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Health Risk Assessment of 1,3-Butadiene

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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
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PREFACE

This Health Risk Assessment of 1,3-Butadiene has been prepared to serve as a source document for Agencywide use. This document was developed primarily for use by the U.S. Environmental Protection Agency's (EPA) Office of Mobile Sources (OMS) to support decision making regarding the Air Toxic Rule's Section 202L2 of the Clean Air Act Amendment. Since OMS requested that this assessment focus on mutagenicity, carcinogenicity, and reproductive/developmental effects, an evaluation of other health hazards has not been included. This document, therefore, is not a comprehensive health assessment. 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, 1997. Key studies have been evaluated to qualitatively describe the mutagenicity, reproductive/developmental effects, and carcinogenicity of 1,3-butadiene. The assessment also includes a summary, conclusions, and risk 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.

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1. INTRODUCTION

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 1,900 mm/Hg at 20°C (Kirshenbaum, 1978). 1,3-Butadiene is highly volatile and has a low solubility in water; thus environmental release results primarily 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).

Approximately 12 billion pounds of 1,3-butadiene are produced annually worldwide and 3 billion pounds 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) (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 in the environment is via emissions from gasoline- and diesel-powered vehicles and equipment. 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. SUMMARY OF PAST CARCINOGEN RISK ASSESSMENTS OF 1,3-BUTADIENE

The purpose of this section is to review past carcinogen risk assessments of 1,3-butadiene. It should be noted that the Toxicological Profile for 1,3-butadiene (ATSDR, 1992), profile of 1,3-butadiene to set the threshhold limit value (TLV) (ACGIH, 1994), and 1,3-Butadiene OEL Criteria Document by the European Center for Ecotoxicology and Toxicology of Chemicals (1997) are not reviewed here as they are not risk assessments.

1.2.1. 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 will not be reviewed in the present document but are briefly summarized below.

EPA reviewed six epidemiological 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 the entire cohort was used to calculate the SMRs for 28 different work areas for the 1977 study. 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), as 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, such as 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 prompted EPA to conclude that the evidence was inadequate for determining a causal association between exposure to 1,3-butadiene and cancer in humans.

Two long-term animal studies presented strong evidence for the induction of cancers at multiple anatomical sites in both rats (HLE, 1981) 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 h/day, 5 d/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. Nonneoplastic effects due to long-term exposure of rats to 1,3-butadiene included clinical signs of toxicity, an 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 h/day, 5 d/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, 1986). 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 was 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 (HLE, 1981) was conducted using pregnant female Sprague-Dawley rats exposed to 200, 1,000, and 8,000 ppm 6 h/day on gestation days 6-15. Developmental effects included slightly decreased fetal weight and mean crown-rump length and increased skeletal variations and malformations. The other study (Carpenter et al., 1944) was inadequately reported.

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 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 h. The upper-limit unit risk of $6.4 \times 10^{-1}(\text{ppm})^{-1}$ was a geometric mean of the values calculated for male and female mice separately. The unit risk extrapolated to humans was $2.5 \times 10^{-2}(\text{ppm})^{-1}$. This value was used to predict human responses in the epidemiologic studies, which were then compared with the actual responses. According to EPA, ". . . 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. . . ." (U.S. EPA, 1985).

1.2.2. Summary of IARC's Evaluation of 1,3-Butadiene (IARC, 1986, 1992)

IARC reported the first evaluation of 1,3-butadiene as a separate chemical in 1986 (IARC, 1986). In an earlier report (IARC, 1982), 1,3-butadiene was evaluated as a chemical used in the rubber industry. IARC's 1986 evaluation of the animal data consisted of the NTP (1984) study using male and female B6C3F$_1$ mice exposed to 625 or 1,250 ppm 1,3-butadiene for 60 or 61 weeks and an abstract description of the HLE (1981) study in rats exposed to 1,000 or 8,000 ppm (Owen et al., 1985). The human data consisted only of a cohort study described by Meinhardt et al. (1982) and a brief mention of the following studies of workers in the rubber industry that were included in IARC's evaluation of the rubber industry: Andjelkovich et al., 1976, 1977; McMichael et al., 1976; and Monson and Nakano, 1976. The supporting evidence considered by IARC consisted of absorption, distribution, metabolism, and excretion (ADME) data. The genotoxicity data showed that 1,3-butadiene was mutagenic in *S. typhimurium* with metabolic activation, and the metabolites (1,2-epoxybutene and 1,2:3,4-diepoxybutane) were mutagenic in *S. typhimurium* without metabolic activation. IARC also evaluated data on acute, reproductive, and developmental toxicity of 1,3-butadiene. IARC (1986) concluded that the supporting evidence for genetic activity was "inadequate," the evidence for carcinogenicity in experimental animals was "sufficient," and the evidence for carcinogenicity in humans was "inadequate" (Group 2B).

IARC reevaluated the data on 1,3-butadiene and reported the results in 1992. Additional animal and human studies were available for evaluation. In addition to the first NTP (1984) study in mice, IARC (1992) evaluated a more recent NTP study reported by Melnick et al. (1990a). In this study, male and female B6C3F$_1$ mice exposed by inhalation to 1,3-butadiene at concentrations of 6.25 to 625 ppm for 2 years developed neoplasms at multiple sites and at all concentrations. IARC also evaluated the published HLE (1981) long-term study showing tumors developing at multiple sites in male and female Sprague-Dawley rats exposed to 1,000 or 8,000 ppm 1,3-butadiene (Owen et al., 1987) and a comparative study in B6C3F$_1$ and NIH Swiss mice examining the role of endogenous retroviruses on the induction of lymphomas by 1,3-butadiene (Irons et al., 1987). IARC also presented some evidence showing that the metabolites 1,3-epoxybutene and 1,2:3,4-diepoxybutane possessed carcinogenic activity.

Epidemiologic studies evaluated by IARC (1992) consisted primarily of the studies published since 1982. The following studies were evaluated: (1) the mortality study conducted by Meinhardt et al. (1982) of workers in two styrene-butadiene rubber plants, but not the most recent update of this study by Lemen et al. (1990); (2) the mortality study by Downs et al. (1987)

of workers who manufactured 1,3-butadiene monomer and the most recent update of this study by Divine (1990); (3) a mortality study by Matanoski et al. (1989) of workers in eight U.S. and Canadian styrene-butadiene rubber plants (update of the study by Matanoski and Schwartz, 1987); (4) a nested case-control study of the 59 workers from the eight U.S. and Canadian plants who died from lymphopoietic cancer (Santos-Burgoa, 1988; Matanoski et al., 1990); (5) a nested case-control study of rubber workers dying from various types of cancer including lymphohematopoietic cancer (McMichael et al., 1976); and (6) a population-based case-control study of various types of cancers (excluding leukemia) conducted in Montreal, Canada (Siemiatycki, 1991).

Supporting evidence evaluated by IARC (1992) included in vitro studies on the metabolism of 1,3-butadiene using human liver and lung homogenates and comparative in vivo and in vitro metabolism studies in mice, rats, and monkeys. A detailed discussion on in vivo and in vitro genetic toxicity of 1,3-butadiene and metabolites (1,2-epoxybutene and 1,2:3,4-diepoxybutane) was presented along with other available information on short-term toxicity and nonneoplastic effects of 1,3-butadiene in humans and experimental animals.

IARC (1992) concluded that the evidence for the carcinogenicity of 1,3-butadiene in humans is "limited" based on (1) a study showing an increased risk for lymphosarcoma and reticulosarcoma among workers who manufacture 1,3-butadiene monomers; (2) a suggested increased risk for leukemia among workers at one of two styrene-butadiene rubber plants studied; (3) no increase of leukemia among the entire cohort of workers at eight U.S. and Canadian styrene-butadiene rubber plants, but a significant risk of leukemia among a subgroup of production workers; and (4) a large excess of lymphohematopoietic cancer nested among workers exposed to 1,3-butadiene in styrene-butadiene rubber plants. IARC also concluded that the evidence for the carcinogenicity of 1,3-butadiene in experimental animals was "sufficient" based on tumor induction at multiple sites in mice and rats, the induction of neoplasms in mice at all concentrations tested (6.25 to 1,250 ppm), the carcinogenicity of two metabolites of 1,3-butadiene, and the detection of activated K-*ras* oncogenes in lymphomas, liver tumors, and lung tumors induced by 1,3-butadiene. Evidence from metabolism and genetic toxicity studies supported the conclusions of the carcinogenicity studies. IARC (1992) concluded that 1,3-butadiene is *probably carcinogenic to humans* (Group 2A).

1.2.3. Summary of the National Institute for Occupational Safety and Health (NIOSH) Evaluation of 1,3-Butadiene (NIOSH, 1991a)

NIOSH (1991a) conducted a qualitative and quantitative assessment of the carcinogenicity of 1,3-butadiene. The evaluation of animal data focused on the studies that could be used for quantitative assessment, namely the studies using Sprague-Dawley rats (Owen et al., 1987) and

$B6C3F_1$ mice (NTP, 1984; Melnick et al., 1990a). The qualitative evaluation of the evidence from human studies focused on the studies by Downs et al. (1987) and updated by Divine (1990); Meinhardt et al. (1982) and updated by Lemen et al. (1990); Matanoski and Schwartz (1987) and updated by Matanoski et al. (1990); and a case-control study of the lymphopoietic cancers (Santos-Burgoa, 1988) from the Matanoski cohort. According to NIOSH, the results of this nested case-control study "provide the strongest human evidence to date for an association between 1,3-butadiene and the risk of lymphopoietic neoplasms, particularly leukemia." NIOSH concluded that overall the epidemiologic studies showed an increase in lymphopoietic neoplasms, which is consistent with the induction of lymphomas in mice exposed to 1,3-butadiene. However, NIOSH reported that the epidemiologic studies had certain limitations, such as the lack of historical exposure levels, the inclusion of both support and production personnel whose exposure would probably be minimal, and the inconsistent diagnosis of the different types of lymphohematopoietic neoplasms.

NIOSH reported on metabolism, pharmacokinetics, and disposition studies; their evaluation focused primarily on studies that provided data for estimating metabolic rates at low concentrations and comparison of metabolic pathways and rates in different species (mice, rats, monkeys, and humans). With respect to genetic toxicity, NIOSH did not focus on details of any studies, but noted that 1,3-butadiene is mutagenic in *Salmonella* with metabolic activation, whereas the metabolites are mutagenic without metabolic activation.

NIOSH (1991a) concluded that the present evaluation supports the conclusion of a previous evaluation (NIOSH, 1984), which stated that "1,3-butadiene should be considered to represent a potential human health hazard with respect to carcinogenicity." The basis for the conclusion was positive evidence of carcinogenicity in three long-term animal bioassays in two species, positive evidence of mutagenicity and genotoxicity, and less conclusive epidemiologic evidence of excess deaths from lymphopoietic neoplasms.

NIOSH used data from the study in $B6C3F_1$ mice (Melnick et al., 1990a) for its quantitative assessment because the lowest concentration (6.25 ppm) was similar to the proposed OSHA standard of 2 ppm. Weibull's one-, two-, and three-stage time-to-tumor models were used to derive the maximum likelihood and 95% confidence limit estimates on excess risk. The models were fit for the individual tumors for which the incidences were significantly higher in exposed groups than in control groups of male and female mice. Hemangiosarcomas of the heart and lymphomas were modeled as fatal lesions and all others as incidental lesions. The equivalent human doses were calculated based on body weight to the three-fourths power ($BW^{3/4}$) and converted back to ppm exposures in the workplace for 45 years of exposure. The excess risk for lifetime occupational exposure at 1 ppm was 305/10,000 based on lung neoplasms in females (highest) and 0.03/10,000 based on heart hemangiosarcomas in females.

NIOSH (1991b) discussed the uncertainties associated with its assessment. The dose-scaling method chosen and species differences in the metabolism of 1,3-butadiene were major sources of uncertainty. Another source of uncertainty was the most relevant tumor site used to predict human risk. The female lung was the most sensitive site, but based on the epidemiologic evidence, lymphomas may be the most relevant neoplasms. Other sources of uncertainty were the model selection: (1) whether the Weibull time-to-tumor model was the most appropriate and which stage model to use, (2) the assumption regarding lethality of tumors and omission of the high-dose group, and (3) estimation of the internal dose and the application of kinetic data.

1.2.4. California Air Resources Board (CARB, 1991)

The CARB (1991) evaluated the data on 1,3-butadiene and presented quantitative estimates of the cancer risk from inhalation exposure to 1,3-butadiene in ambient air. The literature review consisted of toxicokinetic data that focused on information presented by Bond et al. (1986, 1987) for absorption and tissue distribution data and reports published between 1985 and 1991 for metabolism and excretion data. Acute, subchronic, and noncancer chronic toxicity information was obtained from excerpts from EPA's 1985 carcinogen assessment document, and reproductive/developmental toxicity data and genetic toxicity data were reported from the primary literature. Genetic toxicity data focused on mutation tests in *S. typhimurium*, DNA alkylation studies, SCE and chromosome aberration tests, and various in vivo studies.

Animal carcinogenicity studies evaluated by CARB included the two NTP studies in mice (NTP, 1984; Huff et al., 1985; Melnick et al., 1990a), the inhalation study in rats (Owen et al., 1987), the role of retroviruses in 1,3-butadiene-induced carcinogenesis (Irons et al., 1987), and the expression of oncogenes in tumors induced by 1,3-butadiene (Goodrow et al., 1990). Human studies evaluated by CARB started with the 1976 study by McMichael et al. and continued through the 1990 reports by Lemen et al., Divine, and Matanoski et al. CARB discussed several factors that must be considered when interpreting the epidemiologic studies: (1) misclassification of exposure—unexposed individuals classified as exposed would bias the results toward the null; (2) exclusion of most highly exposed workers—studies in which the workers with the highest potential exposure (World War II workers) are excluded would be less likely to see a significant effect; (3) no dose-response effect—the lack of a positive association with duration of exposure should not discredit the study because the most recent NTP animal study (Melnick et al., 1990a) demonstrated that short-term exposure to a high concentration of 1,3-butadiene could be more effective than long-term exposure to low concentrations; and (4) varying health endpoints—there were inconsistencies in the subtypes of lymphopoietic and hematopoietic cancers observed in the various studies, but nomenclature changed over time and there are probably close relationships between the different subtypes. CARB presented four points of evidence for an association

between exposure to 1,3-butadiene and lymphopoietic and hematopoietic cancers in humans. The first point is that the strongest effect was observed in the cohort involved in the production of 1,3-butadiene monomer, and this cohort had the greatest potential for exposure to 1,3-butadiene in the absence of styrene. The second is that the observations of cancers in cohorts having potential exposure to styrene and 1,3-butadiene are consistent with the findings from the cohort from the 1,3-butadiene monomer production facility. The third point is presented in the case-control study by Matanoski and Schwartz (1987) and the cellular study by Checkoway and Williams (1982) in which both attributed the observed effects to 1,3-butadiene exposure and not to styrene exposure. The fourth point is that the cancers observed in humans are consistent with those observed in the mouse experiments. CARB concluded that "the epidemiological studies reported to date give evidence for increased incidences of leukemia and/or lymphohematopoietic neoplasms resulting from exposure to vapors in styrene-butadiene rubber plants or butadiene production plants." They further stated that the evidence for elevated rates of stomach and lung cancers is inconclusive.

CARB conducted an extensive quantitative assessment of the risk from exposure to 1,3-butadiene. The two mouse studies and the rat study were considered suitable for quantitative evaluation. Dose estimations were based on experimental (applied) dose, continuous internal dose, metabolized dose, target tissue dose, and molecular tissue dose. CARB used the retention data from Bond et al. (1986) to estimate the daily dose adjusted for 7-day week exposures (internal dose). The pharmacokinetics model of Hattis and Wasson (1987) was used to estimate internal exposure to metabolites, namely butadiene monoepoxide (metabolized dose). The tissue distribution data of Bond et al. (1986, 1987) were used to estimate the target tissue doses, which were not used for risk estimation because the data were not reliable. Sufficient data on DNA adducts were not available for deriving molecular tissue doses.

CARB fitted the experimental (applied dose), internal, and metabolized doses estimated from the first mouse study (NTP, 1984) to the linearized multistage (Global 86) and the Weibull time-to-tumor models; the cancer potency estimates derived using the linearized multistage model and Weibull's model gave similar results. The multistage model was used to derive cancer potency values using the second mouse study (Melnick et al., 1990a) and the rat study (HLE, 1981). Cancer potency estimates were derived for each anatomical site separately and for the total number of tumor-bearing animals with significantly increased tumors in both males and females. The human cancer potency estimates, based on 70 years of continuous exposures, derived from the first mouse study using the total significant tumors, the internal dose, and the multistage model were 0.32 $(ppm)^{-1}$ or 0.59 $(mg/kg/day)^{-1}$ for male mice and 0.18 $(ppm)^{-1}$ or 0.33 $(mg/kg/day)^{-1}$ for female mice. Cancer potencies derived from applied doses were about 10-fold lower, and those derived from metabolized doses were about 50% lower. The human cancer

potency estimates using the rat data (total significant tumors), internal dose, and the multistage model were 1.8×10^{-3} $(ppm)^{-1}$ or 8.4×10^{-3} $(mg/kg/day)^{-1}$ for male rats and 3.5×10^{-3} $(ppm)^{-1}$ or 1.6×10^{-2} $(mg/kg/day)^{-1}$ for female rats. The estimates based on applied or metabolized doses were much lower. The data from the second mouse study were analyzed extensively; CARB concluded that the best human cancer potency estimates based on internal doses and estimated using the multistage model (Global 86) were 0.37 $(ppm)^{-1}$ or 3.4 $(mg/kg/day)^{-1}$ derived for alveolar/bronchiolar adenoma/carcinoma in female mice. The corresponding unit risk derived from the second mouse study was 1.6×10^{-4} $(\mu g/m^3)^{-1}$ and the exposure for the risk at 10^{-6} was 6.0×10^{-3} $\mu g/m^3$. From their cancer potency values, CARB estimated the lifetime extra risk associated with exposure to 1 ppb 1,3-butadiene to range from 9.8×10^{-6} to 3.7×10^{-4}, which corresponds to 10 to 370 additional cases per 1 million individuals.

1.2.5. Summary of Findings by U.S. Occupational Safety and Health Administration (OSHA)

The most recent analysis of health effects of 1,3-butadiene by a government entity is by OSHA (1996). While the analysis in general is similar to that of NIOSH, OSHA incorporated a recent update of the large SBR polymer retrospective follow-up study that had been started by Matanowski et al. This update, Delzell et al (1995), included not only an additional period of follow-up, but also a detailed exposure history for 1,3-butadiene, styrene, and benzene for more than 15,000 employees who had worked in SBR and related activities at the eight study plants. Delzell et al. concluded that "This study found a positive association between employment in the SBR industry and leukemia. The internal consistency and precision of the results indicate that the association is due to occupational exposure. The most likely causal agent is BD or a combination of BD and [styrene]. Exposure to [benzene] did not explain the leukemia excess." OSHA in its analysis of the Delzell et al. and previous studies recognized these consistencies and similarly concluded that "there is strong evidence that workplace exposure to BD poses an increased risk of death from cancers of the lymphohematopoietic system. The epidemiologic findings supplement the findings from the animal studies that demonstrate a dose response for multiple tumors and particularly for lymphomas in mice exposed to BD" (OSHA, 1996, p. 56764).

OSHA also examined the evidence for reproductive and developmental effects. Analyzing data from both the NTP I and the NTP II studies, OSHA noted the consistency and dose response and concluded "that exposure to relatively low levels of BD resulted in the induction of ovarian atrophy in mice..." (OSHA, 1996, p. 56765). For the total database on these and mutagenic effects, OSHA concluded that "these animal studies taken as a whole, offer persuasive qualitative evidence that BD exposure can adversely affect reproduction in both male and female rodents. The Agency also notes that BD is "mutagenic in both somatic and germ cells" (p. 56767).

