continuous lifetime exposures. An EC_{10} and an LEC_{10} were calculated in each case. All the reproductive responses were treated as incidental, not fatal. Parameter estimates for each reproductive endpoint are presented in Table 10-23.

Results of the Weibull time-to-response model are shown in Table 10-24. The ECs and LECs were similar to those from other models used for ovarian atrophy, uterine atrophy, and testicular atrophy, with the exception of those from the modeling of testicular atrophy including the highest exposure group, for which the Weibull time-to-response model yields results roughly five times lower than the quantal Weibull model. The quantal Weibull model results for uterine and testicular atrophy were for additional risk, whereas the Weibull time-to-response results were for extra risk; however, because of the low background rates of both uterine and testicular atrophy, additional risk and extra risk should be nearly the same. The results of the time-to-response model are used in the calculation of potential RfCs.

The time-to-response model also allows for calculation of extra risks at ages less than full lifetime. For uterine and testicular atrophy, full 70-year lifetimes were used. For ovarian

Table 10-23. Parameters for Weibull time-to-response model used to model reproductive effects observed in mice based on ppm butadiene exposure[a].

Response	625 ppm group included	Q0	Q1	Q2	Z
Ovarian atrophy	no	4.86×10^{-6}	7.06×10^{-6}	—	2.21
	yes	9.01×10^{-7}	1.32×10^{-6}	—	2.58
Uterine atrophy	no	6.73×10^{-5}	5.28×10^{-5}	—	1.0
	yes	9.08×10^{-5}	9.74×10^{-6}	1.31×10^{-6}	1.0
Testicular atrophy	no	4.28×10^{-4}	2.24×10^{-5}	—	1.0
	yes	1.60×10^{-4}	1.52×10^{-4}	—	1.0

[a]Each response was considered to be incidental with induction time, T_0=0. See Section 10.2.2.2 on time-to-tumor modeling of the mouse carcinogenicity data for a discussion of the Weibull model structure and selection.

Table 10-24. Human benchmark 1,3-butadiene exposure concentrations calculated for reproductive effects observed in mice using a Weibull time-to-response model (extra risk).

Response	625 ppm group included[a]	Based on ppm continuous butadiene exposure	
		EC_{10}	LEC_{10}
Ovarian atrophy[b]	No	1.05	0.878
	Yes	1.13	0.958
Uterine atrophy	No	18.8	12.0
	Yes	24.0	15.6
Testicular atrophy	No	44.3	15.9
	Yes	6.54	5.39

[a] Because of the high early mortality in the 625 ppm exposure group, results from the models excluding this group will be used.
[b] Results for ovarian atrophy reflect extra risks only until 50 years of age (see text).

atrophy, however, extra risks were calculated for 50 years to reflect only the time before average age at menopause when follicles are no longer present and available for ovulation, because in the mouse studies of ovarian atrophy, the atrophy occurs as a result of follicular failure (see Chapter 5). For comparison purposes, calculations for the full 70 years yield EC_{10} = 0.50 ppm and LEC_{10} = 0.38 ppm for ovarian atrophy (625 ppm dose group excluded). Prenatal exposures may also

affect ovarian atrophy; however, there are no specific data for such exposures and they were not included explicitly in the analysis.

10.3.5. Summary and Conclusions

ECs and LECs were estimated for three types of exposure scenarios to 1,3-butadiene based on different endpoints:

1. Short-term exposure (10 days)—fetal weight reduction
2. Subchronic exposure (10 weeks)—male-mediated developmental toxicity
3. Chronic exposure—ovarian, uterine, and testicular atrophy

These analyses demonstrate approaches for estimation of ECs and LECs based on continuous and quantal data.

Results of the fetal weight analysis illustrate how both continuous and quantal modeling approaches can be used for continuous data. All of the LECs calculated were below the LOAEL of 40 ppm (i.e., 10 ppm adjusted for continuous exposure), except for two LECs calculated using the continuous power model, which were near this value. Since the log-logistic modeling approach is considered the preferred method for modeling continuous data, the LEC_{05} of 2.9 ppm from this model will be used for calculating the reference concentration for acute exposure.

Results of the combined analysis of subchronic studies of male-mediated developmental toxicity (dominant lethal effects) gave an EC_{05} of 15 ppm for all deaths and an LEC_{05} of 10 ppm. However, since the value for fetal weight is lower, it will also be used as the basis for the subchronic RfC.

Note that the LEC_{05} is selected as the appropriate "point of departure" for the dominant lethal effects and fetal weight reductions, whereas, immediately below, the LEC_{10} is used for ovarian atrophy. This is because the dominant lethal and fetal weight effects occur within litters. Studies with data nested within litters typically have sufficient power to observe levels of response of 5%. [Fetal weight is actually a continuous measure; however, for the log-logistic model recommended here, the data were dichotomized and the LEC_{05} corresponds with a 5% additional risk of obtaining a fetal weight below the 5^{th} percentile of the controls.] The ovarian atrophy data are from a cancer bioassay study, which typically has the power to detect 10% response levels. The "point of departure" is selected to be at the low end of the observable range; thus, a 5% response level is used for the nested data, and a 10% level for the data from the cancer bioassay (see also Section 10.4.2 below).

Modeling of the 2-year ovarian atrophy data, the effect occurring at the lowest chronic exposure level, gave a good fit with the log-logistic model, but only when the highest exposure

level was dropped. This approach was justified because the responses leveled off for the top three exposure groups. The LECs derived for a 10% increase in additional risk or extra risk were five- to sixfold below the LOAEL of 6.25 ppm (i.e., 1.1 ppm adjusted for continuous exposure). When the time-to-response model was applied to account for interim sacrifice data and early mortality, an LEC_{10} of 0.88 ppm (extra risk, up to age 50) was calculated, a value slightly higher than that using the log-logistic model. For the chronic effects of uterine and testicular atrophy, higher LEC_{10}s were obtained: 12 and 16 ppm, respectively.