For quantitative risk assessment, OSHA's analysis was very similar to that of NIOSH (1991a) in its choice of data set (NTP II mouse study), model (multistage Weibull), treatment of tumors (dose-response analysis on an individual basis), treatment of fatal vs. nonfatal in the time-to-tumor analysis, choice of parsimonious model algorithm (fewest parameters of the multistage model that provide an adequate fit to the data) and reporting of the ML estimates. The major difference between the NIOSH and OSHA analyses was that OSHA used (mg/kg bw-day) equivalence for species conversion instead of the $BW^{3/4}$ conversion used by NIOSH. This change of species conversion factors and some minor modifications relating to animal weights and breathing rates decreased OSHA's potency estimates by a factor of approximately 4 from the NIOSH estimates. Based on the female mouse lung tumor response, the OSHA ML estimate of potency was 8.1×10^{-3} $(ppm)^{-1}$ for an occupational lifetime of exposure to 1 ppm, 5 days/week, 50 weeks/year for 45 years. If this potency estimate is extrapolated to be based on a 70-year continuous lifetime exposure, the OSHA estimate would be approximately 36.7×10^{-3} $(ppm)^{-1}$. Based on the OSHA risk assessment, their permissible exposure limit was lowered from 1,000 ppm to 1 ppm with a 15-min short-term exposure limit.

1.3. DISCUSSION

Six different carcinogenicity assessments of 1,3-butadiene, done by five different agencies in different time periods, are summarized in this chapter. The major conclusions of these evaluations are presented in Table 1-1.

Although no apparent agreement is evident from the table among the five agencies' assessments, in fact they are very similar. Both EPA (1985) and IARC (1986) conclude that the carcinogenicity evidence in humans is inadequate and in animals is sufficient. But due to different classification systems, they get different alphabetical assignments, i.e., B2 and 2B, which correspond to "probable" and "possible" descriptors, thus appearing to be in disagreement with each other. NIOSH and OSHA both use the dichotomous descriptors with "potential occupational carcinogen" being the highest ranking.

Table 1-1. Carcinogenicity assessments of 1,3-butadiene

Agency (year)	Cancer classification	Quantitative risk	Remarks
EPA (1985)	"B2-probable human carcinogen" —based on inadequate human and sufficient animal evidence.	Unit risk to humans—2.5 × 10^{-1} $(ppm)^{-1}$ based on NTP (1984) mouse data.	Cancer classification using EPA carcinogen assessment guidelines.
IARC (1986)	"2B-possible human carcinogen"—based on inadequate human and sufficient animal evidence.	No quantitative risk presented.	Cancer classification using IARC system.
IARC (1992)	"2A-probable human carcinogen"—based on limited human and sufficient animal evidence.	No quantitative risk presented.	
NIOSH (1991a)	"Potential human health hazard with respect to carcinogenicity."	Range of excess risk at 1 ppm is MLE of 305/10,000 based on female mouse lung neoplasms to MLE of 0.03/10,000 based on heart hemangiosarcomas in females. Data from Melnick et al. (1990a) used for this quantitation.	OSHA cancer policy classification system used. Quantitative risk is for occupationally exposed populations.
CARB (1991)	No formal classification given	Human cancer potency based on mouse data from Melnick et al. (1990a) range for 1 ppb exposure—9.8×10^{-6} to 3.7×10^{-4}.	No formal cancer classification system used. Quantitative risk is for general population.
OSHA (1996)	Potential occupational carcinogen	Human cancer potency estimate based on female mouse lung neoplasms. MLE is 8.1×10^{-3} $(ppm)^{-1}$.	"Convincing evidence that BD is a probable human carcinogen." Quantitative risk is for occupationally exposed populations.

OSHA, NIOSH, and CARB assessments all state that the human evidence is strongest for an association between butadiene exposure and the occurrence of lymphohematopoietic cancers. The same evidence is described as "limited" human evidence by IARC, which elevates the classification of this compound to "2A-probable human carcinogen." Furthermore, it should be noted that the quantitative risk estimates appear to be different for OSHA/NIOSH and EPA/CARB. NIOSH/OSHA quantitative risk estimates are for occupationally exposed populations, while quantitative estimates of CARB are for general population (lifetime risk), even though they are derived from the same animal data.

The apparent differences in these assessments thus can be explained by availability of the studies at the time of evaluations, different cancer classification systems, and quantitative assessments done for different purposes.

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.

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 a noncorrosive gas 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 is also 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) and is slowly dimerized to 4-vinyl-1-cyclohexene (U.S. Occupational Safety and Health Administration, 1990) and may form peroxide upon exposure to air (Kirshenbaum, 1978). Since 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 h and is expected to be the dominant pathway for atmospheric removal (U.S. Department of Health and Human Services [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).

There are limited data 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. Based on 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 been produced by 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 rubber (synthetic rubber) and of thermoplastic resins. In 1990, 1,3-butadiene was used in the United States for styrene-butadiene rubber (30%), polybutadiene rubber (20%), adiponitrile/ hexamethylenediamine (15%), styrene-butadiene latex (10%), neoprene rubber (5%), acrylonitrile-butadiene-styrene resins (5%), exports (4%), nitrile rubber (3%), and other (including specialty polymers) (8%) (Anon., 1991).

2.2.1. Styrene-Butadiene Latex and Rubber Production

Styrene-butadiene (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 (EPA, 1994a). About 74% of the industrial emissions are from SB latex and rubber production. There are at least 26 facilities in the United States that produce SB latex and rubber (SRI International, 1993).

As stated previously, butadiene has a 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 confirms that, the amount of butadiene found in secondary sources such as waste water and solid waste is minimal or nonexistent. The majority of the butadiene releases during industrial production occurs 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/ton produced (mean of 7.10) measured at 18 facilities (EPA, 1994a).

2.2.2. Polybutadiene Production

The second largest use for butadiene is in the production of polybutadiene, accounting for over 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/ton produced (mean of 6.14) measured at six facilities (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 as belts, cables, hoses, and wires. Three facilities currently produce neoprene, though 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/ton produced (mean of 4.04) (EPA, 1994a).

2.2.4. Acrylonitrile-Butadiene (ABS) Resin Production

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, there are 10 facilities that 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/ton produced (mean of 4.22) measured at three facilities (EPA, 1994a).

2.2.5. Nitrile Elastomer Production

Nitrile elastomer or nitrile-butyl rubber is a specialty elastomer known for its oil-, solvent-, and chemical-resistant properties. Some uses include hoses, belting, and cable manufacturing and 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/ton produced measured at six facilities identified in the 1994 EPA report (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/ton produced (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%) (EPA, 1994a).

Industrial butadiene emissions arise from process vents, equipment leaks, and secondary sources such as waste water treatment. Since 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% (EPA, 1994a).

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 percent 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 since butadiene tends to form a varnish that can be harmful to engines; therefore, refiners try to minimize the butadiene content. Since butadiene is not a component of gasoline, it is not present in mobile source evaporative or refueling emissions and will be found only in exhaust emissions (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 by Lofgren et al. (1991) and Ramnas et al. (1994) also found negligible amounts of 1,3-butadiene in gasoline vapors. When they compared the concentrations of benzene and butadiene in gasoline, they found concentrations to be 3%-5%

and <0.0005%, respectively. Based on these three reports, Nordlinder et al. (1996) concluded that there is no significant amount of 1,3-butadiene 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 (LDGV), light-duty gasoline trucks, heavy-duty gasoline trucks, light-duty diesel vehicles, light-duty diesel trucks, heavy-duty diesel trucks, and motorcycles. On-road mobile sources account for 37.7% national butadiene emissions.

Although data on the butadiene content of motor vehicle exhaust were lacking until the late 1980s, butadiene emissions from LDGV's 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, the 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 gm/mile (EPA, 1994b). A composite emission factor of 0.0156 gm/mile has been calculated for the calendar year 1990 by the Office of Mobile Sources (OMS) 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 (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, and all-terrain vehicles (ATVs) used for off-road recreation and recreational and commercial marine vessels. However, trains and aircraft are not generally included in the nonroad vehicle category.

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 emission factors expected for the three major engines types in this category**C**gasoline-powered two-stroke

engines, gasoline-powered four-stroke engines, and diesel enginesCare generally higher (by a minimum of a factor of 10) than the gasoline engines (EPA, 1991). The EPA 1994 draft denotes that nonroad engines are expected to contribute 41% to the national butadiene emissions (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 take-off (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 percents 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 (EPA, 1994a).

2.3.2. Miscellaneous Sources

This section contains an overview of the miscellaneous sources of butadiene emissions. These sources have been grouped as miscellaneous chemical production, secondary lead smelters, petroleum refining, and combustion sources (especially biomass burning). Emissions from these sources can 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 (EPA, 1994a). This process accounts for 8% of the butadiene use, but only contributes 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/ton produced (product varies) measured at only four facilities.

2.3.2.2. *Secondary Lead Smelters*

Secondary lead smelting involves the reclamation of scrap automobile batteries to produce elemental and lead alloys. There are 23 such facilities in the United States, most of which are 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 (EPA, 1994a).

2.3.2.3. *Petroleum Refining*

The 1992 Toxic Release Inventory contains the emission factor of 437,590 lb/year for petroleum refining. Using this emission factor would make this source the fifth largest emitter of butadiene, contributing 0.3% to the national butadiene emissions. Data are currently being collected to determine the actual contribution of petroleum refining to butadiene emissions.

2.3.3. *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, although only the latter two will be discussed in this section due to the scarcity of data.

2.3.3.1. *Tire Burning*

There are approximately 240 million tires discarded annually, of which only 25% are recycled. The remaining tires are discarded in landfills, stockpiles, or illegal dumps (Lemieux and DeMarini, 1992). 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 therefore it is present in emissions from tire burning. Emission factors have been calculated for the open burning of tires (EPA, 1992; Lemieux and DeMarini, 1992). These emission factors range from 234.28 lb/1,000 tons of chunk tires to 277.95 lb/1,000 tons of shredded tires. No emission factor for butadiene from tire incineration has been located.

2.3.3.2. *Biomass Burning*

Biomass burning includes residential wood combustion in both fireplaces and wood stoves, open burning such as the 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 primarily composed of wood. The relative contribution of biomass burning to the overall national butadiene emissions was calculated at 18.8% in the 1994 EPA report (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 types (flaming or smoldering) and these models correlated butadiene emissions

with CO 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, provides a butadiene:benzene ratio of 0.36 for wood smoke.

2.4. AMBIENT CONCENTRATION OF 1,3-BUTADIENE

2.4.1. Air

In 1989, total emissions of 1,3-butadiene to the air in the United States were estimated at approximately 2,512 tonnes from 158 locations; total land releases were estimated at 6.7 tonnes (U.S. National Library of Medicine, 1991).

2.4.1.1. *Ambient Monitoring Data*

Several EPA databases exist that contain the 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) (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 (EPA, 1989a). EPA uses data from AIRS in order 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 the geographic location of the site and who operates it, the AIRS program also contains extensive information on the ambient levels of many toxic compounds. The AIRS database 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-h 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 in the averaging of 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 can be found in Table 2-1.

Another air monitoring program is the Urban Air Toxic Monitoring Program (UATMP), which the 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 is used in air toxic risk assessment models (EPA 1989b, c; EPA 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-h 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 can be found in Table 2-2.

The monitoring data for the UATMP that were 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-h air samples taken every 24 h between 1970 and 1987 (Shah and Heyerdahl, 1988; Hunt et al., 1988). However, only the most current NAVOC data, taken during 1987, is used in this summary. In addition, samples that had nondetects of 1,3-butadiene were included as one-half the detection limit in averaging the data for this summary. 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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1,3-Butadiene Average Concentration in ppb[b]

[a]The arithmetic average concentration of all samples using half minimum detection limit (MDL) value for samples in which the 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.

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 can be found in Table 2-3.

Table 2-4 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, TX, 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 2-4. This alternate annual average may be more representative of areas that are not near strong point sources.

Tables 2-5, 2-6, and 2-7 regroup and summarize Tables 2-2, 2-3, and 2-4 according to the sampling locations, i.e., rural, suburban, or urban settings. The data obtained from Port Neches, TX, were not included in these averages because of the elevated levels due to 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 h in a 24-h period while the other monitoring networks collected a 24-h air sample every 12 days.

It should also be noted that the ambient levels detected in these three databases are not meant to be indicative of an individual's actual exposure to 1,3-butadiene. Times and concentrations in microenvironments other than the outdoors need to be taken into consideration, i.e., accounting for integrated exposure.

In addition, the ambient levels include contributions from a variety of source categories. Typically, ambient monitoring data are adjusted to represent the amount attributed to a particular source using emissions inventory apportionment. The derivation of an urban annual average exposure estimate for all mobile sources will be used for illustration purposes (EPA, 1993a). The range of ambient data from Table 2-4 (using alternate annual averages when available) is 0.22 to 1.02 $\mu g/m^3$ (0.10 to 0.46 ppb). When this range is adjusted by the estimated proportion of the inventory that is contributed by mobile sources (78.7%) and for integrated exposure to account for time spent indoors and outdoors, the range becomes 0.11 to 0.50 $\mu g/m^3$ (0.05 to 0.23 ppb).

Table 2-3. Summary of outdoor urban data from the National Ambient Volatile Organic Compounds (NAVOC) Database

Sample years	Average concentration used (ppb)	Number of samples	Sampling sites	Land use of monitor location	Number of samples/ sampling time
9/22/87 to 10/4/87	0.30	2	Bakersfield, CA	Urban	1/24-hours
9/16/87 to 9/28/87	0.35	2	Concord, CA	Urban	1/24-hours
10/4/87 to 10/4/87	0.60	1	Fremont, CA	Urban	1/24-hours
10/4/87 to 10/4/87	0.40	1	Richmond, CA	Urban	1/24-hours
9/9/87 to 10/7/87	0.25	2	San Jose, CA	Urban	1/24-hours
9/29/87 to 9/27/87	0.30	1	Stockton, CA	Urban	1/24-hours
Overall 1,3-Butadiene average concentration: 0.344 ppb[a]					

[a]Calculated by averaging all nine samples taken from six cities equally.

Table 2-4. 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		
(ppb)	0.67(1.48)	0.46(1.02)[b]
# Obs.	18	12
# Sites	3	2
1989		
(ppb)	0.23(0.57)	0.25(0.55)[b]
# Obs.	399	369
# Sites	30	29
1990		
(ppb)	0.29(0.64)	0.21(0.46)[b]
# Obs.	101	97
# Sites	7	6
1991		
(ppb)	0.10(0.22)	----[c]
# Obs.	117	
# Sites	6	
1992		
(ppb)	0.16 (0.40)	0.16 (40)[b]
# Obs.	656	650
# Sites	20	19
1993		
(ppb)	0.40(0.88)	0.32(0.71)[d]

# Obs.	2069	1,931
# Sites	64	59
1994		
(ppb)	0.59(1.30)	0.42(0.92)[d]
# Obs.	2666	2,401
# Sites	70	64

Table 2-4. Summary of air monitoring program results for 1,3-butadiene (continued)

	Annual average ppb (µg/m^3)	Alternate annual average[a] ppb (µg/m^3)
UATMP		
1989		
(ppb)	0.21 (0.46)	----[e]
# Obs.	390	
# Sites	13	
1990		
(ppb)	1.02(2.25)	0.12(0.27)[b]
# Obs.	349	293
# Sites	12	10
NAVOC		
1987		
(ppb)	0.34(0.75)[f]	no data
# Obs.	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, Texas, was not monitored during this 4-quarter period.
[f]All urban California sites.

Table 2-5. Summary of 1,3-butadiene ambient data from the EPA Aerometric Information Retrieval System (AIRS) 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 - 0.47	0.46	12/2
1989				0.13 - 0.60	0.25	151/12	0.10 - 0.73	0.27	237/18
1990				0.16 - 0.72	0.29	95/5	0.20 - 0.33	0.27	32/2
1991				0.06 - 0.12	0.10	71/4	0.07 - 0.11	0.09	46/2
1992	0.07-0.45	0.13	271/6	0.05 - 0.19	0.10	154/7	0.09 - 0.72	0.29	176/5
1993	0.05-0.20	0.10	494/10	0.06 - 4.32	0.41	864/31	0.08 - 0.98	0.31	580/21
1994	0.07-0.37	0.18	522/11	0.06 - 1.68	0.45	1135/32	0.05 - 1.54	0.62	780/24

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

Table 2-6. Summary of 1,3-butadiene data from Table 2-2 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.
[b]The arithmetic average concentration of all samples using one-half minimum detection limit value for samples in which the compound was not found.

Table 2-7. Summary of 1,3-butadiene data from Table 2-3 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.

2.4.1.2. *Ambient Source Apportionment*

There are three studies that attempt to apportion sources as to their contribution to ambient levels of 1,3-butadiene. The studies assume that all emissions to the atmosphere contribute proportionally to ambient concentration. These three studies are summarized in Table 2-8.

As observed in Table 2-8, 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 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 database. An actual 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. There is a great deal of uncertainty 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 are primarily dependent on the presence of environmental tobacco smoke (ETS) (CARB, 1992). Several studies indicate that on the average most individuals spend anywhere from about 60% to 70% (Robinson et al., 1989; EPA, 1993b) of their time each day indoors at their residence. 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 $\mu g/m^3$. Further research and measurements are needed to quantify typical indoor 1,3-butadiene exposures.

Table 2-8. Summary of the relative contributions to ambient 1,3-butadiene emissions given as percent 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 included on-road and off-road vehicles and generally excluded trains and aircraft.
[b]Area and point sources generally included 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.

2.4.3. Water

Although 1,3-butadiene has been detected in drinking water in the United States (U.S. EPA, 1978; Kraybill, 1980), it is not clear what happens to the chemical in the body (U.S. DHHS, 1992). Total releases to ambient water in 1989 were estimated to be 65 tonnes (U.S. National Library of Medicine, 1991).

2.4.4. Food

Certain cooking oils release butadiene on heating. 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 U.S. 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 < 5-310 µg/kg (Startin and Gilbert, 1984).

2.5. PATHWAYS OF EXPOSURE

The 1992 U.S. DHHS report states that although 1,3-butadiene undergoes rapid destruction in the atmosphere, it is almost always present at very low concentrations in urban and suburban areas. Automobile exhaust is a constant source of 1,3-butadiene release to the atmosphere. Because of the compound's presence in the atmosphere, the general population is exposed to ppb levels of 1,3-butadiene through inhalation. Exposure to 1,3-butadiene may also

occur from the inhalation of cigarette smoke, or possibly the smoke from wood fires. Possible ingestion of contaminated drinking water may also lead to low levels of exposure, although the concentration of this compound in drinking water has not been well characterized. The levels of 1,3-butadiene in soil are not known. Elevated levels of exposure for the general population may occur for those near its site of manufacture or facilities where it is made into polymeric materials.

Occupational exposure to 1,3-butadiene is expected to be limited to those working at facilities that manufacture 1,3-butadiene or convert it into commercial polymers. Exposure by inhalation is expected to be the dominant pathway for exposure.

3. METABOLISM AND PHARMACOKINETICS

The pharmacokinetics of 1,3-butadiene have been reviewed previously by the U.S. Environmental Protection Agency (U.S. EPA, 1985) and the International Agency for Research on Cancer (IARC, 1986). Data from both in vitro and in vivo studies on the toxic effects of 1,3-butadiene have established that 1,3-butadiene metabolites, not the parent compound, cause these toxic effects. Differences have been noted in the toxic responses to 1,3-butadiene among laboratory species, and understanding the pharmacokinetics of 1,3-butadiene and its metabolites is important in assessing the carcinogenic risk and evaluating other health effects associated with exposure to this chemical. This chapter summarizes the recent research that has provided information on the pharmacokinetics of 1,3-butadiene in several animal species and elucidates the metabolism of 1,3-butadiene, via both in vitro and in vivo studies.

The chemical terminology and units used in the publications reviewed in this chapter have 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; and butene diol (BD) is used for 1,2-dihydroxybut-3-ene and 3-butene-1,2-diol.

3.1. OVERVIEW OF PHARMACOKINETIC STUDIES

In recent years, considerable data have been generated regarding the pharmacokinetics of 1,3-butadiene in various laboratory species. Although in vitro studies can elucidate possible metabolic products and allow measurements of metabolic reaction kinetic constants under controlled conditions, in vivo studies usually encompass several issues of pharmacokinetics and provide an account of the total disposition of the exposed dose. For 1,3-butadiene, because of the toxicity of the 1,3-butadiene metabolites, in vivo pharmacokinetic studies validated the existence of these metabolites and their metabolic rates of activation and detoxification. Absorption of the parent compound was often assessed either from its distribution in the tissue organs or blood or from its excretion in urine, feces, and exhaled air. Absorption and excretion have also been measured from the presence of 1,3-butadiene metabolites in blood, urine, feces, and exhaled air. Species differences have been observed in the toxic effects of 1,3-butadiene in mice, rats, and monkeys and are reflected in the in vitro metabolism and pharmacokinetics of 1,3-butadiene in these species. This section summarizes the metabolic pathways of 1,3-butadiene disposition and the species differences in 1,3-butadiene pharmacokinetics and metabolism from in vitro and in vivo studies.

3.1.1. Pathways Elucidation

Several in vitro and in vivo studies have elucidated the metabolic pathways of 1,3-butadiene metabolism as shown in Figure 3-1 and summarized in Table 3-1 (Himmelstein et al., 1997). Results from in vitro studies show that 1,3-butadiene undergoes cytochrome P-450-mediated biotransformation to the reactive metabolite epoxybutene, which has also been validated from in vivo studies in rats, mice, and monkeys. Epoxybutene can be activated further to another reactive metabolite, diepoxybutane, or detoxified by epoxide hydrolase to butene diol, as shown by in vitro studies and detected in vivo via their glutathione (GSH) conjugates in rats, mice, hamsters, monkeys, and humans. Further metabolism of these two metabolites can be mediated by either the P-450 system or epoxide hydrolase, giving 1,2-dihydroxy-3,4-epoxybutane. The detoxification of epoxybutene occurs by hydrolysis and GSH conjugation and is mediated by the enzymes epoxide hydrolase and glutathione S-transferase (GST), respectively; these reactions have been supported by both in vitro and in vivo studies. Epoxybutene can also form DNA and hemoglobin (Hb) adducts in both rats and mice. Of greater significance is the identification of crotonaldehyde, a DNA-reactive chemical and known mutagen, as a new product of the oxidative metabolism of butadiene. Crotonaldehyde was formed by the tautomerization of 3-butenal formed by chloroperoxidase-dependent oxidation of 1,3-butadiene and was not a metabolic product of epoxybutene. 3-Butanal rapidly tautomerized to crotonaldehyde at room temperature, which may explain its nondetection in in vitro studies. A possible pathway for the metabolism of 3-butene-1,2-diol, a secondary metabolite of 1,3-butadiene, is oxidative dehydrogenation catalyzed by alcohol dehydrogenase. The production of GSH-epoxide conjugates, *S*-(2-hydroxy-3-buten-1-yl)glutathione (compound I) and *S*-(1-hydroxy-3-buten-2-yl)glutathione (compound II), was confirmed using human placental GST. While compound II is chemically stable, compound I tautomerizes to a stable sulfrane. Because these compounds are of low reactivity (including the stable sulfrane), this biotransformation pathway may represent a physiological protective mechanism against the DNA reactivity of epoxybutene.

3.1.2. Species Differences

3.1.2.1. *In Vitro Metabolism*

Species differences for several reactions described in the previous section are shown by measuring their in vitro reaction rates using microsomal and cytosolic preparations from several organs. Himmelstein et al. (1997) gives a comprehensive summary of the in vitro methodology and the studies that measure the reaction rates of the reactions included in the metabolic pathways shown in Figure 3-1. Table 3-2 summarizes the reaction rates and rate constants obtained from the main studies that compare these differences (modified from Himmelstein et

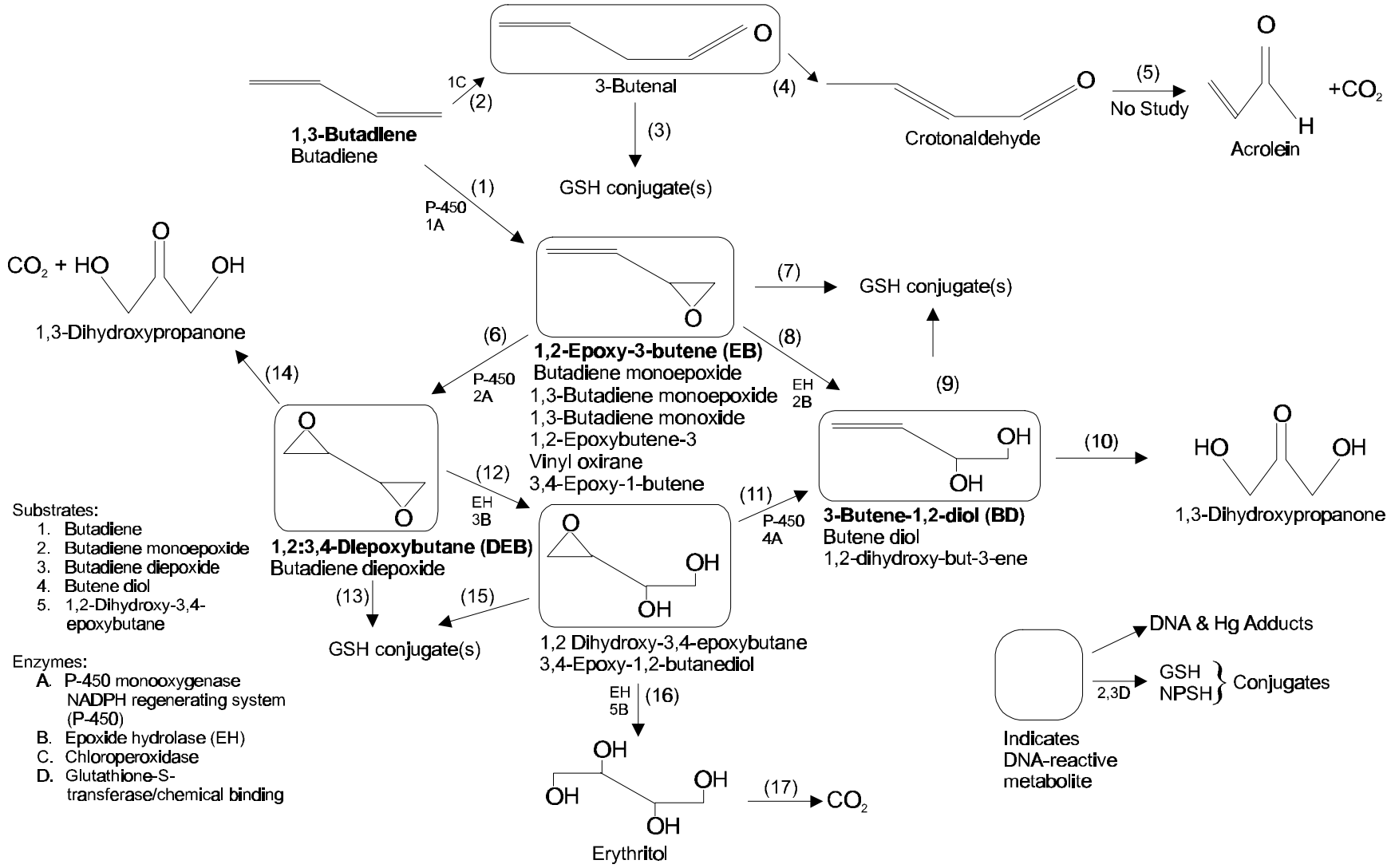

1,3-Butadiene
Butadiene
1C
(2)
3-Butenal
(3)
GSH conjugate(s)
(4)
Crotonaldehyde
(5)
No Study
Acrolein
+CO2
(1)
P-450
1A
CO2 + HO
OH
1,3-Dihydroxypropanone
(6)
P-450
2A
1,2-Epoxy-3-butene (EB)
Butadiene monoepoxide
1,3-Butadiene monoepoxide
1,3-Butadiene monoxide
1,2-Epoxybutene-3
Vinyl oxirane
3,4-Epoxy-1-butene
(7)
GSH conjugate(s)
(8)
EH
2B
(9)
(14)
(12)
EH
3B
1,2:3,4-Diepoxybutane (DEB)
Butadiene diepoxide
(11)
P-450
4A
3-Butene-1,2-diol (BD)
Butene diol
1,2-dihydroxy-but-3-ene
(10)
HO
OH
1,3-Dihydroxypropanone
(13)
(15)
GSH conjugate(s)
1,2 Dihydroxy-3,4-epoxybutane
3,4-Epoxy-1,2-butanediol
EH
5B
(16)
(17)
CO2
Erythritol
Substrates:
1. Butadiene
2. Butadiene monoepoxide
3. Butadiene diepoxide
4. Butene diol
5. 1,2-Dihydroxy-3,4-epoxybutane
Enzymes:
A. P-450 monooxygenase NADPH regenerating system (P-450)
B. Epoxide hydrolase (EH)
C. Chloroperoxidase
D. Glutathione-S-transferase/chemical binding
DNA & Hg Adducts
2,3D
GSH
NPSH
Conjugates
Indicates DNA-reactive metabolite

Figure 3-1. Some pathways in the metabolism of butadiene. Numbers in parentheses represent specific metabolic reactions for which literature references are given in Table 3-1. Reactions (1), (2), (6), and (11) are mediated by cytochrome P-450-dependent monooxygenases. Glutathione (GSH) is a substrate in reactions (3), (7), (9), (13), and (15), which are mediated by glutathione S-transferase or occur spontaneously. A GSH conjugate of reaction(s) is excreted in the urine as *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine. Enzyme-mediated GSH conjugates from reaction (7) include *S*-(2-hydroxy-3-buten-1-yl)glutathione and *S*-(1-hydroxy-3-buten-2-yl)glutathione, which are subsequently excreted in the urine as 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene. The GSH conjugate of reaction (9) is excreted in the urine as 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane. A=*S*-(2-hydroxy-3,4-epoxybut-1-yl)glutathione; B=*S*-(1-hydroxy-3,4-epoxybut-2-yl)glutathione; C=*S*-(1,2,3-trihydroxybut-4-yl)glutathione; D=*S*-(1,3,4-trihydroxybut-2-yl)glutathione. The enzyme-mediated or spontaneous formation of GSH conjugates for reaction 13 form A and B, which are excreted in the urine as C and D, respectively. Reactions (10) and (14) are mediated through the pentose phosphate pathway. Reactions (8), (12), and (16) are mediated by epoxide hydrolase or occur spontaneously.

Sources: Dahl et al., 1990; Laib et al., 1990; Elfarra et al., 1991; Himmelstein et al., 1997.

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism

Reaction	Species and tissue	Reference
(1)1,3-Butadiene→EB		
In vitro experiments		
	Wistar rat liver microsomes.	(1a) Malvoisin et al. (1979a)
	SD rat liver microsomes.	(1b) Bolt et al. (1983)
	B6C3F_1 and NMRI mouse; SD and Wistar rat, rhesus monkey, and human (n = 1) postmitochondrial lung and liver fractions.	(1c) Schmidt and Loeser (1985)
	Liver microsomes of rats (strain not stated), mice (strain not stated), and humans (n = 4).	(1d) Wistuba et al. (1989)
	B6C3F_1 mouse liver microsomes.	(1e) Elfarra et al. (1991)
	SD rat, B6C3F_1 mouse, and human (n = 12) liver and lung microsomes.	(1f) Csanády et al. (1992)
	SD rat and B6C3F_1 mouse liver, lung, kidney, and testis microsomes.	(1g) Sharer et al. (1992)
	Purified human myeloperoxidase from human polymorphonuclear leukocytes and B6C3F_1 mouse liver microsomes.	(1h) Duescher and Elfarra (1992)
	Rat (strain not stated) liver microsomes.	(1i) Cheng and Ruth (1993)
	SD rat, B6C3F_1 mouse, and human (n = 6) liver microsomes.	(1j) Duescher and Elfarra (1994)
	B6C3F_1 mouse bone marrow cells and cell lysates, human bone marrow cells, and purified human myeloperoxidase.	(1k) Maniglier-Poulet et al. (1995)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
In vivo inhalation experiments		
Closed-chamber gas-uptake system	SD rats. EB in exhaled breath.	(1l) Bolt et al. (1983)
	SD rats. Metabolic uptake rate from untreated rats compared with that of rats pretreated with Aroclor 1254 and P-450 inhibitor.	(1m) Bolt et al. (1984)
	$B6C3F_1$ mice. Metabolic uptake rate from untreated rats compared with that of rats pretreated with P-450 inhibitor.	(1n) Kreiling et al. (1986a)
	SD rats and $B6C3F_1$ mice. EB in exhaled breath.	(1o) Kreiling et al. (1987)
	SD rats and $B6C3F_1$ mice, pretreated with 4-methylpyrazole (P-450 inhibitor). Metabolic uptake rate from untreated rats compared with that of rats pretreated with P-450 inhibitor.	(1p) Medinsky et al. (1994)
Nose-only exposure system	SD rats and $B6C3F_1$ mice. EB in blood.	(1q) Bond et al. (1986)
	Cynomolgus monkeys. EB in blood.	(1r) Dahl et al. (1990)
	SD rats and $B6C3F_1$ mice. 1,3-butadiene and EB in blood.	(1s) Himmelstein et al. (1994)
	SD rats and $B6C3F_1$ mice. 1,3-butadiene and EB in blood.	(1t) Bechtold et al. (1995)
	SD rats and $B6C3F_1$ mice. EB in liver and lung tissues.	(1u) Himmelstein et al. (1995)
	SD rats and $B6C3F_1$ mice. EB in blood, fat, heart, liver, lung, spleen, and thymus.	(1v) Thornton-Manning et al. (1995a)
	Female and male SD rats. EB in blood, femur, fat, lung, and mammary tissue.	(1w) Thornton-Manning et al. (1995b)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
(2) 1,3-Butadiene→3-butenal		
In vitro experiments		
EB and crotonaldehyde in reaction mixture. Proposed 3-butenal as intermediate in formation of crotonaldehyde.	$B6C3F_1$ mouse liver microsomes and purified fungal enzyme, chloroperoxidase.	(2a) Elfarra et al. (1991)
3-Butenal detected at optimal pH of 6.0.	Purified fungal enzyme, chloroperoxidase.	(2b) Duescher and Elfarra (1993)
(3) 3-Butenal→GSH conjugates		
***In vivo inhalation experiments: 3-butenal-GSH conjugate*:**		
N-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine.	SD rats and $B6C3F_1$ mice. Urinary metabolite detected in mouse, not in rat.	(3a) Nauhaus et al. (1996)
(4) 3-Butenal→crotonaldehyde		
In vitro experiments		
	Same experiment as (2a) above.	(4a) Elfarra et al. (1991)
	$B6C3F_1$ mouse and SD rat liver, lung, kidney, and testis microsomes. Crotonaldehyde detected in mouse but not in rat.	(4b) Sharer et al. (1992)
	Same experiment as (1h) above.	(4c) Duescher and Elfarra (1992)
	Same experiment as (1i) above.	(4d) Cheng and Ruth (1993)
Crotonaldehyde identified as tautomerization product of 3-butenal.	Same experiment as (2b) above.	(4e) Duescher and Elfarra (1993)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
	Human (n = 6), B6C3F$_1$ mouse, and SD rat liver microsomes and microsomes derived from human β-lymphoblastoid cells containing cDNA-expressed CYP 1A1, 1A2, 2A6, 2B6, 2D6, 2D1, 3A4. Same experiment as (1j) above.	(4f) Duescher and Elfarra (1994)
(5) Crotonaldehyde→acrolein + CO_2		No published literature supporting this reaction in vitro or in vivo.
(6) EB→DEB		
In vitro experiments		
	Wistar rat liver microsomes.	(6a) Malvoisin et al. (1979b)
Detected two stereoisomers of DEB.	Wistar rat liver microsomes.	(6b) Malvoisin and Roberfroid (1982)
	SD rat, B6C3F$_1$ mouse, and human liver (n = 12) and lung (n = 5) microsomes. Identified DEB only in mouse liver microsomes.	(6c) Csanády et al. (1992)
	Human microsomes containing cDNA-expressed CYP isozymes (1A1, 1A2, 2A6, 2D6, 2E1, 2F1, 3A4) and SD rat, B6C3F$_1$ mouse and human (n = 10) liver microsomes.	(6d) Seaton et al. (1995)
In vivo inhalation experiments		
	Same experiments as (1s) above. DEB in blood of mice, not rat.	(6e) Himmelstein et al. (1994)
	Same experiments as (1t) above.	(6f) Bechtold et al. (1995)
	Same experiments as (1u) above. DEB in lungs of mice but not rat.	(6g) Himmelstein et al. (1995)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
In vivo inhalation experiments		
	Same experiments as (1v) above. DEB in blood, heart, and lung, thymus, fat, spleen and liver, higher in mice than in rats.	(6h) Thornton-Manning et al. (1995a)
	Same experiments as (1w) above. DEB in blood, bone marrow, fat, lung, mammary (female only), higher in females than in males.	(6i) Thornton-Manning et al. (1995b)
(7) EB→GSH conjugates		
In vitro experiments		
	Same experiment as (1b) above.	(7a) Bolt et al. (1983)
	SD rat, NMRI mouse, and human (n = 1) liver microsomes and cytosol.	(7b) Kreuzer et al. (1991)
	Purified human placental π class GSH S-transferase.	(7c) Sharer et al. (1991)
	SD rat and B6C3F$_1$ mouse liver, lung, kidney, and testis cytosol. Same experiment as (1g) above.	(7d) Sharer et al. (1992)
	SD rat, B6C3F$_1$ mouse, and human liver (n = 12) and lung (n = 5) microsomes and cytosol. Same experiment as (1f) and (6c) above.	(7e) Csanády et al. (1992)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
***In vivo experiments*: *EB-GSH conjugates*:**		
1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene.	Inhalation exposure of EB to SD and F344/N rats, $B6C3F_1$ mice, Syrian hamsters, and cynomolgus monkeys. Urinary metabolites.	(7f) Sabourin et al. (1992)
S-(2-hydroxy-3-buten-1-yl)glutathione.	i.p. injection of EB to SD rats. Isolated and identified regioisomeric GSH conjugates in bile of rats.	(7g) Sharer and Elfarra (1992)
S-(2-hydroxy-3-buten-1-yl)-*N*-acetyl-L-cysteine and *S*-(1-hydroxy-3-buten-2-yl)-*N*-acetyl-L-cysteine.	SD rats and $B6C3F_1$ mice, by i.p. injection. Urinary metabolites.	(7h) Elfarra et al. (1995)
S-(1-hydroxymethyl)-2-propenyl)-L-cysteine in mouse, not in rat. *N*-acetyl-*S*-(2-(hydroxymethyl)-2-propenyl)-L-cysteine and *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine in both mouse and rats.	Inhalation exposure of rats and mice. Same study as (3a) above. Urinary metabolites.	(7i) Nauhaus et al. (1996)
(8) EB→BD		
In vitro experiments		
	Wistar rat liver microsomes.	(8a) Malvoisin and Roberfroid (1982)
	SD rat liver microsomes treated with inhibitor of epoxide hydrolase. Same experiment as (1b) and (7a) above.	(8b) Bolt et al. (1983)
	SD rat, NMRI mouse, and human (n = 1) liver microsomes.	(8c) Kreuzer et al. (1991)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
	Rat, mouse, and human lung and liver microsomes. Same study as (1f) and (6c) above.	(8d) Csanády et al. (1992)
	Rat (strain not stated) liver microsomes. Same experiment as (4d) above.	(8e) Cheng and Ruth (1993)
***In vivo inhalation experiments: BD-GSH conjugates*:**		
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane. (BD necessary intermediate of this product.)	Same experiment as (7f) above. Urinary metabolites.	(8f) Sabourin et al. (1992)
Human urinary clearance predominantly via epoxide-hydrolase-mediated hydrolysis.	F344/N rats, B6C3F$_1$ mice, and humans in occupational exposure. See (9b) below.	(8g) Bechtold et al. (1994)
	Same study as (3a) and (7i) above.	(8h) Nauhaus et al. (1996)
(9) BD→GSH conjugates		
***In vivo inhalation experiments: BD-GSH conjugates*:**		
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane.	Same experiments as (7f) and (8f) above. Urinary metabolites in monkey, rat, hamster, and mouse.	(9a) Sabourin et al. (1992)
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane.	Same experiments as (8g) above. Urinary metabolites in humans.	(9b) Bechtold et al. (1994)
N-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine in mouse and rat, *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse but not in rat.	Same study as (3a), (7i), and (8h) above. Urinary metabolites.	(9c) Nauhaus et al. (1996)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
(10) BD→1,3-dihydroxypropanone		
In vivo inhalation experiments	Same study as (3a), (7i), (8h), and (9c) above.	(10a) Nauhaus et al. (1996)
(11) BD→3,4-epoxy-1,2-butanediol		
In vitro experiments	Wistar rat liver microsomes. Same experiment as (6b) above.	(11a) Malvoisin and Roberfroid (1982)
	Rat (strain not stated) liver microsomes. Same experiments as (4d) and (8e) above.	(11b) Cheng and Ruth (1993)
(12) DEB→3,4-epoxy-1,2-butanediol		
In vitro experiments	Human (n = 6), SD rat, and B6C3F$_1$ mouse liver and lung microsomes.	(12a) Boogaard and Bond (1996)
(13) DEB→GSH conjugates		
***In vitro experiments: DEB-GSH conjugates*:** *S*-(2-hydroxy-3,4-epoxybut-1-yl)glutathione and *S*-(4-hydroxy-2,3-epoxybut-1-yl)glutathione.	*Salmonella typhimurium* TA1535 transfected with rat GSH S-transferase 5-5 cDNA.	(13a) Thier et al. (1995)
	SD rat, B6C3F$_1$ mouse, and human (n = 6) liver cytosol and SD rat and B6C3F$_1$ lung cytosol.	(13b) Boogaard et al. (1996)
***In vivo experiments: DEB-GSH conjugates*:** *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse, but not in rat.	Same experiment as (3a), (7i), (8h), (9c), and (10a) above. Urinary metabolites.	(13c) Nauhaus et al. (1996)