There is strong evidence suggesting that the diepoxide metabolite (DEB) is responsible for 1,3-butadiene-induced ovarian atrophy (Doerr et al., 1996). Uterine atrophy may be secondary to ovarian atrophy, and thus may also be related to DEB levels. However, an adequate pharmacokinetic model was not available to estimate levels of DEB for dose-response modeling (Chapter 9).

Chronic RfC calculations will be made for both ovarian atrophy, the reproductive effect occurring at the lowest chronic exposure level, and testicular atrophy, the reproductive effect observed in male mice following chronic exposure.

10.4. REFERENCE CONCENTRATIONS FOR REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

10.4.1. Introduction

As discussed in Chapter 5 and Section 10.3, a variety of reproductive and developmental effects have been observed in mice exposed to 1,3-butadiene by inhalation. (No human reproductive or developmental data are available for 1,3-butadiene.) Some of these reproductive and developmental effects represent the most sensitive noncancer endpoints that have been observed in available toxicity studies. In this section, sample chronic reference concentrations (RfCs) are calculated for the most sensitive reproductive and developmental endpoints, that is, those effects exhibiting responses at the lowest exposure concentrations from various exposure scenarios, using both the traditional NOAEL/LOAEL approach and the "benchmark dose" approach (Crump, 1984). A reference concentration (or dose) is an estimate of a daily exposure to humans that is "likely to be without an appreciable risk of deleterious [noncancer] effects during a lifetime" (Barnes et al., 1988). The final reported chronic RfC will be based on the endpoint resulting in the lowest calculated RfC level. An RfC for acute exposure and an RfC for subchronic exposure will also be presented.

10.4.2. Calculation of RfCs

The most sensitive short-term developmental effect was decreased fetal weight in a prenatal developmental toxicity study in the mouse. The most sensitive reproductive effect

observed in subchronic exposure studies was increased prenatal deaths ("all deaths") in dominant lethal studies of mice (i.e., male mice were exposed to 1,3-butadiene and effects on litters were measured after mating to unexposed females). Dominant lethal effects in humans would likely be manifested as infertility (due to reduced fertility or very early deaths) or spontaneous abortions. From chronic exposure studies (2-year bioassays), the most sensitive reproductive endpoints were ovarian atrophy in female mice and testicular atrophy in male mice.

Table 10-25 summarizes the EC_{10}, and in some cases the EC_{05} (i.e., the exposure concentration resulting in a 10% [or 5%] increase in risk based on modeling the exposure-response data in the observable range), the LEC_{10}, and in some cases the LEC_{05} (i.e., the 95% lower confidence limit on the exposure concentration estimated to result in a 10% [or 5%] increase in risk), and the NOAEL (i.e., no observed adverse effect level) or LOAEL (i.e., lowest observed adverse effect level; reported when no NOAEL was observed) for these 1,3-butadiene-induced effects. Table 10-25 also provides sample calculations of RfCs using the NOAEL (or LOAEL), as well as the LEC_{10} (or LEC_{05}), as "points of departure." Uncertainty factors are applied to the "points of departure" to calculate the sample RfCs.

A 10% response level is the standard "benchmark response" for dichotomous data (or continuous data that have been dichotomized for analysis), and the corresponding LEC_{10} is the standard point of departure for such data. This response level is chosen because it is typically at the low end of the range of observation for increased responses in studies of experimental animals. However, in certain cases, other response levels may be more appropriate. For example, studies of developmental effects often have more power to detect effects at lower response levels because of their nested (fetuses within litters) study design, and a response level of 5% is typically within the range of observation (Allen et al., 1994b). Therefore, the LEC_{05} is used here for the point of departure for decreased fetal weight. Similarly, the dominant lethal data have a nested design, and an LEC_{05} is used as the point of departure for this endpoint as well.

For interspecies uncertainty, a factor of 10 is generally used when the "point of departure" is based on nonhuman data. However, when ppm equivalence across species is assumed for inhalation studies, as was done here, a factor of 3 is used instead. (Note that the assumption of ppm equivalence is identical to using EPA's inhalation dosimetry methodology with $RGDR_r$=1 [U.S. EPA, 1994c]). This convention assumes that pharmacokinetic differences have been accounted for but not pharmacodynamic differences. Thus, in Table 10-25 an interspecies uncertainty factor of 3 was used for all endpoints. For ovarian atrophy, evidence is convincing that the diepoxide metabolite (1,2:3,4-diepoxybutane, DEB) is required to elicit the effect (see Chapter 5), and it is expected, based on pharmacokinetic data, that humans produce less DEB than mice (see Chapter 3). However, DEB levels cannot be quantified without an adequate

Table 10-25. Points of departure and sample chronic RfC calculations for reproductive and developmental effects of 1,3-butadiene.

Effect	NOAEL[a] (or LOAEL) (ppm)	EC_{10}[a] (ppm)	LEC_{10}[a] (ppm)	Interspecies uncertainty factor	Intraspecies uncertainty factor	Acute/ subchronic-to-chronic uncertainty factor	LOAEL-to-NOAEL uncertainty factor	Effect level extrapolation factor[b]	Modifying factor for incomplete database[c]	RfC based on NOAEL (ppm)	RfC based on LEC_{10} (or LEC_{05}) (ppm)
Decreased fetal weight		EC_{05}=6.9 (EC_{10}=10)	LEC_{05}=2.9 (LEC_{10}=4.7)	3	10	1[d]		4	3		0.007
	10 (LOAEL) (GD 6-15)			3	10	1[d]	10		3	0.01	
All prenatal deaths (dominant lethal effect)		EC_{05}=15 (EC_{10}=31)	LEC_{05}=10 (LEC_{10}=21)	3	10	1[f]		5	3		0.02
	2.2[e] (10 week)			3	10	1[f]	1		3	0.02	
Ovarian atrophy		1.0[g]	0.88[g]	3	10	1		10	3		0.0009
	1.1 (LOAEL) (2 year)			3	10	1	10		3	0.001	
Testicular atrophy		44	16	3	10	1		10	3		0.02
	36 (2 year)			3	10	1	1		3	0.4	

[a] Adjusted to 24-h daily exposure for the exposure period. Ppm equivalence across species was assumed; this is the same as using U.S. EPA's inhalation dosimetry methodology with $RGDR_r$=1 (U.S. EPA, 1994c).