Table 3-2. Species comparison of reaction rates for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene

Reaction and tissue	Species	Strain	Reaction rate[a] or V_{max}	K_M (mM)[b]	Reference (exposure concentration)[c]
(1) 1,3-Butadiene→EB					
Liver microsomes	Mouse	All	0.24-9.9		All studies with species differences data
	Rat	All	0.36-45		
	Monkey		----		
	Human		0.121-22.8		
	Mouse	$B6C3F_1$, NMRI	0.24-0.40[d]		(1c) Schmidt and Loeser (1985) (30,000 ppm 1,3-butadiene)
	Rat	SD, Wistar	0.08-0.1[d]		
	Monkey	rhesus	0.73[g]		
	Human		0.12[d]		
	Mouse	$B6C3F_1$	2.6	2.0	(1f) Csanády et al. (1992) (600-25,000 ppm 1,3-butadiene)
	Rat	SD	0.59	3.74	
	Monkey		----		
	Human		1.18	5.14	
	Mouse	$B6C3F_1$	6.4		(1g) Sharer et al. (1992) (330,000-660,000 ppm 1,3-butadiene)
	Rat	SD	3.0		
	Monkey		----		
	Human		----		
	Mouse	$B6C3F_1$	9.2	160	(1j) Duescher and Elfarra (1994) (30,000-660,000 ppm 1,3-butadiene)
	Rat	SD	2.0	120	
	Monkey		----		
	Human		10.4-22.8	200-400	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (μM)[b]	Reference (exposure concentration)[c]
Lung microsomes	Mouse	All	2.3-6.1		All studies
	Rat	All	0.16-1.5		
	Human		0.15		
	Mouse	$B6C3F_1$, NMRI	4.4-5.6[e]		(1c) Schmidt and Loeser (1985)
	Rat	SD, Wistar	0.6-0.91[e]		(30,000 ppm 1,3-butadiene)
	Human		----		
	Mouse	$B6C3F_1$	2.3	5.01	(1f) Csanády et al. (1992)
	Rat	SD	0.16	7.75	(600-25,000 ppm 1,3-butadiene)
	Human		0.15	2.0	
	Mouse	$B6C3F_1$	6.1		(1g) Sharer et al. (1992)
	Rat	SD	1.5		(330,000-660,000 ppm 1,3-butadiene)
	Human		----		
Kidney microsomes	Mouse	$B6C3F_1$	23.8		(1g) Sharer et al. (1992)
	Rat	SD	0.5		(330,000-660,000 ppm 1,3-butadiene)
(6) EB → DEB					
Liver microsomes	Mouse	$B6C3F_1$	1.4	141	(6d) Seaton et al. (1995)
	Rat	SD	0.41	145	(5-1,000 μM EB)
	Human		0.38-1.2	304-880	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (µM)[b]	Reference (exposure concentration)[c]
(7) EB →GSH conjugates					
Liver cytosol	Mouse	$B6C3F_1$	107	3,100	(7d) Sharer et al. (1992)
	Rat	SD	71	3,100	(2-10 mM EB)
	Human		----		
	Mouse	$B6C3F_1$	500	35,300	(7e) Csanády et al. (1992)
	Rat	SD	241	13,800	(20-200 ppm EB)
	Human		45.1	10,400	
Lung cytosol	Mouse	$B6C3F_1$	12	3,100	(7d) Sharer et al. (1992)
	Rat	SD	3	3,100	(2-10 mM EB)
	Human		----		
	Mouse	$B6C3F_1$	273	36,500	(7e) Csanády et al. (1992)
	Rat	SD	44.2	17,400	(20-200 ppm EB)
	Human		$2.56 \cdot 10^{-4}$ [f]		
Kidney cytosol	Mouse	$B6C3F_1$	16	3,100	(7d) Sharer et al. (1992)
	Rat	SD	7	3,100	(2-10 mM EB)
Testis cytosol	Mouse	$B6C3F_1$	30	3,100	(7d) Sharer et al. (1992)
	Rat	SD	51	3,100	(2-10 mM EB)

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (µM)[b]	Reference (exposure concentration)[c]
Purified placental GSH S-transferase	Human		500	10,000	(7d) Sharer et al. (1992)
Spontaneous[g]			$2.01 \cdot 10^{-4}$		
(8) EB →BD					
Liver microsomes	Mouse	NMRI	19	1,500	(8c) Kreuzer et al. (1991)
	Rat	SD	17	700	(30, 300, 3,000 ppm EB)
	Human		14	500	
	Mouse	$B6C3F_1$	5.79	1,590	(8d) Csanády et al. (1992)
	Rat	SD	2.48	260	(20-200 ppm EB)
	Human		9.2-58.1	240-1,650	
Lung microsomes[h]	Mouse	$B6C3F_1$	$1.86 \cdot 10^3$		(8d) Csanády et al. (1992)
	Rat	SD	$1.32 \cdot 10^3$		(20-200 ppm EB)
	Human		$3.19\text{-}7.55 \cdot 10^3$		
Spontaneous[g]	Human		$7.75 \cdot 10^{-4}$		(8d) Csanády et al. (1992)
(12) DEB → hydrolysis products					
Liver microsomes	Mouse	$B6C3F_1$	32.0	8,100	(12a) Boogaard and Bond (1996)
	Rat	SD	52.9	2,760	(0.185-15 mM DEB)
	Human		156	4,800	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (μM)[b]	Reference (exposure concentration)[c]
Lung microsomes	Mouse		49.3	7,500	(12a) Boogaard and Bond (1996)
	Rat		19.3	7,100	(0.558-5 mM EB)
	Human		21.7	2,830	
(13) DEB → GSH conjugates					
Liver cytosol	Mouse	$B6C3F_1$	162	6,400	(13b) Boogaard et al. (1996)
	Rat	SD	186	24,000	(0.1-25 mM DEB)
	Human		6.4	2,100	
Lung cytosol	Mouse		38.5	1,700	(13b) Boogaard et al. (1996)
	Rat		17.1	4,200	(0.1-25 mM DEB)
Spontaneous[g]			$1.65 \cdot 10^{-3}$		(13b) Boogaard et al. (1996) (0.1-25 mM DEB)

[a]Reaction rates are either from the reported reaction rates (units of nmol · min^{-1} · mg microsomal or cytosolic $protein^{-1}$ unless otherwise noted) or from maximum reaction rate (V_{max} in units of nmol · min^{-1} · mg microsomal or cytosolic $protein^{-1}$ unless otherwise noted), when corresponding K_M is given.
[b] Concentration at one-half maximum reaction rate.
[c] Numbers and letters in parentheses preceding reference refer to appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.
[d] Values reported were corrected from nmol · min^{-1} · g $tissue^{-1}$ to nmol · min^{-1} · mg microsomal $protein^{-1}$ assuming values of 11.6 (mouse), 16.8 (rat), and 14.5 (human) mg microsomal protein · g $tissue^{-1}$ reported by Csanády et al. (1992).
[e] Value has units of nmol·min^{-1} · g $tissue^{-1}$.
[f] None of the human lung cytosolic fractions displayed Michaelis-Menten reaction kinetics. The reactions are described by first-order k_{gsh} with units of L · nmol · min^{-1} · mg $protein^{-1}$.
[g] Spontaneous conjugation rate of EB or DEB with GSH is described by first-order k_{gsh} with units L · min^{-1} · $mmol^{-1}$.
[h] Hydrolysis of epoxybutene in lung microsomes is described as first-order k_{hy} with units of min^{-1} · mg $protein^{-1}$.
[I] Spontaneous hydrolysis rate of epoxybutene or diepoxybutane in 0.1 M phosphate buffer is described as first-order k_{hy} with units of min^{-1}.

Source: Modified from Himmelstein et al., 1997.

al., 1997). In general, the range of reaction rates or maximal reaction velocity (V_{max}) for different reactions in different tissues do not provide a clear pattern of species differences; in particular, the range of values for human tissues spans the range of values for both rats and mice. However, within any single study, for the oxidation of 1,3-butadiene to epoxybutene, the reaction rates of liver and lung microsomes are higher in rats than in mice. Multiple cytochrome P-450 enzymes are involved in the metabolism of 1,3-butadiene. For example, in human liver microsomes, the metabolic oxidation of 1,3-butadiene to epoxybutene is principally mediated by P-450 isoenzymes 2A6 and 2E1. Biotransformation of 1,3-butadiene to the non-DNA-reactive butene diol is the predominant pathway observed in in vitro metabolism studies that used hepatic microsomes from rats and humans, and formation of the DNA-reactive diepoxybutane is relatively minor in these species. However, the latter pathway is significant in mouse hepatic microsomes. 1,3-Butadiene can also be metabolized to epoxybutene by human myeloperoxidase and by mouse and human bone marrow cells.

In the Csanády et al. (1992) study, the authors also extrapolated the kinetic constants obtained from in vitro experiments to equivalent in vivo rates by adjusting the in situ protein content and organ weights across species, as shown in Table 3-3. However, for GST, Kohn and Melnick (1993) pointed out that the rate constants should be adjusted to the mg cytosolic protein/g liver instead of to the mg microsomal protein/g liver as done by Csanády et al. (1992). The corrected values are also included in Table 3-3. These can all be used in pharmacokinetic models as hepatic and lung metabolic clearance.

3.1.2.2. *In Vivo Pharmacokinetics*

In vivo pharmacokinetic studies examine absorption, distribution, metabolism, and/or elimination. Most studies report results on several of these four components. Absorption is often measured either by the distribution of 1,3-butadiene and/or its metabolites in tissue organs or by the elimination of 1,3-butadiene metabolites in excreted urine, feces, and exhaled air. In vivo metabolism studies include measurements of concentration profiles of the various metabolite pools after exposure to butadiene. Metabolic kinetic constants are usually calculated from the rate of formation of the metabolites or from the clearance rate evaluated from excretion data. This section summarizes the in vivo pharmacokinetic studies. Because inhalation is the principal route of exposure to 1,3-butadiene, most of the absorption data for the chemical have been derived from inhalation exposure studies. Based on the blood:air partition coefficient for 1,3-butadiene (0.603 in vitro; 0.645 in vivo), the passage of 1,3-butadiene from the air into the blood is by simple diffusion (Carpenter et al., 1944).

Table 3-3. Rate constants for in vivo hepatic clearance of 1,3-butadiene and EB[a] (extrapolated from in vitro)

	Cytochrome P-450 monooxygenase[b]	Epoxide hydrolase[b,c]	Cytosolic glutathione S-transferase[b]	Microsomal conjugation of EB with GSH[d]	First-order hydrolysis
Mouse	55.9 0.55[e]	0.16	4.4	0.011	0.0028
Rat	7.92	0.48	5.7	0.024	0.0022
Human	6.19	0.86	0.46	0.069	0.0014

[a]Values are in units of L/h/kg.
[b]In vivo V_{max} values were calculated from in vitro V_{max} (tables 3-1 through 3-3) and adjusted for interspecies differences in microsomal and cytosolic protein concentrations and liver volume. Mouse, rat, and human liver microsomal concentrations were 11.6, 16.8, and 14.5 mg/g liver, respectively. Mouse, rat, and human liver cytosolic concentrations were 11.6, 16.8, and 14.5 mg/g liver, respectively. Mouse, rat, and human liver cytosolic concentrations were 82.8, 108, and 58 mg/g liver, respectively. Liver organ volumes for mice, rats, and humans were 6.2, 5.0, and 3.1% of body weight, respectively. In vivo hepatic clearance values (V_{max}/K_M expressed in L/h/kg) were estimated by dividing the in vivo V_{max} values by the apparent in vitro K_M's for the reaction.
[c]Modified according to Kohn and Melnick, 1993.
[d]For nonenzymic hydrolysis and reaction with glutathione, in vivo clearance was calculated using the organ fractions in footnote b. To estimate the in vivo clearance for reaction with glutathione, a concentration of 10 mM GSH was used.
[e]Rate constant for metabolism of EB to DEB.

Sources: Csanády et al., 1992; Kohn and Melnick, 1993.

Two main in vivo inhalation systems are used to conduct inhalation studies. The first one is the closed-system inhalation chamber, and the second one is the nose-only exposure inhalation system. These studies are reviewed by Himmelstein et al. (1997) and summarized in Tables 3-4 to 3-6 for the closed inhalation chamber studies and Tables 3-7 to 3-10 for the nose-only inhalation studies.

In the closed-system inhalation chamber study, rats or mice are placed in a desiccator jar chamber. Two rats or up to eight mice per experiment are exposed to different initial 1,3-butadiene chamber concentrations. Air samples from the desiccator are measured directly by gas chromatography-mass spectrometry (GC-MS) through an air valve. With the use of a two-compartment pharmacokinetic model (Filser and Bolt, 1981), shown in Figure 3-2, uptake and clearance kinetic constants of 1,3-butadiene and epoxybutene can be evaluated, as shown in Tables 3-5 and 3-6, which give the results of these studies. Because the metabolic elimination rate constant (k_{el}) cannot be determined accurately from the gas uptake studies, 1,3-butadiene and epoxybutene were administered intraperitoneally to the mice and rats, and exhaled 1,3-butadiene and epoxybutene concentrations were monitored in the chamber and used to evaluate k_{el} (Bolt et al., 1984). Tables 3-5 and 3-6 show that for both 1,3-butadiene and epoxybutene, uptake ($k_{12}V_1$) and clearance (Cl_{tot}) in mice are about twofold greater than in rats. Although the exhalation rate constant (k_{21}) and metabolic elimination rate constant (k_{el}) are comparable for 1,3-butadiene in both mice and rats, mice exhaled epoxybutene about twice as much as rats (k_{21}), whereas the metabolic rate constant (k_{el}) is about fivefold higher in rats than in mice (Laib et al., 1990). Under these conditions, the steady-state epoxybutene concentration in mice is about sixfold that in rats (Melnick and Huff, 1992; Himmelstein et al., 1994).

A second inhalation experimental system is the nose-only exposure, where exhaled breath is sampled by placing the animals in plethysmography tubes. Additional blood and tissue samples can also be obtained by sacrifice of the animals after different exposure durations. However, while the air samples are measured at real time, all blood and tissue samples are subjected to some time delay due to processing of the samples. These studies are summarized in Table 3-7 (modified from Himmelstein et al., 1997). Table 3-8 summarizes the results of the studies showing that 1,3-butadiene and its epoxide metabolites (epoxybutene and diepoxybutane) have been found in blood at different inhalation exposure concentrations to 1,3-butadiene in rats, mice, and monkeys.

Thornton-Manning et al. (1995a) also examined the disposition of epoxybutene and diepoxybutane in various tissues following nose-only inhalation exposure of male Sprague-

Table 3-4. Summary of closed-chamber inhalation studies

Reaction and reference[a]	Description of experiment	Finding
(1) 1,3-Butadiene→EB		
(1l) Bolt et al. (1983)	Exposure of SD rats to butadiene at 6,000 to 7,000 ppm initial concentration.	Quantified epoxybutene in exhaled breath. Peak concentrations of epoxybutene were 2 to 4 ppm at 15 h after exposure.
(1m) Bolt et al. (1984)	Exposure of SD rats to butadiene at initial concentrations ranged from 90 to 12,000 ppm.	Metabolic uptake rate = 220 μmol · h^{-1} · kg^{-1} for untreated rats when the butadiene chamber concentration was >1,500 ppm. Pretreatment with Aroclor 1254 caused a linear increase in the metabolic uptake rate from 220 to 1,200 μmol · h^{-1} · kg^{-1}; P-450 inhibitor diethyldithiocarbamate completely inhibited metabolism.
(1n) Kreiling et al. (1986a)	Exposure of $B6C3F_1$ mice to butadiene at initial concentrations ranged from 10 to 5,000 ppm.	Maximal metabolic uptake rate = 400 μmol · h^{-1} · kg^{-1}. Pretreatment of mice with P-450 inhibitor diethyldithiocarbamate completely inhibited uptake of butadiene.
Filser and Bolt (1984)	Exposure of SD rats to epoxybutene at initial concentration ranged from 500 to 5,000 ppm (not reported by author, estimated from Figure 3-2 in their paper).	Linear metabolic uptake occurred up to 5,000 ppm. V_{max} > 2,600 umol · h^{-1} · kg^{-1} (see text for description of model used in calculation).

Table 3-4. Summary of closed-chamber inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
Uptake of EB[b]		
Kreiling et al. (1987)	Exposure of B6C3F$_1$ mice to epoxybutene at initial concentration ranging from 10 to 5,000 ppm (estimated from Figure 1 of Kreiling et al., 1986b).	Saturated metabolic uptake occurred between 100 and 500 ppm. V_{max} = 350 umol $\cdot h^{-1} \cdot kg^{-1}$.

Abbreviations: ppm = parts per million; SD = Sprague-Dawley rat; V_{max} = Michaelis-Menten enzyme kinetic constant expressing maximum metabolic rate.

[a] Numbers and letters preceding reference refer to the appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.
[b] Reaction rates measured as uptake of epoxybutene, which could involve several reactions shown in Figure 3-1 and summarized in Table 3-1.

Source: Modified from Himmelstein et al., 1997.