[b] For benchmark concentrations - to decrease risk to below the benchmark response level; analogous conceptually to the LOAEL-to-NOAEL uncertainty factor.

[c] Reflects primarily the absence of a multigenerational reproductive study and neurodevelopmental studies.

[d] Although from acute study, only exposure during gestation is assumed to be relevant to fetal weight.

[e] Although this exposure level was a LOAEL in one study, it was a NOAEL in the other two, and the combined analysis of the three studies did not find the increase in prenatal deaths at this level to be statistically significant.

[f] Although from subchronic studies, exposure appears to affect only post-meiotic stages of spermatogenesis, thus dominant lethal effects are not expected to be cumulative with exposure.

[g] Extra risk has been calculated for a 50-year exposure up to the time of menopause, after which ovarian atrophy from follicular loss is not expected to be an adverse health effect for humans.

physiologically based pharmacokinetic (PBPK) model, thus default dosimetry (i.e., 1,3-butadiene exposure concentration) was used for dose-response modeling and the default value of 1 for the pharmacokinetic portion of the interspecies uncertainty factor was retained.

A large degree of human variability has been observed in metabolic activities that could affect 1,3-butadiene toxicity. For example, Seaton et al. (1995) measured a 60-fold variation in the initial rate of oxidation of 1,2-epoxy-3-butene (EB) to DEB in microsomes from 10 different human livers. However, overall variability in total metabolism and susceptibility is unknown, thus the conventional intraspecies uncertainty factor of 10 for human variability was used for each endpoint in Table 10-25.

With respect to the acute/subchronic-to-chronic uncertainty factor, none was needed for ovarian or testicular atrophy because these effects were based on chronic studies. No acute-to-chronic uncertainty factor was used for fetal weight either, because only exposures during gestation are relevant. Similarly, the dominant lethal effects appear to occur with exposure during a specific time period of spermatogenesis (i.e., only postmeiotic stages of developing sperm appear susceptible), and results after a 4-week exposure were similar to those from a 10-week exposure. Thus, even though these effects were observed in subchronic studies, no subchronic-to-chronic uncertainty factor is used for the dominant lethal effects either, since the effects are not expected to be cumulative with exposure.

Under the NOAEL/LOAEL approach, the NOAEL is defined as the exposure level for which there is no observed adverse effect, although it is circumscribed by the detection limit of the study. For endpoints for which there is no NOAEL, an uncertainty factor of 10 is typically used to attempt to extrapolate from the LOAEL to a level at which there are presumed to be no detectable effects. In the benchmark dose approach, the "points of departure" used here correspond to 5% and 10% increased response levels, which are explicitly not no-effect levels, and an analogous uncertainty factor is needed. EPA is planning to develop guidance for calculating an "effect level extrapolation factor" to be used as an uncertainty factor to decrease the risk below the effect level at the point of departure. Pending final guidance on this uncertainty factor, in this risk assessment we use a formula that accounts for the slope of the dose-response curve and generates a factor between 3 and 10 for use with 10% response levels and between 3 and 5 for a 5% response level. The formula is as follows: uncertainty factor = $x \times$ (slope of the line from the EC_x to 0)/(slope of the dose-response curve at the EC_x), where x% is the response level. Results of the formula are confined within a minimum value of 3 and a maximum value of x.

A modifying factor of 3 was applied to all the sample calculations to reflect the fact that the database for calculating the RfCs is incomplete. In particular, a multigeneration reproductive study and neurodevelopmental studies are lacking.

The sample chronic RfCs in Table 10-25 suggest that ovarian atrophy is the most sensitive reproductive/developmental endpoint (i.e., the "critical" effect), and thus, should be the basis for the final chronic RfC. Based on the critical effect of ovarian atrophy and the benchmark dose methodology, a final chronic RfC of 0.0009 ppm, or 0.9 ppb, is adopted.

In addition to the chronic RfC, two "RfC"s were calculated for acute and subchronic exposures. (EPA is currently developing guidance for deriving reference values for acute and subchronic exposure scenarios. These values are not technically RfCs, because RfCs are by definition for chronic exposure scenarios.) An "RfC" for acute exposure of 0.007 ppm, or 7 ppb, was calculated for decreased fetal weight, based on the benchmark dose methodology and using the same factors as those depicted in Table 10-25. This acute "RfC" is identical to the sample chronic RfC calculated for decreased fetal weight because no subchronic-to-chronic uncertainty factor was used in that calculation. A multigenerational reproductive study would not be considered essential for a complete database for an acute reference value; however, an uncertainty factor of 3 for incomplete database is still warranted because of the absence of neurodevelopmental studies. Effects observed in such studies may result from acute exposures during a sensitive period of development. Finally, an "RfC" for subchronic exposure was calculated. As shown in Table 10-25, the sample chronic RfC for the prenatal death endpoint from the subchronic dominant lethal studies, using the benchmark dose methodology, was 0.02 ppm. Because no subchronic-to-chronic uncertainty factor was used here either, this value is can be considered a sample "RfC" for subchronic exposure, without adjustment. However, this value is higher than the acute "RfC" of 7 ppb from the fetal weight data. Therefore, 7 ppb is used for the "RfC" for subchronic exposure as well.

10.4.3. Discussion

There are substantial uncertainties in estimating low-exposure human risks for noncancer effects observed in animals exposed to high concentrations of an agent. It is generally believed that there is a sublinear low-dose exposure-response relationship for noncancer effects, and perhaps a threshold, although this is difficult to demonstrate empirically. The shape of this low-dose exposure-response relationship is unclear, however, and thus RfCs are calculated for most noncancer effects rather than exposure-based risk estimates. Uncertainties related to the shape of the exposure-response curve still pertain to RfCs, however. It is anticipated that by using NOAELs or LECs coupled with an effect level extrapolation factor, as well as the other uncertainty factors, the ultimate chronic RfC represents an exposure level "likely to be without appreciable risk of deleterious effects during a lifetime." Note, however, that in situations in which most of the uncertainty factors do not apply, e.g., if the chronic RfC is based on good-quality human data from a diverse population, the resulting value may not actually be far enough

down the sublinear part of the exposure-response curve to ensure that it is "likely to be without appreciable risk."

Major uncertainties considered in deriving a chronic RfC include extrapolation of effects observed in laboratory animals to humans (interspecies extrapolation), potential existence of sensitive human subpopulations resulting from human (intraspecies) variability, extrapolation of effects observed from acute or subchronic exposures to chronic exposure scenarios, and various deficiencies in the database. These areas of uncertainty are addressed to some extent by the uncertainty factors. Other methodological uncertainties arise in determination of the "point of departure" and in selection of the relevant exposure metric for equating experimental animal exposure-response relationships to humans.

There are a number of limitations in using the NOAEL/LOAEL approach for obtaining a "point of departure"; these have inspired development of an alternative "benchmark dose" (or concentration) methodology. First, the NOAEL/LOAEL approach relies on one exposure level and ignores the rest of the exposure-response data. Second, the NOAEL/LOAELs depend explicitly on the specific exposure levels selected for the study. They are also a function of study power because a LOAEL is the lowest exposure level with a statistically significant increase in an adverse effect, whereas a NOAEL could represent an increase that lies below the study's limit of detection. Finally, NOAEL/LOAELs are not readily comparable across endpoints or studies because they can refer to different response levels.

The alternative benchmark concentration approach involves modeling the full exposure-response curve in the observable range and calculating an effective concentration (EC) corresponding to some level of response (e.g., 10%) that can be used as a point of comparison across endpoints and studies (the 10% effect level is typically at the low end of the observable range, although sometimes a lower level of response can be estimated). The 95% lower bound on the EC is used as the "point of departure" to take into account statistical variability around the EC estimate. While the benchmark concentration approach alleviates some of the limitations of the NOAEL/LOAEL approach, there are still uncertainties regarding the appropriate exposure-response model to use. It is generally expected that models that provide a good fit to the data in the observable range should yield reasonably similar EC_xs, as shown for quantal models by Allen et al. (1994b).

As shown in Table 10-25, these two approaches yielded nearly identical sample RfCs for decreased fetal weight, prenatal deaths (dominant lethal effect), and ovarian atrophy. For testicular atrophy, the NOAEL-based sample RfC is about 20 times higher than the LEC_{10}-based sample RfC. At least part of this discrepancy is likely attributable to the fact that the time-to-response modeling conducted to derive the LEC_{10} took into account the decreased survival times in the higher exposure groups in the chronic study, thus increasing the effective percent with

testicular atrophy in the midrange of the exposure-response curve, which otherwise is fairly flat. This assessment adopts the use of the benchmark dose/concentration approach as the superior methodology in general.

Uncertainties also exist in the choice of exposure metric. Ideally, NOAELs or LOAELs and LEC_xs should be converted to appropriate human equivalent exposures before using these exposure levels as "points of departure." Theoretically, this is best accomplished by using a PBPK model to convert animal exposures to biologically effective doses to the target organ and then to convert these tissue concentrations back to human exposures to the parent compound. Unfortunately, current PBPK data and models are inadequate for use in risk assessment; therefore, exposure concentrations of 1,3-butadiene are used as the default exposure metric (this risk assessment assumes equivalence of effects from equivalent ppm exposures across species). For the lifetime chronic exposure study, demonstrating ovarian and testicular atrophy, mouse exposure concentrations were adjusted to human equivalent continuous chronic exposures.

For the subchronic and acute studies, however, the appropriate timeframe for exposure averaging is less clear. In this assessment, daily exposures have been adjusted to an equivalent 24-h exposure for the exposure period (e.g., exposure to 10 ppm, 6 h/day, 5 days/week, would be adjusted to 10 ppm (6/24)(5/7) = 1.8 ppm continuous daily exposure). However, the exposure concentrations for these subchronic and acute effects have not been adjusted to reflect total duration of exposure because the critical timeframes are unknown. Thus, for example, a 1-day exposure is treated equivalently to a 10-week exposure to the same daily level.

10.4.4. Conclusions

A chronic RfC of 0.9 ppb was adopted for the critical endpoint of ovarian atrophy, based on mouse data. This reference concentration, the uncertainties discussed above notwithstanding, is presumed to represent a daily exposure to humans that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. In addition, an "RfC" for acute exposure of 7 ppb and an "RfC" for subchronic exposure of 7 ppb were calculated based on fetal weight data for mice. Each of these RfCs was obtained using benchmark concentration methodology.

10.5. CONCLUSIONS ON QUANTITATIVE RISK ESTIMATES

In this chapter, a lifetime extra unit cancer risk of 0.027 per ppm of continuous 1,3-butadiene exposure was calculated based on linear modeling and extrapolation of the excess leukemia mortality reported in a high-quality occupational epidemiology study of male workers. Applying the same linear relative rate model and actuarial program to background leukemia *incidence* rates resulted in an excess cancer unit risk estimate of 0.04 per ppm for leukemia incidence. However, results in experimental animal studies suggest that 1,3-butadiene is a

multisite carcinogen and that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males; therefore, the leukemia results based on male workers may underestimate the total cancer risk to the general population, in particular females. Thus, an adjustment factor of 2 was used, yielding a ***lifetime extra cancer unit risk estimate of 0.08/ppm***. Using this cancer potency estimate, the chronic exposure level resulting in an increased cancer risk of 10^{-6} can be estimated as follows:

$$(10^{-6})/(0.08/\text{ppm}) = 1 \times 10^{-5} \text{ ppm} = 0.01 \text{ ppb}.$$

A range of human cancer potency estimates from 4×10^{-3}/ppm to 0.29/ppm was also calculated based on a variety of tumors observed in mice and rats exposed to 1,3-butadiene. These risk estimates are considered inferior to those based on the epidemiologic data, primarily because of the large uncertainties in extrapolating 1,3-butadiene cancer risks across species in light of the large unexplained differences in responses of rats and mice.