Table 3-5. Toxicokinetic parameters for uptake and elimination of 1,3-butadiene in mice and rats

Parameter (units)	Mouse	Rat	Definition of parameter
$K_{12}V_1$ (mL/h)	10,280	5,750	Equilibrium constant between chamber volume and test animals; first order, $V_1 \rightarrow V_2$.
K_{21} (h^{-1})	3.2	2.5	Equilibrium rate constant between chamber volume and animals; first order, $V_2 \rightarrow V_1$.
K_{eq} (NA)	2.7	2.3	Static equilibrium constant representing virtual absence of metabolism.
K_{st} (NA)	1	0.5	Steady-state concentration; ratio of concentration in animal to chamber concentration.
k_{el} (h^{-1})	7.6	8.8	First-order metabolic elimination rate constant.
Cl_{tot}[a,b] (mL/h)	7,300	4,500	Total clearance of chemical from chamber.
V_{max} (µmol/h/kg)	400	220	Maximum rate of metabolism of chemical.

[a]Calculated for $V_1 \rightarrow \infty$.
[b]Valid for linear range of metabolism (up to 1,000 ppm for both species).
NA = not applicable.
Source: Filser and Bolt, 1981; Kreiling et al., 1990.

Table 3-6. Toxicokinetic parameters for the uptake and elimination of epoxybutene in rats and mice

Parameter (units)	Mouse	Rat	Definition of parameter
$k_{12}V_1$ (mL/h)	33,500	13,800	Equilibrium constant between chamber volume and test animals; first order, $V_1 \rightarrow V_2$.
K_{21} (h^{-1})	0.79	0.37	Equilibrium rate constant between chamber volume and animals; first order, $V_2 \rightarrow V_1$.
K_{eq} (NA)	42.5	37	Static equilibrium constant representing virtual absence of metabolism.
K_{st} (NA)	10.2	1.16	Steady-state concentration; ratio of concentration in animal to chamber concentration.
k_{el} (h^{-1})	2.3	11.5	First-order metabolic elimination rate constant.
Cl_{tot}[a,b] (mL/hr)	24,900	13,400	Total clearance of chemical from chamber.
V_{max} (µmol/h/kg)	350	>2,600	Maximum rate of metabolism of chemical.
Metabolic saturation (ppm)	500	>5,000	Concentration resulting in saturated metabolism.

[a]Calculated for $V_1 \rightarrow \infty$.
[b]Valid for linear range of metabolism (up to 1,000 ppm for both species).
NA = not applicable.
Source: Filser and Bolt, 1981; Kreiling et al., 1987; Laib et al., 1990.

Table 3-7. Summary of nose-only inhalation studies

Reaction and reference[a]	Description of experiment	Finding
(1) 1,3-Butadiene→EB		
(1q) Bond et al. (1986)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (7, 70, or 1,000 ppm for up to 6 h).	Epoxybutene tentatively identified in blood after 2, 4, or 6 h of exposure. EB blood concentrations were 0.4 and 3.3 µM in rats exposed to 70 and 1,000 ppm 1,3-butadiene and 0.5, 1.6, and 13 µM in mice exposed to 7, 70, and 1,000 ppm, respectively. Concentration of EB in blood of mice 2 to 3 times > rats.
(1r) Dahl et al. (1990)	Exposure of cynomolgus monkeys to 1,3-butadiene (10, 300, or 8,000 ppm for 2 h).	Blood samples collected at single time immediately after exposure. Quantitation of EB in blood by vacuum-trap distillation method. Results shown in Table 3-8. Total butadiene metabolites in blood were 5 to 50 times lower in monkey than in the mouse and 4 to 14 times lower than in the rat (rat and mouse data were from Bond et al., 1986).
(1s) Himmelstein et al. (1994)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (62.5, 625, or 1,250 ppm up to 6 h; blood collected at 2 to 6 h of exposure and up to 30 min postexposure).	1,3-Butadiene and EB pharmacokinetics characterized in blood by GC (butadiene) and GC-MS (EB). Butadiene steady-state concentrations (µM) ranged from 2.4 to 58 (mice) and 1.3 to 37 (rats). EB steady-state concentrations (µM) ranged from 0.56 to 8.6 (mice) and 0.07 to 1.3 (rats); EB blood concentration in mice was 4 to 8 times > rats.
(1t) Bechtold et al. (1995)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (100 ppm for 4 h; blood collected at end of exposure).	Quantitated butadiene and EB in blood by GC-GC-MS. Blood levels (µM) of butadiene were 4.1 (rat) and 2.9 (mouse). Blood levels of EB (µM) were 0.10 (rat) and 0.38 (mouse).
(1u) Himmelstein et al. (1995)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (625, 1,250, or 8,000 [rats only] ppm for 6 h; tissue samples collected at 3 and 6 h of exposure and 6 and 12 min postexposure).	Quantitated EB concentration by GC-MS in mouse lung 14 times > rat lung, mouse liver 5 to 8 times > rat liver. Peak concentrations of EB (nmol · g tissue^{-1}) during exposures were 2.6 to 3.7 (mouse lung), 0.16 to 1.3 (rat lung), 0.58 to 0.93 (mouse liver), and 0.06 to 1.2 (rat liver).

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(1v) Thornton-Manning et al. (1995a)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (62.5 ppm for 4 h; samples collected at 2 and 4 h of exposure and at 0.5 and 1 h postexposure included blood, fat, heart, liver, lung, spleen, and thymus).	Quantitated EB by GC-GC-MS. Detection limits were 0.031, 0.037, and 0.062 nmol · g tissue^{-1} in blood, heart, and lung, respectively. EB concentrations were 3 to 74 times higher in tissues of mice compared with rats. EB was not detected in lung or liver of rats.
(1w) Thornton-Manning et al. (1995b)	Exposure of female and male SD rats to butadiene (6.25 ppm for 6 h, samples collected at end of exposure included blood, femur, fat, lung, and mammary tissue).	Quantitated EB by GC-GC-MS. EB concentrations were similar for males and females. At 6 h exposure. Results summarized in Table 3-10.
(1y) Thornton-Manning et al. (1996)	Exposure of female SD rats and female B6C3F$_1$ mice after either a single 6 h (daily 6 h exposure for 10 days to 62.5 ppm 1,3-butadiene).	Quantitated EB by GC-GC-MS. EB levels were 5- and 1.6-fold higher in mammary tissue and 2- and 1.4-fold higher in fat tissue in rats and mice, respectively, after repeated exposures. DEB levels were 7.7 ± 2.2 and 12.5 ± 0.8 pmol/g in fat of rats and 265 ± 19 and 191 ± 29 pmol/g in mammary tissue of mice after single and repeated inhalation exposures, respectively.
(3) 3-Butenal→GSH conjugates		
(3a) Nauhaus et al. (1996)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-[^{13}C]-butadiene (800 ppm up to 5 h; urine collected during exposure and for up to 20 h postexposure).	Quantitated the urinary metabolite *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine in mouse urine using ^{13}C-NMR. This metabolite represented 3.7% of total urinary metabolites excreted by mice but was not detected in rat urine.
(6) EB→DEB		
(6e) Himmelstein et al. (1994)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1s) above.	Quantitated time course of DEB in blood of mice by GC-MS. Peak concentrations of DEB in the blood of mice were 0.65, 1.9, and 2.5 µM after 6 h of exposure to 62.5, 625, or 1,250 ppm 1,3-butadiene; DEB not quantitated in rats. Detection limit = 0.13 µM.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(6f) Bechtold et al. (1995)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1t) above.	Same finding as (6d) above. DEB detected in mouse and not rat blood; DEB concentration = 0.33 µM in mouse blood after 4 h exposure to 100 ppm 1,3-butadiene. Detection limit = 0.1 µM.
(6g) Himmelstein et al. (1995)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1u) above.	Quantitated time course of DEB in lungs of mice by GC-MS. Peak concentrations in mice exposed to 625 and 1,250 ppm 1,3-butadiene were 0.71 and 1.5 nmol · g tissue^{-1}, respectively. Detection limit = 0.04 µM.
(6h) Thornton-Manning et al. (1995a)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1v) above.	Quantitated DEB by GC-GC-MS. Detection limits were 0.0016, 0.031, and 0.026 nmol · g tissue^{-1} in blood, heart, and lung, respectively. DEB concentrations were 40- to 163-fold lower in rat tissues compared with mice. DEB was not detected in liver of rats. Results summarized in Table 3-9.
(6i) Thornton-Manning et al. (1995b)	Exposure of female and male SD rats to 1,3-butadiene. Same conditions as (1w) above.	Quantitated DEB by GC-GC-MS. DEB concentrations were 3.6- to 7.1-fold greater in tissues of female rats compared with tissues of male rats. Results summarized in Table 3-9.
(7) EB→GSH conjugates		
(7f) Sabourin et al. (1992)	Exposure of SD and F344/N rats, B6C3F$_1$ mice, Syrian hamsters, and cynomolgus monkeys to 1,3-[^{14}C]-butadiene (8,000 ppm, 0.78 µCi/mmol).	Products identified in urine included 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene.
(7i) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a) above.	Quantitated urinary metabolites (as percentage of total ^{13}C) using NMR. *S*-(1-(hydroxymethyl)-2-propenyl)-L-cysteine (4.7%) present in mouse urine but not detected in rat urine. *N*-acetyl-*S*-(2-(hydroxymethyl)-2-propenyl)-L-cysteine was present in mouse (22%) and rat (53%) urine. *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine was present in mouse (44%) and rat (18%) urine.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(8) EB→BD		
(8g) Bechtold et al. (1994)	Exposure of F344/N rats and B6C3F $_1$ mice to 1,3-butadiene (11.7 ppm for 4 h by nose-only exposure). In vivo inhalation exposure of humans occupationally exposed to 1,3-butadiene (human study described in more details in text).	Predominant pathway for clearance of epoxybutene in humans is by epoxide hydrolase-mediated hydrolysis rather than direct conjugation with GSH. See (9b) below.
(8h) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a) and (7i) above.	Quantitated BD in mouse and rat urine using ^{13}C-NMR. This metabolite represented 2.9% and 5.0% of total ^{13}C-metabolites excreted by mice and rats, respectively.
(9) 3-Butene→BD		
(9b) Bechtold et al. (1994)	Same experiment as (8g) above.	Quantitated 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane in human urine using isotope-dilution GC-MS. Metabolite represents 97% of GSH conjugates derived directly from EB (see (7g) above) or indirectly by epoxide hydrolase-mediated hydrolysis and GSH conjugation of BD (see (8e) above).
(9c) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), and (8h) above.	Quantitated *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine in mouse (7.1%) and rat (26.4%) urine using ^{13}C-NMR. *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine occurred in mouse (7.1%) urine but was not detected in rat urine. This latter metabolite was also surmised to be a product of DEB as described in reaction 13 below.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(10) BD→1,3-dihydroxypropanone		
(10a) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), and (9c) above.	Quantitated 1,3-dihydroxypropanone in rat urine as 5.3% of total ^{13}C-metabolites excreted. It was not detected in mouse urine. Transformation of DEB to 1,3-dihydroxypropanone may also contribute to excretion of this compound (see reaction 14 below). This reaction would also be expected to contribute to the formation of CO_2.
(13) DEB→GSH conjugates		
(13c) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), (9c), and (10a) above.	Quantitated *N*-acetyl-*S*-(1-hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse (7.1%) urine. This metabolite was not detected in rat urine and also may be formed as a product of BD as described in study (9c) above.
(14) DEB→1,3-dihydroxypropanone		
(14a) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), (9c), (10a), and (13c) above.	Quantitated 1,3-dihydroxypropanone in rat urine as 5.3% of total ^{13}C-metabolites excreted. It was not detected in mouse urine. Transformation of BD to 1,3-dihydroxypropanone may also contribute to excretion of this compound (see study (10a) above). This reaction would also be expected to contribute to the formation of CO_2.
(15) 3,4-Epoxy-1,2-butanediol→GSH conjugates	No published literature supporting this reaction.	GSH conjugates of epoxybutanediol have not been quantitated in either in vitro or in vivo studies.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(17) Erythritol→CO_2	No published literature supporting this reaction.	Bond et al. (1986) showed that CO_2 was exhaled in the breath of SD rats and B6C3F$_1$ mice exposed to 1,3-[^{14}C]-butadiene, although the chemical reactions leading to CO_2 formation are unknown. Mass balance of $^{14}CO_2$ from exhaled breath and residual $^{14}CO_2$ from carcass showed that mice had greater uptake of butadiene than rats. Assumption is made that CO_2 derives from erythritol. Dahl et al. (1991) also measured $^{14}CO_2$ in exhaled breath of monkeys exposed to 1,3-[^{14}C]-butadiene. Uptake and retention of ^{14}C in mice > rat > monkey.

Abbreviations: BD = 3-butene-1,2-diol; DEB = 1,2:3,4-diepoxybutane; CO_2 = carbon dioxide; EB = 1,2-epoxy-3-butene; GC = gas chromatography; GSH = glutathione; MS = mass spectrometry; NMR = nuclear magnetic resonance spectrometry; ppm = parts per million; SD = Sprague-Dawley rat.

[a] Numbers and letters preceding reference refer to the appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.

Source: Modified from Himmelstein et al., 1997.

Table 3-8. Comparison of 1,3-butadiene, epoxybutene, and diepoxybutane blood concentration data from different species of laboratory animals exposed to 1,3-butadiene by inhalation

Species	Butadiene exposure concentration (ppm) Mean (SE)[a]	Concentration of analyte in blood (nM analyte · ppm^{-1}) Mean (SE) 1,3-Butadiene	EB	DEB	Reference
Mouse	7.83 (0.02)	72 (20)	68 (9)	8.5 (0.8)	Bond et al. (1986)[b]
	80 (0.2)	5.4 (0.6)	20 (2)	2.1 (0.2)	Bond et al. (1986)
	1,031 (13)	5.8 (0.6)	12.9 (0.5)	2.3 (0.3)	Bond et al. (1986)
	71 (7)	34 (4)	7.9 (1)	9.2 (1.7)	Himmelstein et al. (1994)[c]
	603 (44)	61 (7)	6.1 (0.8)	3.2 (0.4)	Himmelstein et al. (1994)
	1,282 (33)	45 (3)	6.7 (0.5)	2.0 (0.4)	Himmelstein et al. (1994)
	101 (4)	29 (5)	3.8 (0.6)		Bechtold et al. (1995)[d]
	93 (5)			3.5 (0.9)	
Rat	73.9 (0.4)	1.8 (0.2)	5.4 (0.4)	1.1 (0.2)	Bond et al. (1986)[b]
	949 (12)	3.2 (0.2)	3.5 (0.4)	0.8 (0.1)	Bond et al. (1986)
	63 (2)	21 (1)	1.1 (0.2)		Himmelstein et al. (1994)[c,e]
	616 (8)	29 (1)	1.5 (0.1)		Himmelstein et al. (1994)

Table 3-8. Comparison of 1,3-butadiene, epoxybutene, and diepoxybutane blood concentration data from different species of laboratory animals exposed to 1,3-butadiene by inhalation

	Butadiene exposure concentration (ppm)	Concentration of analyte in blood (nM analyte · ppm^{-1})			
		Mean (SE)[a]			
Species	Mean (SE)	Butadiene	EB	DEB	Reference
Rat	1,249 (3)	30 (1)	1 (0.1)		Himmelstein et al. (1994)
	7,938[f]	32 (1)	0.18 (0.01)		Himmelstein et al. (1995)
	97 (2)	42 (4)	0.6 (0.1)		Bechtold et al. (1995)[d]
Monkey	10.1 (0.1)	0.8 (0.4)	0.16 (0.05)	0.19 (0.06)	Dahl et al. (1990)[f] for EB
	310 (10)	1.8 (1.3)	1.6 (0.9)	0.9 (0.5)	Dahl et al. (1991)[f,j] for DEB
	7,760 (170)	4.1 (0.5)	0.14 (0.06)	0.08 (0.03)	

[a] Standard error (SE) combines variation of butadiene exposure concentration and blood concentration data.
[b] Pooled mean ± SE of samples (n = 9) collected after 2, 4, and 6 h of exposure; animals removed from 1,3-butadiene exposure and exhaled 1,3-butadiene before blood was collected; ^{14}C-labeled analytes were recovered by vacuum-line cryogenic distillation and quantitated by liquid scintillation counting.
[c] Values are means ± SE (n = 6-33 samples) for blood collected between 2 and 6 h of exposure; animals continued to inhale 1,3-butadiene as blood was collected; 1,3-butadiene was quantitated by a vial headspace equilibrium technique using GC-flame ionization detection; detection limit = 0.3 µM.
[d] Values are means ± SE (n = 6) for samples collected at 4 h of exposure; animals continued to inhale 1,3-butadiene as blood was collected; analytes were recovered by vacuum-line cryogenic distillation and analyzed by GC-GC-MS; detection limit = 0.1 µM.
[e] One exposure was conducted at this 1,3- butadiene concentration, therefore no SE reported.
[f] Blood (n = 3) collected immediately following 2 h exposure using indwelling catheter; 1,3-butadiene recovered by vacuum-line cryogenic distillation and quantitated as ^{14}C-labeled equivalent using liquid scintillation counting.
[g] EB and DEB were recovered by extraction into methylene chloride and quantitated by GC-MS; detection limits = 0.03 µM for EB and 0.13 µM for DEB.
[h] Detection limits for EB and DEB = 0.02 µM and 0.01 µM, respectively.
[i] The authors exposed the monkeys to 1,3-[^{4}C]-butadiene and vacuum-line cryogenic distillation for this compound includes ^{14}C-labeled DEB, BD, and potentially other unidentified ^{14}C-metabolites. Since this study looked at products resulting from several reactions, it was not included in Table 3-7 (which described single reactions in the pathways).

Source: Modified from Himmelstein et al., 1997.

Table 3-9. Tissue levels of epoxybutene and diepoxybutane (pmol/g tissue) in male rats and male mice exposed by inhalation to 62.5 ppm 1,3-butadiene for 4 h

Tissue[a]	EB		DEB[a]	
	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[c]	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[c]	95 ± 12
Thymus	12.5 ± 3.2	104 ± 55	2.7 ± 0.7[c]	109 ± 19
Bone marrow[d]	0.2 ± 0.1	2.3 ± 1.5	ND	1.4 ± 0.3

[a]Mean ± SE; n = 3 or 4.
[b]ND = not detected; indicates that analyte was not detected or was not above control level.
[c]Includes at least one ND value.
[d]As mean pmol/mg protein ± SE.

Source: Modified from Thornton-Manning et al., 1995a.

Table 3-10. Tissue levels of epoxybutene and diepoxybutane (pmol/g tissue) in male and female rats exposed by inhalation to 62.5 ppm 1,3-butadiene for 6 h

Tissue[a]	EB		DEB	
	Males	Females	Males	Females
Blood	25.9 ± 2.9	29.4 ± 2.0	2.4 ± 0.4	11.4 ± 1.7[c]
Femur	9.7, 9.3	10.4 ± 1.0	1.1, 1.8	7.1 ± 1.3[c]
Lung	12.7 ± 5.0	2.7 ± 4.3	1.4 ± 0.8[b]	4.8 ± 0.7[c]
Fat	175 ± 21	203 ± 13	1.1 ± 0.1	7.7 ± 1.3[c]
Mammary	ND	57.4 ± 4	ND	10.5 ± 2.4[c]

[a]n = 3, except for male femur, where n = 2.
[b]One value was not detectable; instrument detection limit/2 was substituted to calculate the mean.
[c]Statistically greater than male tissue value, $p \leq 0.05$.

ND = not determined.
Source: Modified from Thornton-Manning et al., 1995b.

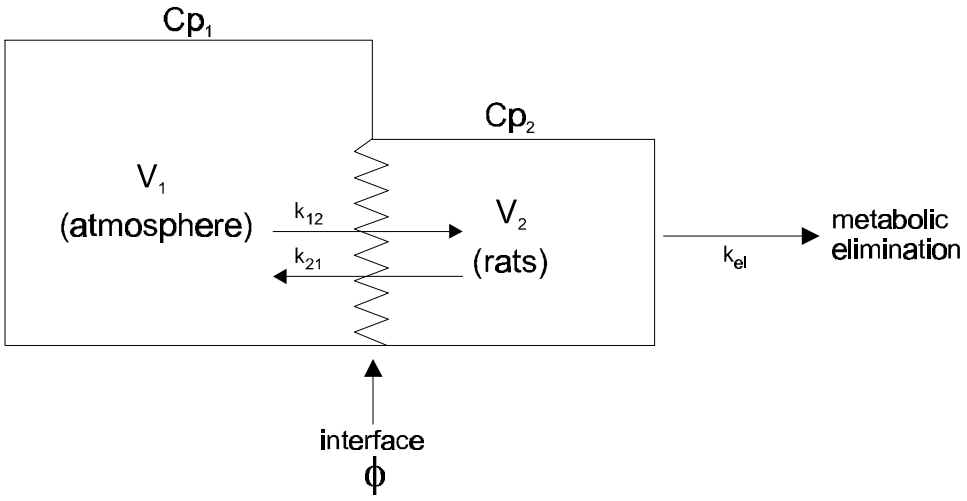


Figure 3-2. Two-compartment pharmacokinetic model for inhalation chamber.

Source: Filser and Bolt, 1981.

Dawley rats and male B6C3F$_1$ mice to 62.5 ppm 1,3-butadiene for 4 h, as described in Table 3-7, with the results shown in Table 3-9. The same group of investigators (Thornton-Manning et al., 1995b) also examined gender differences in the production and disposition of epoxybutene and diepoxybutane by determining tissue concentrations of the two butadiene metabolites in male and female Sprague-Dawley rats, as described in Table 3-7, with the results shown in Table 3-10. The concentrations of epoxybutene did not differ significantly between male and female rats in any of the tissues examined. The highest concentrations were observed in the fat tissues of both sexes. Tissue levels of the diepoxybutane, however, were consistently greater in females than in males. Blood diepoxybutane levels of female rats were 4.75-fold greater than those of male rats. The greatest gender difference was in the levels of the diepoxybutane in fat tissue, with females having a sevenfold greater tissue concentration than males. The mammary tissue of females also contained relatively high levels of the diepoxybutane. The authors suggest that the greater production of the highly mutagenic diepoxybutane 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).

Dahl et al. (1991) exposed cynomolgus monkeys to nose-only inhalation of 1,3-butadiene and measured the levels of diepoxybutane and 3-butene-1,2-diol. Results for diepoxybutane are included in Table 3-9 for comparison to 1,3-butadiene and epoxybutene levels measured in their previous study (Dahl et al., 1990). Exhaled air and excreta were collected during exposure and for 96 h after exposure and are summarized in Table 3-11.

Two in vivo studies provided data on the urinary excretion of butadine metabolites by humans. In the first study (included in Table 3-7 and described in more detail here), Bechtold et al. (1994) identified and measured two metabolites of 1,3-butadiene, 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*-)-butane (M-I) and 1-hydroxy-2-(*N*-acetylcysteinyl-*S*-)-3-butene (M-II) in the urine of workers employed at the Texaco Chemical Co. in Port Neches, Texas, a 1,3-butadiene extraction plant. The study population included (1) 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; (2) an intermediate exposure group spending variable time periods in low- and high-exposure areas; (3) nonexposed employees who worked in areas with historical time-weighted average concentrations of less than 0.1 ppm 1,3-butadiene; and (4) outside controls who had no known exposure to 1,3-butadiene. Urine samples were analyzed from 7, 3, 10, and 9 subjects, respectively, from the above four groups. The assay was based on isotope-dilution GC-MS. After addition of deuterated internal standards, the metabolites were isolated from urine samples by solid-phase extraction and selective precipitation. M-I but not M-II could be readily identified and quantitated in the urine samples

Table 3-11. Excretion of ^{14}C by monkeys exposed to 1,3-[^{14}C]-butadiene[a]

Exposure concentration (ppm)	Exhalants			Uptake	
	CO_2	Other[b]	Urine	Feces	Total metabolites recovered[c]
10.1	1.5 ± 0.2	0.45 ± 0.33	0.9 ± 0.1	0.021 ± 0.005	2.88 ± 0.22
310	0.21 ± 0.04[d]	0.40 ± 0.21	0.8 ± 0.2	0.011 ± 0.003[d]	1.40 ± 0.42[d]
7760	0.08 ± 0.02[d,e]	1.00 ± 0.35	0.58 ± 0.06[d]	0.002 ± 0.001[d,e]	1.65 ± 0.29[d]

[a]Values are mean percentage of total inhaled ± SE measured for 96 h after 2-h exposure.
[b]Includes all material (except CO_2) exhaled during the 2-h exposure and 96-h postexposure.
[c]Mean ± SE of the sums of CO_2, other, urine, and feces values for individual monkeys; does not include residues, if any, in monkeys' bodies.
[d]Significantly different from low-level exposure ($p<0.05$).
[e]Significantly different from mid-level exposure ($p<0.05$).
Source: Dahl et al., 1991.

(limits of sensitivity for this assay, 100 ng/mL). The average values of M-I for exposed, intermediately exposed, nonexposed, and outside control employees were 3,200 ± 1,600, 1,390± 550, 630 ± 190, and 320 ± 70 ng/mL, respectively. Although the levels of exposure for each individual were not known, the urinary levels of M-I for the exposed groups were significantly higher ($p<0.05$) compared with the outside control group. The implications of M-I in the urine from individuals with no known exposure to 1,3-butadiene are not known.

In the second study that provided human data, Ward et al. (1996a) reported increased levels of the urinary metabolite 1,2-dihydroxy-4-(*N*-acetyl-cysteinyl)-butane (a human urinary metabolite also idenfified by Bechtold et al., 1994) and somatic mutations in workers at a styrene-butadiene rubber plant. Exposure was assessed in workers from areas of higher exposures (reactor, recovery, tank farm, laboratory) and lower exposure (blend,coagulation, bailers, shipping, utilities, shops) using badge dosimeters; the concentration of the metabolite was measured in urine; and the frequency of *hprt* mutant lymphocytes was determined by autoradiography. 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. Sixteen high- and nine low-exposure urine and blood samples were analyzed. Expressed as ng/mg creatinine, metabolite concentrations were 2,363 ± 1,880 and 937 ± 583 ($p<0.05$), respectively, for the

high- and low-exposure groups. The respective mean mutant frequencies were $7.09 \pm 5.2 \times 10^{-6}$ and $2.26 \pm 1.34 \times 10^{-6}$ ($p<0.05$).

Other in vivo studies that further confirm the pathways shown in Figure 3-1 but use different intermediate endpoints than those shown or with noninhalation exposure are described below. Deutschman and Laib (1989) studied the effects of 1,3-butadiene exposure on nonprotein sulfhydryl (NPSH) content of lung, heart, and liver tissue of rats and mice. In these experiments, male $B6C3F_1$ mice and Sprague-Dawley rats were exposed to 1,3-butadiene at concentrations of 10, 50, 100, 250, 500, 1,000, or 2,000 ppm for 7 h. For rats, a reduction (≈70%; significance level not stated) occurred in liver NPSH for animals exposed to 1,000 to 2,000 ppm. A reduction of approximately 20% was observed in lung NPSH of rats, and no appreciable depletion of NPSH was observed for heart tissue of rats. For mice, depletion of hepatic NPSH was observed at exposure concentrations of 100 to 250 ppm and declined to 20% of control for the 2,000 ppm exposure group. Similarly, the NPSH content of mouse lung tissue also declined by 80% to 90% at the two highest exposure levels. For heart NPSH content in mice, minor declines were noted for exposure levels up to 500 ppm, but a rapid decrease was observed between 1,000 and 2,000 ppm that resulted in an ≈75% depletion. Kreiling et al. (1988) suggested that the greater susceptibility of mice to the carcinogenic effects of inhaled 1,3-butadiene might reasonably be explained by the higher rate of formation of the epoxide intermediate and its limited detoxification and subsequent accumulation in mice. The authors applied the concentration-response data to the exposures used in earlier bioassays (HLE, 1981) and noted that, for rats exposed chronically to 1,3-butadiene concentrations of 1,000 or 6,000 ppm, a daily hepatic NPSH depletion of about 25% and 60% and lung NPSH content depletion of 20% and 30% for the low and high exposures, respectively, was calculated. However, these values were based on assumptions that 1,3-butadiene metabolism and NPSH resynthesis remained constant throughout the duration of the chronic exposure. Applying the same methods and assumptions for mice, daily NPSH depletions for the low-exposure (625 ppm) and high-exposure (1,250 ppm) levels, respectively, were estimated for liver (50% and 70%), lung (70% and 90%), and heart (25% and 40%). In studies assessing the effects of 1,3-butadiene exposure on NPSH content of various tissues, Deutschmann and Laib (1989) reported that depletion of cardiac NPSH content in mice after inhalation exposure was an indicator of systemically available epoxide intermediates of 1,3-butadiene that reach the heart by efferent blood flow from the lungs or liver.

The reduction and/or depletion of NPSH content in mice is also indicative of saturation of conjugation of the epoxide metabolites of 1,3-butadiene by glutathione. Glutathione conjugation of epoxybutene and metabolism by glutathione S-transferase was shown by Malvoisin et al. (1981) and Bolt et al. (1983), respectively, and a reduction in hepatic NPSH content in mice

exposed to 1,3-butadiene was shown by Kreiling et al. (1987). A more recent study by Kreiling et al. (1988) suggested that glutathione conjugation may be important in the detoxification of this reactive intermediate. Air-exposed control animals exhibited a moderate time-dependent decrease in hepatic NPSH content, whereas those animals exposed to 1,3-butadiene exhibited a significantly greater reduction in NPSH. After 7 h of exposure, the hepatic NPSH content in mice was reduced to approximately 20% and was reduced further to about 4% after 15 h of exposure. For Sprague-Dawley and Wistar rats, hepatic NPSH content was initially reduced to 80% and 65%, respectively, but remained stable thereafter. Concurrent with the significant depletion of NPSH in the mice were signs of acute toxicity (specifics not noted); no toxicity was observed in any of the rats tested. This experiment clearly demonstrates species variability in the magnitude and time course of hepatic NPSH depletion after inhalation exposure to high concentrations of 1,3-butadiene. Furthermore, the progressive decline in hepatic NPSH content in mice correlates with a reduction in epoxide exhalation and a decline in 1,3-butadiene metabolism. The accumulation of epoxide intermediates (epoxybutene and diepoxybutane) in mice (Bond et al., 1986) is consistent with the observed depletion of hepatic NPSH in this species and the increased metabolism (i.e., production of epoxide intermediates) observed for mice.

Nauhaus et al. (1996) indicated that metabolites were detected in mouse urine that are also seen following exposure to acrolein and acrylic acid, suggesting that these compounds may arise directly from 1,3-butadiene oxidation or indirectly from further metabolism of crotonaldehyde. Rats excreted 1,3-dihydroxypropanone, a metabolite that may be derived from hydrolysis of diepoxybutane. Metabolites derived from diepoxybutane were similar in rats and mice when expressed as a percentage of total metabolites; however, when normalized to body weight, the amount of diepoxybutane-derived metabolites was four times greater in mouse urine than in rat urine. The greater body burden of diepoxybutane in the mouse and the greater ability of rats to detoxify diepoxybutane through hydrolysis may be related to the greater toxicity of 1,3-butadiene in mice. The metabolites derived via reactive aldehyde intermediates in mice also suggest a role of these aldehydes in the toxicity of 1,3-butadiene.

Following i.p. injection of 14.3 or 143 µmol/kg of epoxybutene, 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). At either dose, the amount of conjugates excreted in 30 min was at least 85% of that excreted in 120 min. When the epoxybutene dose 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. 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.

Although the study showed that epoxybutene GSH conjugates are formed in vivo after administration of epoxybutene, biliary excretion of GSH conjugates account for only a small portion of the administered dose.

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 with epoxybutene intraperitoneally (Elfarra et al., 1995). When rats were injected with epoxybutene at doses ranging from 71.5 to 285 µmol/kg, the 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 of epoxybutene administration exhibited a linear dose-relationship; the total amount of the two mercapturic acids combined averaged 17% ± 4%. No metabolites were detected in urine samples collected 8 to 24 h after dosing. Mice excreted similar amounts of mercapturic acids (26% ± 13%) at 285 µmol/kg within 24 h of dosing. However, at 143 and 71.5 µmol/kg, excretion accounted for only 7% ± 3% and 9% ± 3% of the dose, respectively. 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). The study showed that at low exposure levels, rats excrete higher levels of epoxybutene mercapturic acids than mice.

In summary, the inhalation studies show the uptake of 1,3-butadiene exhibits first-order kinetics at exposure concentrations <1,000 ppm, but at higher concentrations, the process becomes saturated and exhibits zero-order kinetics; mice exhibit saturation kinetics at lower exposure concentrations than do rats. At exposure concentrations up to 1,800 ppm, the uptake of 1,3-butadiene is approximately fourfold greater in mice than in rats. In addition, mice accumulate a greater amount of 1,3-butadiene or its metabolites or both than do rats exposed similarly. Limited data on monkeys indicate that the metabolic uptake rate is less than that for rats or mice. After inhalation of 1,3-butadiene, mice appear to have greater levels of radioactivity (15- to 100-fold greater at all time points after exposure) in all tissues than do rats exposed similarly, but no significant qualitative differences have been observed regarding storage depots or target tissues. However, immediately after a 2 h inhalation exposure, mice exhibited higher levels of 1,3-butadiene metabolites (including the reactive epoxybutene) in the blood than did rats. A comparison of butadiene epoxide levels in target tissues (blood, bone marrow, heart, lung, fat, spleen, and thymus) of rats and mice following inhalation of low levels of 1,3-butadiene showed consistently higher epoxide levels in mouse than in rat tissues. Other in vivo experiments demonstrated gender differences in the production of butadiene metabolites in rats, with tissues from female rats containing higher concentrations of the diepoxybutane than tissues from male rats. The experiment also showed that the levels of epoxybutene were similar in males and females. In vivo experiments have confirmed the role of cytochrome P-450 in the metabolic

activation of 1,3-butadiene observed in in vitro studies. Epoxybutene, a reactive intermediate, may undergo epoxide hydrolase-mediated hydroxylation or conversion via P-450 to another reactive intermediate, diepoxybutane. Conjugation with glutathione represents a detoxification process.

Although 1,3-butadiene may be metabolized by microsomal cytochrome P-450 in both rats and mice, species-related quantitative differences in the fate of inhaled 1,3-butadiene are well documented. The greater susceptibility of mice to the carcinogenic effects of 1,3-butadiene may be related to the higher rate of epoxybutene formation and the limited detoxification and, hence, the greater accumulation of this reactive intermediate in this species. At concentrations >2,000 ppm, the metabolism of 1,3-butadiene follows saturation kinetics in both rats and mice, but the rate of metabolism in mice is greater (about twice); furthermore, the metabolism of epoxybutene is saturable in mice but not in rats. With increasing exposure concentration, the metabolic capacity for epoxybutene becomes rate-limiting in mice but not in rats. Data available from studies with nonhuman primates show that at low-exposure concentrations ($\leq$10 ppm), the steady-state tissue levels of reactive 1,3-butadiene metabolites are lower in monkeys than in rats or mice. The lower uptake rate of inhaled 1,3-butadiene by monkeys suggests that, for comparable exposures, monkeys will receive a lower internal dose of reactive butadiene metabolites. The uptake and retention of 1,3-butadiene appears to be nonlinear in the concentration ranges used in long-term exposure studies, and repeated exposures to 1,3-butadiene do not appear to induce its metabolism.

1,3-Butadiene may be excreted via the respiratory tract, urine, or feces. The rate of 1,3-butadiene excretion by rats and mice was shown to be unaffected by exposure concentration (0.14 to 13,000 µg/L). Half-lifes for urinary excretion of radioactivity were similar for both rats and mice (5.6 and 4.6 h, respectively), but fecal excretion was somewhat greater in rats (22 h) than in mice (8.6 h). A shift to excretion of 1,3-butadiene-derived [^{14}C] via the lungs was noted for rats but not mice at high (13,000 µg/L) exposure concentrations. Approximately 2% of the total inhaled dose was excreted as $^{14}CO_2$ or in the urine of monkeys exposed for 2 h to 1,3-[^{14}C]-butadiene at concentrations ranging from 10 to 8,000 ppm. At the higher concentrations, the proportion of CO_2 decreased, whereas exhaled metabolites (diepoxybutane and butene diol) increased. Elimination of radioactivity from the blood and tissues of rats and mice after inhalation exposure to 1,3-[^{14}C]-butadiene was biphasic; half-lifes for initial removal were 2 to 10 h and for slower elimination were 5 to 60 days. Excretion of epoxybutene via the lungs by rats and mice also has been studied and notable differences between the species observed. For rats, exhaled epoxybutene concentrations at 10 h attained a plateau of about 4 ppm and remained at this level for >12 h. For mice, however, the plateau level was about 10 ppm but declined to 6 ppm at 15 h, a decline that coincided with signs of acute toxicity in the mice.

Studies on the urinary excretion of 1,3-butadiene metabolites in mice, rats, hamsters, monkeys, and humans have shown that all these species predominantly produce two urinary metabolites, 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*)-butane (M-I) and 1-hydroxy-2-(*N*-acetylcysteinyl-*S*)-3-butene (M-II), but in different proportions. The M-II is a mercapturic acid formed by conjugation of GSH with epoxybutene, while M-I is a mercapturic acid that appears to form by GSH conjugation with butene diol, the hydrolysis product of diepoxybutane. M-I but not M-II was also found in the urine of workers exposed to low levels of 1,3-butadiene.

3.2. MOLECULAR DOSIMETRY

In addition to data on absorption, metabolism, and excretion, a complete dosimetry model for 1,3-butadiene should incorporate information on molecular dosimetry, which links exposure to some internal biomarkers of exposure. This last component is best evaluated by assessing adduct formation.

The use of Hb adducts as biomarkers of exposure to 1,3-butadiene was investigated by Sun et al. (1989a). In this study, male B6C3F$_1$ mice and male Sprague-Dawley rats were injected intraperitoneally with 1,3-[^{14}C]-butadiene at doses of 1, 10, 100, or 1,000 µmol/kg, and adduct formation was monitored. Hb adduct formation was linearly related to dose up to 100 µmol/kg for both species. The Hb adducts accumulated linearly after repeated injections of 100 µmol/kg for 3 days. The 1,3-butadiene-derived Hb adducts showed lifetimes of ≈24 and ≈65 days in mice and rats, respectively, which correlates with the lifetimes of red blood cells. Assuming that adduct formation is a function of the extent of 1,3-butadiene metabolism, the similarity in the degree of Hb adduct formation between mice and rats does not reflect the species variability in toxicity of this compound. Therefore, Hb adducts may not serve as accurate indicators of levels of reactive metabolites in the blood and, thus, as indicators of toxicity. However, Hb adduct formation may be useful as an indicator of 1,3-butadiene exposure.

Similar findings of exposure-dependent Hb adduct formation and stability of the adducts were reported by Osterman-Golkar et al. (1991) for Wistar rats exposed to 1,3-butadiene at concentrations of 250, 500, or 1,000 ppm, 6 h/day, 5 days/week for 2 weeks. In this study, the Hb adduct formation also increased linearly with exposure up to the highest exposure level. The investigators also concluded that Hb adducts were useful for assessing dosimetry of long-term exposure to 1,3-butadiene.

Osterman-Golkar et al. (1996) studied Hb 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. Using stationary and personal monitoring devices, the ambient 1,3-butadiene level for workers handling 1,3-butadiene containers was 11.2 ± 18.6 mg/m^3 and ≤1.2 mg/m^3 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 epoxybutene. Higher concentrations of Hb adducts (0.16 ± 0.099 pmol/g) were recorded in the workers handling 1,3-butadiene containers compared with those in maintenance, laboratory workers, and nine unexposed controls (≈0.05 pmol/g).