In addition, benchmark concentrations and reference concentrations were calculated for an assortment of reproductive and developmental effects observed in mice exposed to 1,3-butadiene. These effects represent the most sensitive noncancer endpoints that have been observed in available toxicity studies. A ***chronic RfC of 0.9 ppb*** was adopted for the critical effect of ovarian atrophy observed in mice, using a benchmark concentration approach to obtain the "point of departure." The RfC is presumed to be a chronic exposure level without "appreciable risk" of deleterious noncancer effects. This chronic RfC of 0.9 ppb is about 90 times higher than the chronic exposure level estimated for a 10^{-6} cancer risk.

Finally, an ***"RfC" for acute exposure of 7 ppb*** and an ***"RfC" for subchronic exposure of 7 ppb*** were calculated from mouse fetal weight data, each using benchmark concentration methodology.

11. DOSE-RESPONSE CHARACTERIZATION

Cancer potency estimates of lifetime extra cancer risk in humans were derived from both human and animal data. In addition, after benchmark dose modeling of a variety of reproductive and developmental effects, which are the most sensitive noncancer endpoints that have been observed in available toxicity studies, a chronic reference concentration (RfC) was calculated based on the critical effect of ovarian atrophy, observed in female mice. "RfC"s for acute and subchronic exposures were also calculated, based on reduced fetal weight in mice.

Several physiologically based pharmacokinetic (PBPK) models have been developed to attempt to explain the interspecies differences in the potency and site specificity of the carcinogenic response between mice and rats and to provide a dosimetric basis for quantitatively extrapolating carcinogenic (or reproductive) risks from rodents to humans. While progress has been made in recent years, our understanding of the bases for the species differences in 1,3-butadiene carcinogenicity is still inadequate, and major uncertainties in the existing PBPK models preclude their use in human risk assessment at this time (see Chapter 9).

11.1. QUANTITATIVE RISK ESTIMATION FOR CANCER

Lifetime extra cancer risk is estimated to be about 0.08 per ppm continuous 1,3-butadiene exposure, based primarily on human data. When adequate human data are available, as is the case with 1,3-butadiene, it is generally preferable to base cancer risk estimates on the human data rather than on data from experimental animals because of the inherent uncertainties associated with interspecies extrapolation. Cancer risk estimates were calculated for increased leukemia risk from the Delzell et al. (1995) study of 1,3-butadiene polymer workers. There were insufficient exposure-response data to calculate a lymphoma risk estimate from the monomer cohorts. Cancer risk estimates based on the experimental animal data are also presented for comparison purposes (see below).

The Delzell et al. (1995) retrospective cohort study of more than 15,000 male styrene-butadiene rubber production workers provides high-quality epidemiologic data on leukemia mortality risk from 1,3-butadiene exposure. In this study, 1,3-butadiene exposure was estimated for each job and work area for each study year, and these estimates were linked to workers' work histories to derive cumulative exposure estimates for each individual worker. Subsequent to the Poisson regression analyses by Delzell et al. (1995), which used four different mathematical models to fit the exposure-response data, Health Canada obtained the data on this cohort and performed their own analyses. The Health Canada analyses were similar to those of Delzell et al., but involved some minor refinements, and it is the Health Canada analyses that are used for this risk assessment (Health Canada, 1998).

The results were adjusted for age, calendar period, years since hire, and cumulative styrene exposure. Benzene exposure was also estimated for each worker but was not found to be a confounder and, hence, was not included in the models. Risk estimates were made using the relative rate models and an actuarial program that accounts for the effects of competing causes of death. U.S. age-specific mortality rates for all race and gender groups combined (NCHS, 1996) were used to specify the leukemia and all-cause background rates. Excess leukemia mortality risks were computed up to age 85 for continuous 1,3-butadiene exposures. The occupational exposures in the epidemiology study were converted to continuous exposures by adjusting for the differences in the number of days exposed per year (240/365 days) and differences in the amount of (contaminated) air inhaled per day (10/20 m^3).

Consistent with EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986) and evidence of the genotoxicity of 1,3-butadiene, the linear relative rate exposure-response model was used to calculate a maximum likelihood estimate (MLE) of 1.3×10^{-2}/ppm (or 1×10^{-2}/ppm, rounded to one significant figure) for lifetime extra risk of leukemia mortality from continuous environmental 1,3-butadiene exposure. The corresponding 95% upper limit on unit risk is 0.03/ppm.

Alternatively, interpreting the proposed new carcinogen risk assessment guidelines (U.S. EPA, 1996, 1999), linear extrapolation from the LEC_{01} (i.e., the 95% lower confidence limit of the exposure concentration associated with a 1% increased risk) is warranted given the clear genotoxicity of 1,3-butadiene and the fact that a 1% increase in risk is within the range of the epidemiologic data. The models presented by Health Canada yield LEC_{01} values ranging from 0.037 to 0.87 ppm. Using a linear extrapolation and the LEC_{01} as the "point of departure," the corresponding cancer potency estimates range from 0.011/ppm to 0.27/ppm. While various models adequately fit the data in the range of observation, there is no compelling reason to deviate from the linear model historically used for human data. Therefore, the potency estimate of 0.027/ppm from the linear model (LEC_{01} = 0.375 ppm) was used to represent the extra risk of leukemia mortality from the occupational data. Applying the same linear relative rate model and actuarial program to background leukemia *incidence* rates resulted in an excess cancer unit risk estimate of 0.04 per ppm for leukemia incidence.

An adjustment factor of 2 was then applied to this unit risk estimate to reflect evidence from rodent bioassays suggesting that extrapolating the excess risk of leukemia in a male-only occupational cohort may underestimate the total cancer risk from 1,3-butadiene exposure in the general population. First, studies in both rats and mice indicate that 1,3-butadiene is a multisite carcinogen. It is possible that humans exposed to 1,3-butadiene may also be at risk of cancers other than leukemia and that the epidemiologic study had insufficient power to detect excess

Table 11-1. Estimates of upper bounds on human extra unit cancer risk (potency) from continuous lifetime exposure to 1,3-butadiene based on animal inhalation bioassays.

Species	Sex	Tumor sites/types	Upper bound on potency (ppm^{-1})
Rat[a]	M	Leydig cell, pancreatic exocrine cell, Zymbal gland	4.2×10^{-3}
	F	Mammary gland, thyroid follicular cell, Zymbal gland	5.6×10^{-2}
Mouse[b]	M	Lymphocytic lymphomas, histiocytic sarcomas, heart hemangiosarcomas, lung, forestomach, Harderian gland, liver, preputial gland	0.22
	F	Lymphocytic lymphomas, heart hemangiosarcomas, lung, forestomach, Harderian gland, liver, ovary, mammary gland	0.29
	M	Lymphocytic lymphomas	6.4×10^{-3}
	F	Lymphocytic lymphomas	2.4×10^{-2}

[a]From U.S. EPA's 1985 assessment, based on the Hazleton (1981b) study; linearized multistage model.
[b]Based on 1993 NTP study; Weibull multistage time-to-tumor model.

risks at other sites. Second, both the rat and mouse studies suggest that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males (see, e.g., Table 11-1), and the female mammary gland was the only 1,3-butadiene-related tumor site common to both species. For the female mouse, which is the most sensitive sex and species tested, the MLE of cancer potency for the mammary gland is 0.02/ppm and the 95% upper confidence limit is 0.03/ppm (see Section 10.2.2.2). Applying a twofold adjustment to the potency estimate of 0.04/ppm derived for leukemia incidence from the occupational epidemiologic study yields a cancer potency estimate of 0.08/ppm, which roughly corresponds to a combination of the human leukemia and mouse mammary gland tumor risk estimates, addressing the concerns that the leukemia risk estimated from the occupational data may underestimate total cancer risk to the general population, in particular females. It is this excess cancer unit risk estimate of 0.08/ppm, derived using the 1996 and 1999 proposed guidelines linear default methodology and a twofold adjustment factor, that is adopted in this assessment. (See Section 10.1 for additional details about the derivation of this risk estimate.)

For comparison purposes, human unit cancer risk estimates based on extrapolation from the results of lifetime animal inhalation studies are summarized in Table 11-1. These potency estimates are 95% upper confidence limits on unit extra cancer risk calculated from incidence data on all significantly elevated tumor sites using a linearized low-dose extrapolation model. Such estimates are generally considered by EPA to represent plausible upper bounds on the extra

unit cancer risk to humans. The rat-based estimates are from EPA's 1985 assessment and use the linearized multistage model, consistent with the 1986 Guidelines. The mouse-based estimates use a Weibull multistage time-to-tumor model with the low-dose extrapolation methodology of the 1986 Guidelines because it is more amenable to the methodology used for combining risk estimates from different tumor sites (see Section 10.2.2.2). Table 11-1 also includes unit risk estimates based only on the lymphocytic lymphomas in mice, because this was the tumor type in rodents most analogous to the lymphohematopoietic cancers observed in workers exposed to 1,3-butadiene.

Human health risk estimates based on extrapolation from high-quality epidemiologic results are preferable to those based on rodent data because the uncertainties inherent in extrapolating across species are avoided and, typically, the human exposures in epidemiologic studies are closer to anticipated environmental exposures than the high exposures used in animal studies, thus the extent of low-dose extrapolation is reduced. In the case of 1,3-butadiene, while the rat exposures were at least 100-fold higher than human exposures, the lowest exposure in the 1993 NTP mouse study (4.7 ppm, 8 h time-weighted average [TWA]) is within the range of occupational exposures (0.7-1.7 ppm median and 39-64 ppm max 8 h TWAs for work-area groups). However, interspecies differences in tumor sites and susceptibilities between rats and mice are especially pronounced, and the biological bases for these differences are unresolved. A review of available pharmacokinetic data and models revealed that the state of the science is currently inadequate for explaining interspecies differences or improving on default dosimetry assumptions. Therefore, the quantitative extrapolation of rodent risks to humans is highly uncertain for 1,3-butadiene.

Even though high-quality human data were used for the quantitative cancer risk estimation for 1,3-butadiene, there are inevitable uncertainties in the calculated risk estimate. *First, there are uncertainties inherent in the epidemiologic study itself.* In particular, there are uncertainties in the retrospective estimation of 1,3-butadiene exposures, which could have resulted in exposure misclassification. Nondifferential exposure misclassification would tend to bias estimates of effect toward the null, resulting in an underestimate of risk. Differential misclassification could bias results in either direction.

Nonetheless, a great deal of effort and detail went into estimating exposures in the Delzell et al. (1995) study. The investigators reviewed relevant plant records and conducted plant walk-throughs and interviews with plant experts and long-term employees (at least 2 employees with work experience for each work area and time period) (Macaluso et al., 1996). They also assessed determinants of exposure for specific component tasks (e.g., frequency and duration of task, work practice, distance from emission source) in each job at different time periods. Then they developed exposure models to estimate exposure intensities associated with specific tasks in

different time periods. There was some validation of these exposure models in one plant. Finally, the job-exposure matrices were linked to each worker's work history to calculate cumulative exposure estimates for each individual worker. Furthermore, these individual cumulative exposure estimates were derived with the investigators "blind" to the mortality data for the individuals.

However, as discussed in Section 10.1.3, some concerns were later raised by the investigators about the accuracy of the exposure estimates (letters from Elizabeth Moran, CMA, March 25, 1996, and Delzell and Macaluso, University of Alabama, April 2, 1996, to Aparna Koppikar). In 2000, Delzell et al. completed a re-assessment of exposure estimates. Analyses performed by Robert Sielken, a consultant to the American Chemistry Council who had access to the exposure-response data, suggest that the revised exposure estimates result in about a 70% decrease in the leukemia risk estimate (Sielken and Valdez-Flores, 2001). It should be noted, though, that Sielken's analyses differ somewhat from those of EPA (e.g., Sielken based his linear extrapolation on the EC_{01} rather than on the LEC_{01}), thus the Agency's results with the revised exposure estimates may differ slightly from the 70% decrease.