Citti et al. (1984) conducted an in vitro study that examined the reactivity of epoxybutene (referred to as epoxybutene by the authors) with isolated nucleosides and DNA. They reported that two adducts were formed: 7-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine. The authors indicated that the epoxide reacted similarly with either free DNA or DNA-bonded deoxyguanosine and that the half-life of these adducts under physiological conditions was 50 h.

Kreiling (1987) reported the in vivo formation of the DNA adduct 7-(1-hydroxy-3-buten-2-yl)guanine in the liver of mice exposed to 1,3-[^{14}C]-butadiene (exposure concentration and duration not specified). No DNA adducts were detected in the livers of 1,3-butadiene-exposed rats. Note that this adduct was one of two reported by Citti et al. (1984) for the in vitro reaction of 3,4-epoxybutene with DNA and deoxyguanosine. Additional details were not available in the abstract by Kreiling nor was additional information reported in later publications by Kreiling and coworkers.

Jelitto et al. (1989) reported species-dependent differences in the in vivo formation of DNA adducts by male B6C3F$_1$ mice and male Sprague-Dawley rats exposed to 1,3-[^{14}C]-butadiene at concentrations of 250, 500, or 1,000 ppm for 7 h. Analysis (alkaline elution and comparison of HPLC profiles with synthesized adduct standards) of liver DNA from the mice showed that two adducts had been formed: 7-*N*-(1-hydroxy-3-buten-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine, the latter being derived from diepoxybutane. These products were not detected in rat liver DNA. Alkaline elution curves showed that protein-DNA and DNA-DNA cross-linking occurred in mice, but not in rats, after a 7 h exposure to 1,3-butadiene at concentrations of 250 ppm and above. These findings provide additional evidence at the molecular level for explaining the difference in the carcinogenic response between mice and rats.

3.3. STRUCTURE-ACTIVITY RELATIONSHIPS

Studies by Del Monte (1985) and Dahl et al. (1987) have shown that the metabolism of structurally related isoprene (2-methyl-butadiene) may be qualitatively similar to that of 1,3-butadiene. Although the diepoxybutane metabolite of isoprene has been shown to be genotoxic in *Salmonella*, data are unavailable regarding the carcinogenic potential of isoprene.

Del Monte et al. (1985) showed that mouse hepatic microsomal monooxygenases converted isoprene to epoxides and diepoxides and that the biotransformation was inhibited by cytochrome P-450 inhibitors such as CO, SKF 525-A, and metyrapone. Specifically, 3,4-epoxy-3-methyl-

butene and 3,4-epoxy-2-methyl-1-butene were major and minor metabolites, respectively, with the latter representing about 20% of the former. The 3,4-epoxy-2-methyl-1-butene metabolite was metabolized further in microsomal incubations to the mutagenic isoprene dioxide (diepoxide). Data from these in vitro metabolism studies were used to calculate the K_M and V_{max} for the production of the diepoxide. The resulting K_M (mM) and V_{max} (nmol diepoxide/mg protein/min) values for diol production by microsomes from control, phenobarbital-induced, and 3-methylcholanthrene-induced mice were 0.24 and 1.7, 0.29 and 5.1, and 0.22 and 2.0, respectively. The V_{max} for the formation of the diepoxide was significantly increased ($p<0.01$) in incubations using hepatic microsomes from phenobarbital-treated mice.

Gervasi and Longo (1990) provided additional information on the metabolism of in vitro isoprene by hepatic microsomal preparations from rats, mice, rabbits, and hamsters. Hepatic microsomal preparations from these species metabolized isoprene to epoxybutene, 3,4-epoxy-3-methyl-1-butene, and 3,4-epoxy-2-methyl-1-butene. The former was the major metabolite and was found to have a half-life of 85 min. Microsomal preparations from all species further metabolized the 3,4-epoxy-2-methyl-1-butene to isoprene dioxide (2-methyl-1,2,3,4-diepoxybutane), which was found to be mutagenic and to have alkylating ability. The K_M (mM) and V_{max} (nmol/mg/protein/h) for the rat, mouse, rabbit, and hamster microsomal metabolism of isoprene were 0.08 and 0.24, 0.09 and 1.79, 0.2 and 0.66, and 0.06 and 1.20, respectively. Unlike 1,3-butadiene, isoprene exhibited the same pattern of metabolism in all species tested and did not result in mutagenic epoxybutene intermediates.

In the 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 highest exposure), and urine, feces, and exhalants were collected over a 66 h postexposure period. During this period, >75% of the nonisoprene (metabolites) radioactivity was excreted in the urine. Except for the highest exposure group where greater amounts of radioactivity were excreted in the feces, a pattern of predominantly urinary excretion was consistent among the various exposure groups. 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). 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 and that it probably represented covalently bound metabolites, conjugates of isoprene metabolites, or tetrols. Only at the two highest exposure concentrations were materials detected that possessed volatilities matching those of isoprene and isoprene monoepoxides. The percentage of inhaled isoprene-derived ^{14}C present as diepoxide or diol in the blood remained fairly constant with time but decreased with exposure concentration. Assessing the distribution of isoprene and its metabolites in some animals of the 1,480 ppm exposure group revealed that the liver and blood

contained the majority of the radioactivity. Relatively large amounts of metabolites were present in respiratory tract tissues after 20 min of exposure. The mutagenic metabolite, isoprene diepoxide, was identified in all tissues examined and, in the blood, represented between 0.0018% and 0.031% of the inhaled ^{14}C label. Although exposure to high concentrations of 1,3-butadiene result in CO_2 as the major metabolite, this study suggested that the major route of excretion for isoprene is in the urine. The authors noted, however, that this finding is tentative and may be the result of a labeling artifact. Although no evidence for metabolic saturation was detected for the isoprene concentrations used, the uptake and fate of inhaled isoprene are similar to that of butadiene.

Peter et al. (1987) also studied the pharmacokinetics of isoprene in male Wistar rats and male $B6C3F_1$ mice. Animals were exposed in closed systems to concentrations 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. The V_{max} for the metabolism of isoprene in rats and mice was 130 and 400 µmol/h/kg, respectively. Exhalation of the parent compound was approximately 15% and 25% in rats and mice, respectively.

Chloroprene (2-chloro-butadiene) is also structurally similar to 1,3-butadiene. Studies have shown that the biotransformation of chloroprene results in the formation of peroxides that may interact with tissue thiols (Haley, 1978). Furthermore, cytochrome P-450 mixed-function oxygenases may form an epoxide intermediate similar to that formed during 1,3-butadiene metabolism.

In summary, in vitro metabolism studies have shown that the structurally similar isoprene is metabolized in a similar fashion by several different species and that epoxybutene intermediates are formed, one of which may be epoxidized further to a genotoxic diepoxybutane. In vivo inhalation studies that used rats and mice exposed to isoprene showed that its uptake and fate are similar to that of 1,3-butadiene and that a genotoxic diepoxybutane metabolite, but not a genotoxic epoxybutene intermediate, is formed.

Preliminary data indicate that Hb adducts may be useful as biomarkers of exposure for 1,3-butadiene exposure. Research efforts are focusing on dosimetry modeling for extrapolating from high- to low-dose exposures and for interspecies extrapolation. Furthermore, on validation, dosimetry models may be useful in predicting levels of 1,3-butadiene and its reactive metabolites in various tissues.

3.4. DISCUSSION AND CONCLUSIONS

Species variability in the metabolism and disposition of 1,3-butadiene may explain, in part, species variability in the toxicity of the compound. Current data indicate that the toxicity of 1,3-

butadiene depends on the metabolic activation to reactive intermediates such as epoxybutene and diepoxybutane and that these biotransformation processes vary quantitatively and qualitatively among species. The mutagenic epoxybutene and diepoxybutane metabolites have been shown to occur in the blood of rats and mice exposed to 1,3-butadiene, and their concentrations are two- to fivefold greater in the blood of mice. Limited data for humans have shown that liver microsomes have a higher capacity for the formation of epoxybutene than do rodent liver microsomes but that the metabolism of epoxybutene to 1,3-butadiene epoxide by human liver microsomes was 20-fold greater than that observed in rat or mouse microsomes. These data suggest that levels of this reactive intermediate in humans may be substantially less than in the rodent species. The oxidation of epoxybutene to diepoxybutane (also a reactive metabolite) appears to be negligible in humans and rats (formation of the non-DNA-reactive butene diol 1,2-dihydroxybut-3-ene is the preferred pathway) and is substantial in mice. Study results have shown species-related differences in the uptake and retention of inhaled 1,3-butadiene. Uptake and retention by mice is greater than for rats, and saturation kinetics are observed in mice at exposure concentrations of 500 ppm but not in rats at exposures as high as 5,000 ppm. These differences may be used to support the hypothesis that the greater sensitivity of mice to the toxic effects of 1,3-butadiene may be a function of a greater internal dose, greater production of reactive metabolites, and lower detoxification potential.

Although the previous findings provide considerable insight into the understanding of 1,3-butadiene toxicity, some researchers have indicated the need for examining additional, although quantitatively minor, metabolic pathways (e.g., glutathione S-transferase-mediated detoxification processes and formation of toxic metabolites such as butene diol and crotonaldehyde) and the possible effects of pulse exposures on the metabolism and disposition of 1,3-butadiene.

Molecular dosimetry studies have also shown species-related differences in the formation of various adducts. Additional work in this area will be useful in assessing these adducts as either biomarkers of exposure or effects.

Dosimetry models are being developed or refined to extrapolate the relatively high exposures and doses used in animal tests to the low exposure concentrations in human exposure situations. These models will be especially useful in predicting blood and tissue concentrations of butadiene metabolites.

4. MUTAGENICITY

4.1. INTRODUCTION

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). The last of these reviewed publications through 1994 are on the genetic effects associated with butadiene (and metabolites). There is extensive evidence that butadiene and the two primary epoxide metabolites (epoxybutene and diepoxybutane) induce genotoxic effects in a variety of in vitro and in vivo test systems. 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 where mice are more responsive than rats. 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 butadiene metabolites.

4.2. GENE MUTATIONS

Most of the earlier in vivo genotoxicity studies used cytogenetic endpoints (aberrations, micronuclei, or sister chromatid exchange [SCE]). It is recognized that this reflected the dearth of in vivo assays measuring gene mutations and limited the interpretation of in vitro versus in vivo findings. 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 (1993, 1994a) used $B6C3F_1$ mice and human TK6 cells to evaluate the mutagenic potential of butadiene and the two major metabolites. In the in vivo studies, mice were exposed for 6 h/day, 5 days/week for 2 weeks to butadiene at 625 ppm. The induced *hprt* mutant frequency was 6.2×10^{-6} compared with 1.2×10^{-6} from unexposed controls. For the metabolites, mice received three daily intraperitoneal (i.p.) injections of 60, 80, or 100 mg/kg of epoxybutene or 7, 14, or 21 mg/kg of diepoxybutane. Mutant frequencies in *hprt* from splenic T cells were dose related for both metabolites, with maximal responses of 8.6×10^{-6} and 13×10^{-6} for epoxybutene and diepoxybutane, respectively. Similarly, they found diepoxybutane about 100 times more effective than epoxybutene when human lymphoblastoid TK6 cells were treated in vitro.

In a recent meeting presentation, Meng et al. (1996) reported on a study in which both mice and rats were exposed by inhalation to 1,250 ppm butadiene for 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 in both tissues of both species increased for several weeks and then declined. Maximal frequencies were: in thymus, 1.3×10^{-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 7.18 for mice compared with 2.04 for rats.

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 several experiments in which male B6C3F$_1$ *lacI* transgenic mice were exposed to 62.5, 625, and 1,250 ppm 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. Mutations 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. These findings are consistent with those observed in butadiene-induced *hprt* mutant T lymphocytes from B6C3F$_1$ mice (Cochrane and Skopek, 1994b).

Several studies of genetic effects in exposed workers have recently been reported. Ward et al. (1994, 1996b) measured the frequency of *hprt* mutations in lymphocytes of workers in a butadiene production plant (two studies) and in a styrene-butadiene rubber plant. In the first study exposure estimates were based on 8 h samples in two production areas and in a central control area. Mean butadiene concentration in the production areas was 3.5 ppm, but the majority of samples showed concentrations below 1 ppm; mean butadiene concentration in the control 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., 1996b). Measured 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. (1996b) 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 ± 0.85 × 10^{-6} for the high and low groups, respectively. While 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 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. The autoradiographic procedure for detecting *hprt* variants was used in these studies. The limitation of this method is that it is not possible to distinguish between several independent mutations and a single mutation giving rise to a clone of cells with the mutant phenotype. The procedure using the T lymphocyte cloning assay and subsequent DNA sequence analysis of clones as described by Albertini et al. (1982), and Recio et al. (1990) provide sufficient data for ascertaining independent mutational events.

Hayes et al. (1996) employed the T cell cloning assay to detect mutant frequencies in lymphocytes of workers in a rubber production factory. Butadiene levels were measured using personal samplers during the 6-h work shift and expressed as 6-h time-weighted average. 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, 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 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 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 butadiene detected in the personal samplers.

The results in both of the T cell cloning assay groups are clearly in conflict with the Ward et al. (1994, 1996b) findings both for exposed versus nonexposed and for smokers versus nonsmokers. A simple explanation would be that the increase in the autoradiographic assay was due to clones of mutants having arisen from earlier mutations. Even if true, the increase is clearly exposure related because 7 of the 8 exposed workers exhibited higher variant frequencies than the highest of the nonexposed controls. As indicated by Hayes et al. (1996), there are many differences between the two studies and currently no basis for rejecting either finding.

4.3. CYTOGENETIC EFFECTS—HUMAN

There have been four 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 butadiene-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, micronuclei, and sister chromatid exchange (SCE) frequencies were not elevated above samples from unexposed persons. They did note that smoking had a slight effect in micronuclei and SCE but not chromosome aberrations. Preliminary data measuring chromosome aberrations and micronuclei in blood samples from the 1994 group of workers was 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.

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 when treated with 50 or 250 µM 1,2-epoxy-3-butene. 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 diepoxybutane; however, they observed a 60% increase

in SCE in lymphocytes from GSTT1-null individuals when treated with 2 or 5 µM diepoxybutane. Neither GSTM1 nor GSTT1 deficiency affected the induction of SCE by 250 or 500 µM of 3,4-epoxybutane-1,2-diol (Bernadini et al., 1996). In a separate study, Kelsey et al. (1995) found that GSTT1 deficiency significantly increased the frequency of SCE induced by diepoxybutane in lymphocyte cultures of workers exposed to butadiene. Hence while all three epoxides of butadiene metabolism are effective inducers of SCE in cultured human lymphocytes, there are differences in the role of at least two of the GST genes (GSTM1 and GSTT1) in the detoxification of the three metabolites.

4.4. CYTOGENETIC EFFECTS—RODENT

Most of the rodent in vivo cytogenetic studies on 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. Recent efforts have focused on cytogenetic effects in germ cells of butadiene as well as effects of the two primary epoxides of butadiene.

Butadiene induced dominant lethal effects in studies of male mice exposed by inhalation (Adler and Anderson,1994); the details are described in Chapter 4. That study was followed by an experiment measuring heritable translocations induced in exposed males (Adler et al., 1995). Males were exposed by inhalation to butadiene at 1,300 ppm for 5 days for 6 h/day. Offspring were tested for translocations by both litter size and cytogenetic analysis of meiotic and somatic cells. The translocation frequency from treated males was 2.7% compared with 0.05% for historical controls.

Xiao and Tates (1995) evaluated the cytogenetic effects of 1,2-epoxybutene (EB) and 1,2:3,4-diepoxybutane (DEB) in both somatic and germ cells of mice and rats. Male animals of both species received single i.p. injections of 40 or 80 mg/kg of EB. Animals were sacrificed at various time intervals after treatment and spleen and testes were processed for scoring of micronuclei. In splenocytes, EB was almost four times more effective in the mouse as in the rat. 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 spermatocytes) 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. For DEB, mice were injected with 15 or 30 mg/kg and rats received single i.p. 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. In mouse germ cells, DEB increased the frequency of micronuclei only on day 3 after treatment. Significant increases of micronuclei were observed in rat germ cells at all doses and all time periods. The results in somatic cells are

consistent with all other reports of greater sensitivity in mice than in rats. This difference is contradicted in germ cells with rats equally (or more) sensitive to micronuclei induction by both EB and DEB. The authors offered no explanation for this, stating that more research is needed to better understand the organ and species differences. It is noted that the strains of both species are different from those used in most other endpoint measurements. The mice were F_1 males of a (102 × C3H) cross of parent stocks from Adler's laboratory in Germany. The rats were Lewis rats supplied by Harlan CPB, the Netherlands.

Sjöblom and Lähdetie (1996) used an in vitro meiotic micronucleus assay to examine the effects of EB, DEB, and 1,2-dihydroxy-3,4-epoxybutane (diolEB) in seminiferous tubule sections of male Sprague-Dawley rats. Tissue sections were cultured for 4 days with EB at 100, 500, or 1,000 mol/L; DEB at 5, 10, or 20 µmol/L; or diolEB at 10, 50, or 100 µmol/L. The frequency of micronuclei was increased only by DEB and the increase was clearly dose-related. That EB was not effective is contrasted with the findings of Xiao and Tates (1995) above. The authors suggest that EB requires further metabolism by P450 enzymes, which they indicate does not occur in rat testes microsomes.

4.5. SUMMARY

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 due to the mono- and diepoxide. While the difunctional DEB is clearly more effective than the monofunctional EB for most endpoints, it is not possible to ascribe the effects observed to one or the other when animals are exposed to butadiene. Where both 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 GST is also clearly established for the genotoxic effects of butadiene in human lymphocytes. That the two glutathione S-transferases (GSTM1 and GSTT1) react differently with the three epoxide metabolites suggests that the relative concentrations of these metabolites will vary depending on the individual's genotype.

5. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

5.1. REPRODUCTIVE EFFECTS

Several reproductive toxicity studies for 1,3-butadiene have been undertaken, starting with a study in rats, guinea pigs, rabbits, and dogs by Carpenter et al., 1944. Two studies by Owen and coworkers were done in rats (Owen et al., 1987; Owen and Glaister, 1990). NTP conducted two chronic reproductive toxicity studies in mice (NTP, 1984; 1993). Hackett and co-workers undertook an acute "sperm head morphology" study in B6C3F1 mice (Hackett et al., 1988a) and a dominant lethal study in CD-1 male mice (Hackett et al., 1988b). Dominant lethal studies, both acute and subchronic, have also been done in CD-1 male mice (Anderson et al., 1993, 1995) and in (102/E1xC3H/E1)F1 mice (Adler and Anderson, 1994).

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. Body weights were measured weekly; blood was analyzed monthly; and urinalysis, blood chemistry, organ weights (kidney and liver), and gross and histopathologic examinations were performed at termination. Males and females were mated, but the authors did not indicate when this occurred relative to the treatment period. No deaths were noted in the exposed animals. 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 control body weights. A similar trend was noted for male guinea pigs, and weights for dogs and rabbits fluctuated. No effects on organ weights that could be attributed to exposure to 1,3-butadiene were observed. There were no abnormal findings for hematology values or blood chemistry. Microscopic lesions were not observed in the testes, ovaries, or other organs examined (heart, kidney, skeletal muscle, pancreas, or spleen) except for the liver, in which mild, cloudy swelling was noted in 68% of the animals exposed to 6,700 ppm.

Carpenter et al. (1944) provided a few results regarding fertility of rats, guinea pigs, and rabbits exposed to 1,3-butadiene. 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 had an effect on fertility in rats. Furthermore, fertility in rats was not affected by exposure to 1,3-butadiene when litter size (8.4 pups/litter at 600 ppm, 7.9 pups/litter at 2,300 ppm, and 7.8 pups/litter at 6,700 ppm) was used as the measure; the

average litter size of the two higher exposure groups was similar to that of the control group. Two male and two female offspring from rats exposed to each concentration were exposed along with the parents. According to the authors, the F_1 controls and the 660-ppm group produced three times as many pups as did the F_1 groups exposed to 2,300 or 6,700 ppm. Too few animals were used to adequately evaluate the fertility of the exposed offspring. Guinea pigs in each exposure group produced 16, 13, 10, and 13 pups, respectively. Rabbits exposed to 600 or 2,300 ppm produced no pups, whereas the controls produced 24 pups and the 6,700 ppm group produced 27 pups. Considering that the highest concentration had no effect on fertility in rabbits, it is doubtful that the lack of fertility at the lower concentrations was due to exposure to 1,3-butadiene.