EPA is considering conducting a critical review of both the revised exposure estimates as well as the a priori estimates. If EPA concludes that the exposure re-assessment yields more credible exposure estimates, the Agency will consider modifying the cancer risk estimate, provided that the revised exposure estimates have a substantial impact. EPA does not at this time have the data needed to conduct a comparison cancer risk analysis based on the revised exposure estimates. If the revised exposure estimates are an improvement over the a priori estimates and EPA's analysis yielded the same results as Sielken's, then the leukemia risk estimate would decrease about 70%. However, EPA would still apply an adjustment factor that would effectively increase the leukemia risk estimate by 0.03-0.04/ppm to account for possible cancer risk at other sites, in particular the female mammary gland. Thus, under the above assumptions, the decrease to the total cancer unit risk estimate would be closer to 40%.

Second, there are uncertainties regarding the appropriate dose metric for dose-response analysis. Although the dose surrogate of cumulative exposure (i.e., ppm × years) yielded highly statistically significant exposure-response relationships, cumulative exposure is strongly correlated with other possible exposure measures, and there may be a dose-rate effect (e.g., risk at high exposures may be more than proportionately greater than at lower exposures) obscured in the analysis, or operative at exposures below the observable range but relevant to low-dose extrapolation.

Third, there are uncertainties pertaining to the model to use for the epidemiologic data. Several mathematical models adequately fit the exposure-response data from the epidemiology study, and because the specific mechanisms of 1,3-butadiene carcinogenesis are unknown, there

is no biological basis for choosing one model over another. The linear model was chosen in this risk assessment to derive the "point of departure" for low-dose extrapolation because there was no compelling reason to deviate from historical approaches.

Fourth, it is uncertain which potential modifying or confounding factors should be included in the model. The linear model of Health Canada, which is used in this risk assessment, adjusted for age, calendar year, years since hire, race, and exposure to styrene. Plant and benzene exposure were ruled out as potential confounders. However, there may be other relevant factors that were not included in the models.

Fifth, there are uncertainties in the parameter estimates used in the models. The study of Delzell et al. (1995, 1996) is large, providing some degree of reliability in the parameter estimates; however, especially given the large human variability that has been observed in metabolic activities that could affect cancer risk from 1,3-butadiene exposure, the generalizability of the occupational results is unclear.

Sixth, there are uncertainties in extending the relative rate models from the epidemiology study to derive lifetime excess leukemia incidence unit risk estimates for the U.S. population. Notwithstanding, the actuarial-type analysis that was used is a well-established methodology, and the background leukemia incidence rates and mortality rates used in the analysis are from large national databases.

Seventh, the precise model for low-dose extrapolation is unknown. The linear default extrapolation procedure in the 1996 proposed guidelines (U.S. EPA, 1996) was used in this assessment because of the well-established genotoxicity of 1,3-butadiene, via its metabolites.

In addition, important concerns are raised by comparison with the rodent data. First, the rodent studies suggest that 1,3-butadiene is a multisite carcinogen (see Chapter 6). It is possible that humans may also be at risk of 1,3-butadiene-induced carcinogenicity at other sites and that the epidemiologic study had insufficient power to detect the other excess risks. In the mouse, for example, the lung is the most sensitive tumor site. Significant excesses of lung cancer may not have been detectable in the epidemiologic study because of the high background rates of lung cancer in humans. A crude "power" calculation presented in Section 10.1.3 suggested that if humans were as sensitive as female mice to the lung cancer effects of 1,3-butadiene, one might have expected to see 26 *excess* lung cancer cases in the epidemiology study. However, the statistical power of the study to detect a corresponding SMR of 108 is estimated to be only 42%. The epidemiology-based excess cancer risk estimate of 0.04/ppm, which is based only on leukemias, may be an underestimate if other sites are also at risk.

Second, both the rat and mouse studies suggest that females are more sensitive to 1,3-butadiene-induced carcinogenicity than males, and the mammary gland in females was the only tumor site common to both species. If female humans are also more sensitive than males,

then the male-based risk estimates calculated from the epidemiology study would underestimate risks to females. Because of these concerns, an adjustment factor of 2 is used, as discussed above, yielding a cancer potency estimate of 0.08/ppm.

Despite these uncertainties, confidence in the excess cancer risk estimate of 0.08/ppm is moderate. First, the estimate is based primarily on human data. Furthermore, these data are from a large, high-quality epidemiologic study in which 1,3-butadiene exposures were estimated for each individual *a priori* to conducting the exposure-response analysis. Although there are uncertainties in the exposure estimation, a serious attempt was made to reconstruct historical exposures for specific tasks and work areas. It is virtually unprecedented to have such a comprehensive exposure assessment for individual workers in such a large occupational epidemiologic study. In addition, the assumption of linearity for low-dose extrapolation is reasonable given the clear evidence of genotoxicity by 1,3-butadiene metabolites.

Using the cancer potency estimate of 0.08/ppm, the lifetime average exposure level resulting in an increased cancer risk of 10^{-6} (i.e., one in a million) can be estimated as follows: $(10^{-6})/(0.08/\text{ppm}) = 1 \times 10^{-5}$ ppm = 0.01 ppb.

11.2. QUANTITATIVE ESTIMATION (RfC) FOR NONCANCER EFFECTS

A chronic RfC of 0.9 ppb was obtained for the critical effect of ovarian atrophy, from a chronic lifetime bioassay of female mice. In addition, an "RfC" for acute exposures of 7 ppb and an "RfC" for subchronic exposures of 7 ppb were calculated based on fetal weight data for mice. A chronic RfC is an estimate of the daily exposure to humans that is "likely to be without appreciable risk of deleterious (noncancer) effects during a lifetime." The RfC is calculated for the "critical (noncancer) effect," that is, the effect for which an increased response is observed at the lowest concentration used in the study, or for which benchmark concentration modeling yields the lowest EC_{10}, or the effect which yields the lowest concentration after application of the relevant uncertainty factors. In this assessment, sample RfC calculations were performed for several reproductive and developmental effects, because these represent the most sensitive noncancer endpoints observed in the available toxicity studies.

Of the 1,3-butadiene noncancer effects, the critical effect was ovarian atrophy, from a chronic lifetime bioassay of female mice. The RfC calculation was based on the LEC_{10} (0.88 ppm), which was derived from data from the 1993 NTP 2-year bioassay using benchmark concentration methodology (Weibull time-to-response model), and uncertainty factors for interspecies extrapolation (3), intraspecies variability (10), "effect level extrapolation" to extrapolate to a level below the 10% effect level (analogous to the LOAEL-to-NOAEL uncertainty factor) (10), and incomplete database (3). The LEC_{10} was derived from a chronic bioassay; therefore, an acute/subchronic-to-chronic factor was not required. A factor of 3 (the

standard factor for the pharmacodynamic portion) was used for the interspecies uncertainty factor because the use of "ppm equivalence" in determining the human equivalent exposures is assumed to account for the pharmacokinetic portion. Note that the assumption of ppm equivalence is the same as using EPA's inhalation dosimetry methodology with $RGDR_r$=1 (U.S. EPA, 1994c). There is strong evidence that the ovarian atrophy is caused by the diepoxide metabolite of 1,3-butadiene (DEB; see Chapter 5), and it is expected, based on pharmacokinetic data, that humans produce less DEB than mice (see Chapter 3). However, PBPK models are inadequate to quantify levels of DEB (see Chapter 9), thus default dosimetry (i.e., 1,3-butadiene exposure concentration) was used for dose-response modeling and the default value of 1 for the pharmacokinetic portion of the interspecies uncertainty factor was retained. The effect level extrapolation factor was derived from a formula that takes into account the slope of the exposure-response curve at the "point of departure." However, because the slope was supralinear at the LEC_{10}, a maximum factor of 10 for the 10% level was used (see Section 10.4). The default factor of 10 was used for intraspecies variability, and a factor of 3 was used to reflect an incomplete database, in particular the absence of a multigenerational reproductive study and a developmental neurotoxicity study. The resulting chronic RfC is 0.9 ppb (i.e., 0.88 ppm/(3 × 10 × 10 × 3)).

Thus, a chronic RfC of 0.9 ppb is adopted for the critical effect of ovarian atrophy. The actual risks at low exposure levels are unknown; the chronic RfC merely provides a bound on chronic exposure below which no "appreciable risk" of noncancer effects is expected.

In addition, an "RfC" for acute exposures of 7 ppb and an "RfC" for subchronic exposures of 7 ppb were calculated from the mouse fetal weight data (Hackett et al., 1987b), using benchmark concentration methodology (log-logistic model) to obtain the "point of departure" for applying uncertainty/modifying factors. For the fetal weight data, an LEC_{05} = 2.9 ppm was obtained, and uncertainty/modifying factors for interspecies extrapolation (3), intraspecies variability (10), "effect level extrapolation" to extrapolate to a level below the 5% effect level (analogous to the LOAEL-to-NOAEL uncertainty factor) (4), and incomplete database (3) (e.g., the absence of neurodevelopmental studies), were applied, yielding 7 ppb (i.e., 2.9 ppm/(3×10×4×3)). These RfC values are summarized in Table 11-2.

11.3. SUMMARY AND CONCLUSIONS

The estimate of human lifetime extra cancer risk from chronic exposure to 1,3-butadiene is 0.08 per ppm based primarily on linear modeling and extrapolation of the increased leukemia risks observed in occupationally exposed workers. Although there is uncertainty in extrapolating from occupational exposures to lower environmental exposures, this risk estimate has the advantage of being based on a large, high-quality *human* study, and linear extrapolation is warranted by the known genotoxicity of 1,3-butadiene metabolites. This cancer potency estimate

Table 11-2. Reference concentrations (RfCs) for 1,3-butadiene, based on mouse data.

Type of RfC	Critical effect	Point of departure[a]	Uncertainty factors[b]	RfC (ppb)
Chronic	Ovarian atrophy	LEC_{10} = 0.88 ppm	3 × 10 × 1 × 10 × 3 (1000)	0.9
Subchronic	Fetal weight	LEC_{05} = 2.9 ppm	3 × 10 × NA × 4 × 3 (400)	7
Acute	Fetal weight	LEC_{05} = 2.9 ppm	3 × 10 × NA × 4 × 3 (400)	7

[a]The point of departure (or "benchmark dose") is the 95% lower bound on the exposure concentration corresponding to an effect level at the low end of the range of observations (in this case a 5% or 10% effect level).
[b]Interspecies × intraspecies × acute/subchronic-to-chronic × effect level extrapolation × incomplete database (total); see text and Section 10.4 for explanations.
NA: not applicable.

incorporates an adjustment factor of 2 to address concerns that the leukemia risk estimate from a male worker population may underestimate total cancer risk to the general population. The corresponding estimate of the chronic exposure level of 1,3-butadiene resulting in an extra cancer risk of 10^{-6} (i.e., one in a million) is 0.01 ppb.

1,3-Butadiene also causes a variety of reproductive and developmental effects in mice; no human data on these effects are available. The most sensitive effect was ovarian atrophy observed in a lifetime bioassay of female mice (LEC_{10} = 0.88 ppm). Based on this critical effect and using benchmark concentration methodology, an RFC (i.e., a chronic exposure level presumed to be "without appreciable risk" for noncancer effects) of 0.9 ppb was calculated. The actual risks at low exposure levels are unknown; this RfC merely provides a bound on chronic exposure below which no "appreciable risk" of noncancer effects is expected. An acute "RfC" and a subchronic "RfC" were also calculated (7 ppb) based on reduced fetal weight.

In summary, the primary changes in EPA's conclusions about the health effects of 1,3-butadiene from the 1985 document are:

- The cancer characterization has been changed from probable human carcinogen to carcinogenic to humans by inhalation.
- The unit cancer risk estimate has been changed from 0.64/ppm (upper bound based on mouse data) to 0.08/ppm (based primarily on linear modeling and extrapolation of human data).
- For the first time, a (chronic) RfC (0.9 ppb), an acute "RfC" (7 ppb), and a subchronic "RfC" (7 ppb) are calculated for noncancer effects.

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