5.1.2. Owen et al., 1987; Owen and Glaister, 1990

This 2-year toxicological and carcinogenicity study is the same as the Hazleton Laboratories Europe, Ltd. (HLE, 1981), 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. Ten males and 10 females were killed at 52 weeks. The average weekly concentration of 4-vinyl-1-cyclohexene (a 1,3-butadiene dimer) was 413 ppm (v/v). A comprehensive postmortem examination, including necropsy and histopathologic examination, was conducted of 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 Leydig cell tumors were observed.

5.1.3. NTP, 1984

The first inhalation toxicological and carcinogenicity 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 toxicological and carcinogenicity 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, 312 ppm for 52 weeks, or 625 ppm for 13 or 26 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 5-1. 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 gland like structures. At the 15-month interim evaluation, ovarian atrophy was observed in mice exposed to 20 ppm or higher; the incidence at 62.5 ppm or higher was significant compared with concurrent controls. Hyperplasia of the germinal epithelium was seen at 200 and 625 ppm at nonsignificant incidences. 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. The incidence of angiectasis was significantly elevated only at 62.5 and 200 ppm, and the incidence of germinal epithelial hyperplasia was significantly elevated at 20 to 625 ppm. The occurrence of ovarian atrophy and germinal epithelial hyperplasia showed significant dose-related trends, whereas ovarian

Table 5-1. 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%)	—	—	0 (0%)	9 (90%)[b]	8 (100%)[b]
Germinal epithelial hyperplasia (NOS)	0 (0%)	—	—	0 (0%)	0 (0%)	1 (13%)
Uterus[a]	10	—	—	10	10	8
Atrophy[c]	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%)[b]	7 (70%)[b]	2 (100%)[d]
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[c]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
2-Year study[e]						
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%) $p<0.001$
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[c]	1 (2%)	0 (0%)	1 (2%)	1 (2%)	8 (16%)	41 (53%)

[a]Number of animals for which this site was examined microscopically.
[b]$p<0.01$, pairwise comparison with controls by Fisher's exact test.
[c]Statistical tests were not conducted for these lesions.
[d]$p<0.05$, pairwise comparison with controls by Fisher's exact test.
[e]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.

angiectasis did not. 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 5-2. The testes 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. Among male mice exposed to 1,3-butadiene in the stop-exposure studies, testicular atrophy was observed in only five mice exposed to 200 ppm (40 weeks), five exposed to 625 ppm (26 weeks), three exposed to 312 ppm (52 weeks), and three exposed to 625 ppm (13 weeks). It is not possible to determine if the lack of a more prominent response in mice exposed to 625 ppm for 26 weeks was due to insufficient time for induction of testicular atrophy or if atrophy had been induced during exposure and the lesion repaired before termination of the stop-exposure study.

5.1.5. 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. Twenty male $B6C3F_1$ mice (12 to 13 weeks old) per group 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. The animals were exposed in a 2.3 m^3 stainless steel chamber with a mixing volume of 1.7 m^3. Positive controls received intraperitoneal injections of 167 mg/kg of ethyl methane sulfonate daily for 5 consecutive days. After exposure, the mice were observed twice daily for mortality, morbidity, and signs of toxicity; body weights were determined weekly. 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).

Final body weights for the unexposed, treated, and positive control groups were similar, and net body weight gain over the period of the experiment was also similar for all groups. Piloerection and dyspnea were observed within the first 20 to 30 min after exposure in mice

Table 5-2. 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%)	C[e]	C	C	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%)	C	0 (0%)	C	0 (0%)	4 (57%)
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.

receiving 5,000 ppm; no signs of toxicity were noted for the other groups. Exposure-related gross toxicity was not observed in the reproductive tract. 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 ($p \leq 0.05$). The percentage 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.

5.1.6. Hackett et al., 1988b

This dominant lethal study was conducted using proven breeder male CD-1 mice (20 per group) exposed to 0, 200, 1,000, or 5,000 ppm 1,3-butadiene 6 h/day for 5 successive days. Measured concentrations (mean ± SD) were 200 ± 5.73, 1,010 ± 13.9, and 5,000 ± 85.4 ppm, respectively. The purity of the 1,3-butadiene was 99.88%, and the headspace dimer concentration was 215 ± 49 ppm. For mating, one exposed or control male mouse was placed with two unexposed female mice for 1 week for 8 successive weeks; the two females were replaced each week. Male mice were sacrificed at termination of matings, and female mice were sacrificed 12 days after the last cohabitation day. The reproductive status, total number, position and status of implantations, the number of early and late resorptions, and the number of live and dead fetuses were recorded.

No animals died during the study, and body weights of the exposed groups were similar to those of the control group. All males exposed to 1,3-butadiene were fertile during the 8-week exposure period. During the first week of mating (postexposure week), the total number of dead implants was significantly elevated for the group exposed only to 1,000 ppm ($p \leq 0.05$) compared with that of controls. Early resorptions accounted for most of the dead implants. In addition, the percentage of dead implants relative to the total implants was significantly elevated in groups exposed to 1,000 ppm ($p \leq 0.05$), and the percentage of females with more than one intrauterine death was significantly elevated in all exposed groups ($p \leq 0.05$) relative to controls. During the second postexposure week, the total number of dead implants was also significantly elevated at 200 and 1,000 ppm relative to controls. The percentage of dead implants and the percentage of females with more than one intrauterine death were elevated, but not significantly. For postexposure weeks 3, 5, 6, 7, and 8, the number of dead implantations, percentage of dead

implantations, and percentage of females with more than one intrauterine death in all exposed groups were similar to those of controls (i.e., not statistically significant). For postexposure week 4, the percentage of dead implants (5,000 ppm) and the percentage of females with more than one intrauterine death (200 and 5,000 ppm) were significantly reduced ($p \leq 0.05$) relative to the control value. However, the control values for these parameters were unusually high compared with control values at other postexposure weeks. Thus, the significantly reduced values for treated mice were probably not treatment related.

The results indicate that exposure to 1,3-butadiene may affect mature spermatozoa and spermatids assessed by preimplantation deaths for postexposure weeks 1 and 2. Interpretation of these results is complicated because the effects occurred in the 200 and 1,000 ppm groups but not in the 5,000 ppm group, which showed no indications of toxicity.

5.1.7. Anderson et al., 1993

The ability of 1,3-butadiene to induce dominant lethal mutations in male mice following acute and subchronic inhalation exposure was assessed by evaluating the number of dead implants in females mated to exposed males. For acute exposures, male CD-1 mice were exposed to 0, 1,250, and 6,250 ppm 1,3-butadiene for 6 h; 5 days later, each male was mated to two females. Males used for subchronic exposures were treated with 0, 12.5, or 1,250 ppm, 6 h/day, 5 days/week for 10 weeks. Following mating in both experiments, one female was killed on gestation day (gd) 17 and the other was allowed to litter for evaluation of long-term effects on the offspring. Results of long-term carcinogenic effects on the live offspring are not yet available. The female killed on gd 17 was examined for number of live fetuses, number and type of malformations in the fetuses, and number of postimplantation deaths. The only effect seen in the acute study was a decrease ($p \leq 0.05$) in the number of implantations in females mated to males exposed to 1,250 ppm. In the subchronic study, females mated to males exposed to 12.5 ppm had an increase in the number of late postimplantation deaths ($p \leq 0.01$; both fetal and placental tissue were present); females mated to males exposed to 1,250 ppm had a decrease in mean implantations per dam ($p \leq 0.01$) and an increase in both early ($p \leq 0.001$; resorption) and late postimplantation deaths ($p \leq 0.001$). 1,3-Butadiene appears to affect the male germ cell line, resulting in late postimplantation death of the fetuses. It is unknown whether the mutations/alterations of the germ cells resulting in a reduction in live fetuses are due to an effect on reproductive ability or a teratogenic effect resulting in death.

5.1.8. Adler et al., 1994

To assess the stage at which male germ cells are affected by 1,3-butadiene, male (102/E1 × C3H/E1)F_1 mice 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 appears to induce dominant lethality in spermatozoa and late spermatids.

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 coworkers 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.

5.2.1. IISRP, 1982

Female Sprague-Dawley CD rats were mated with male rats of the same strain (2f:1m) to produce 138 sperm-positive females. Groups of mated females (220 to 266 g) were exposed by inhalation to 1,3-butadiene at target concentrations of 0, 200, 1,000, or 8,000 ppm 6 h/day on 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 animals were exposed in stainless steel chambers. Twenty-four pregnant females were exposed to each concentration of 1,3-butadiene, 40 were exposed to filtered air (controls), and 26 were given 250 mg acetylsalicylic acid/kg body weight by gavage on gd 6 to 15 (positive controls). The purity of the 1,3-butadiene was not reported; the mean concentration of the dimer, 4-vinyl-1-cyclohexene, was 108.6 ± 53.59, well below the target of 300. The rats were weighed on gd 0, 3, 6, 9, 12, 15, 18, and 20. Various parameters of maternal and developmental toxicity were evaluated and analyzed using the litter as the statistical unit.

Maternal effects of 1,3-butadiene are summarized in Table 5-3. No animals died of exposure to 1,3-butadiene. One animal exposed to 1,000 ppm was killed because of morbidity unrelated to treatment. Clinical signs of toxicity were not observed in any group, and the

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

Parameter	Concentration (ppm)			
	0	**200**	**1,000**	**8,000**
No. dams assigned	40	24	24	24
No. of deaths	0	0	1[a]	0
No. pregnant (%)	90	91.7	100	95.8
Whole body weight (g)				
Day 0	239	238	240	239
Day 20	362	357	355	347
Body weight gain[b] (g)				
Days 0-6	24	24	23	23
Days 6-9	13	9	1[c]	1[c]
Days 9-12	16	13	14	11[c]
Days 12-15	15	15	16	15
Days 15-20	54	58	61	60
Gravid uterine weight (g)	63.9	61.1	66.5	62.8
Extragestational weight[d] (g)	297.9	296.2	280.8	283.9
Extragestational weight gain[e] (g)	59	58	49[f]	45[f]
Significant clinical signs	None	None	None	None

[a]This animal was killed in moribund state on day 19; morbidity was not related to exposure to butadiene.
[b]Determined from differences in group mean body weights reported for specific days of gestation.
[c]$p<0.01$, compared with corresponding control; analysis of variance and *t* test.
[d]Body weight on gd 20 minus gravid uterine weight.
[e]Extragestational weight minus body weight on gd 0.
[f]$p<0.05$, compared with corresponding control; analysis of variance and *t* test.

Source: IISRP, 1982.

pregnancy rates were similar in all groups. Terminal body weights showed a dose-related decrease (no statistical analysis). 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. Developmental effects of 1,3-butadiene are summarized in Tables 5-4 and 5-5. 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

For the experiment with rats, 208 female Sprague-Dawley CD rats and 108 male Sprague-Dawley CD rats (all 7 to 8 weeks old) were used. The rats were mated by placing two females with one male rat overnight for 5 consecutive nights or until a sperm-positive vaginal smear was obtained; gd 0 was the day sperm were detected. Thirty sperm-positive female rats per group were exposed to 0, 40, 200, or 1,000 ppm 1,3-butadiene (99.84% purity; 197 ± 6 ppm mean headspace dimer concentration). The measured concentrations (mean ± SD) were 40.1 ± 0.62 (mean ± SD), 199.8 ± 2.61, and 1,005 ± 11.9 ppm, respectively. On gd 6 to 15, the females were exposed for 6 h/day in stainless-steel chambers having a total volume of 2.3 m^3 and a mixing volume of 1.7 m^3. The females were weighed 1 week before mating and on gd 0, 6, 11, 16, and 20 (the day of sacrifice). Various parameters of maternal and developmental toxicity were evaluated. The experimental design is summarized in the upper section of Table 5-6.

The effects of inhalation exposure to 1,3-butadiene on maternal endpoints in rats are summarized in Table 5-7. All females survived to the end of the study. No clinical signs of toxicity were observed. Final body weights were similar to those of controls; body weight gain,

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

Parameter	Concentration (ppm)			
	0	**200**	**1,000**	**8,000**
No. pregnant (%)	90	91.7	100	95.8
No. litters with live fetuses	36	22	23	23
No. implantations/dam[a]	13.0	12.8	14.1	13.8
Preimplantation loss (%)	15.4	17.1	11.5	12.4
Postimplantation loss (%)	3.6	6.0	4.9	7.3
Total no. resorptions	17	17	16	23
Early resorptions	16	13	16	20
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	450/36	265/22	308/23	294/23
Fetal body weight[a] (g)	3.3	3.2	3.2	3.1[b]
Females[a]	3.2	3.1	3.1	3.0
Males[a]	3.4	3.3	3.3	3.2
Crown/rump length (mm)	37.8	37.2	37.2	35.9[c]
Sex ratio (% males)	49.8	54.7	51	50

[a]Mean values.
[b]$p<0.05$, Wilcoxon test.
[c]$p<0.01$, Wilcoxon test.

Source: IISRP, 1982.

Table 5-5. Malformations and variations in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	200	1,000	8,000
Total no. fetuses/no. litters examined	450/36	265/22	308/23	294/23
External/visceral defects				
Minor defects	76 (16.9)[a]	63 (23.8)	75 (24.4)[b]	75 (23.3)
Major defects	0	0	0	2 (0.7)
Unilateral lens opacity	19 (4.2)	11 (4.2)	8 (2.6)	13 (4.4)
Bilateral lens opacity	18 (4.0)	24 (9.1)	30 (9.7)	31 (9.5)[b]
Bilateral ureter dilation	13 (2.9)	8 (3.0)	3 (1.6)	19 (6.5)
Skeletal defects				
Minor defects	72 (23.2)	49 (26.9)[c]	45 (20.9)	43 (21.1)
Major defects	2 (0.6)	4 (2.2)	6 (2.8)[b]	12 (5.9)[d]
Any thoracic center (10-13) bipartite	4 (1.29)	11 (6.04)[d]	14 (6.51)[d]	8 (3.92)[d]
Wavy ribs (marked to severe)	2 (0.6)	4 (2.2)	3 (2.3)	7 (3.4)[b]
Wavy ribs (slight to moderate)	3 (1.6)	3 (1.7)	7 (3.3)	8 (3.9)
Variations (abnormal ossification)	267 (85.9)	164 (90.1)	185 (84.0)	199 (97.5)[c]
Skull (occipital)	79 (25.4)	58 (31.9)	69 (32.1)	71 (34.8)
Skull (interparietal)	82 (26.4)	66 (36.3)	79 (36.7)	75 (36.7)
Sternebrae no. 6	152 (48.9)	107 (58.8)	126 (58.6)	147 (72.1)
Ribs	0	1 (0.55)	2 (0.93)	6 (2.9)[b]
Metacarpals	207 (66.6)	140 (76.9)	141 (65.6)	172 (84.3)
Phalanges	141 (45.3)	114 (62.6)	139 (64.6)	123 (60.3)

[a]Numbers of fetuses affected; numbers in parentheses denote the percentage of affected fetuses/fetuses examined.
[b]$p<0.05$, Fisher's randomization test based on frequencies of affected litters.
[c]$p<0.05$, Wilcoxon's test.
[d]$p<0.01$, Fisher's randomization test based on frequencies of affected litters.

Source: IISRP, 1982.

Table 5-6. Design of the developmental toxicity studies on 1,3-butadiene

Species/strain/route of exposure	Exposure (ppm)	No. of animals/ group	Gestation days of exposure	Gestation day of sacrifice
Rat/ Sprague-Dawley CD/ Inhalation	0	30	6-15	20
	40	30	6-15	20
	200	30	6-15	20
	1,000	30	6-15	20
Mouse/ CD-1/Inhalation	0	32	6-15	18
	40	33	6-15	18
	200	31	6-15	18
	1,000	33	6-15	18

Source: Hackett et al., 1987a, b.

Table 5-7. 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.

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 5-7). Fetal measures, including the numbers of implantations/dam, resorptions/litter, dead fetuses/litter, fetal body weights, sex ratios, malformations, and variations, were not affected by exposure to 1,3-butadiene (Tables 5-8 and 5-9). Overall, no developmental toxicity was observed in rats exposed to 40 to 1,000 ppm during gd 6 to 15; a slight maternal toxicity, manifested as reduced extragestational weight gain, was observed at the 1,000 ppm level.

5.2.3. Hackett et al., 1987b

Because 1,3-butadiene is more toxic in mice than in rats, a study was also conducted in CD-1 mice using a protocol similar to that used for the rats. Groups of 31 to 33 sperm-positive females were exposed to 0 (filtered air), 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 (Table 5-6, bottom section). Measured concentrations were 39.9 ± 0.06, 199.8 ± 3.0, and 1,000 ± 13.1 ppm (mean ± SD). The dams were weighed on gd 0, 6, 11, 16, and 18 (day of sacrifice).

The effects of 1,3-butadiene on maternal toxicity in CD-1 mice are summarized in Table 5-10. 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 was 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 5-11 and 5-12. 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 ($p\leq0.05$), and 22.4% at 1,000 ppm ($p\leq0.05$). Significant differences from controls were seen at all treatment concentrations for fetal males and

Table 5-8. Developmental toxicity in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. pregnant (%)	28 (93)	24 (80)	26 (87)	28 (93)
No. litters with live fetuses	28	24	26	27[a]
No. implantations/dam	14.4 ± 0.55[b]	14.0 ± 0.71	15.3 ± 0.45	14.8 ± 0.63
No. resorptions/litter	0.46 ± 0.17	0.58 ± 0.17	0.96 ± 0.26	0.67 ± 0.14
Early resorptions/litter	0.39 ± 0.15	0.54 ± 0.16	0.88 ± 0.25	0.63 ± 0.14
Dead fetuses/litter	0	0	0	0
No. fetuses/no. litters examined	389/28	321/24	372/26	382/27
Fetal body weight (g)	3.49 ± 0.04	3.44 ± 0.05	3.40 ± 0.05	3.50 ± 0.06
Females	3.40 ± 0.05	3.36 ± 0.05	3.29 ± 0.06	3.38 ± 0.06
Males	3.59 ± 0.05	3.52 ± 0.05	3.51 ± 0.06	3.59 ± 0.06
Sex ratio (% males)	50.2 ± 2.281	52.5 ± 2.95	50.5 ± 2.77	52.5 ± 2.58

[a]One rat had only one implant; this animal was excluded from statistical evaluations.
[b]Mean ± standard error.

Source: Hackett et al., 1987a.

Table 5-9. Malformations and variations in Sprague-Dawley CD rats exposed to 1,3-butadiene by inhalation

Parameter	Concentration (ppm)			
	0	40	200	1,000
No. fetuses/no. litters examined	389/28	321/24	372/26	382/27
No. fetal heads examined	196	161	185	191
Malformations[a]				
Generalized edema	1/1	3/1	1/1	3/1
Hydrocephalus	--[b]	3/3	--	--
Meningoencephalocele	--	--	--	2/1
Missing rib	--	--	2/2	--
Variations				
Low ear set	--	--	2/1	--
Hydroureter	36/17	35/15	39/14	31/12
Misaligned sternebrae	--	--	1/1	1/1
Extra rib	--	1/1	4/2	--
Reduced ossification				
Skull	27/13	22/13	18/10	29/11
Sternebrae no. 1-4	60/15	48/13	95/21	66/15
Ribs	1/1	2/2	5/3	2/2
Thoracic vertebrae (centra)	109/23	97/21	75/21	81/25
Pelvis	9/7	--	6/5	5/5
Phalanges	1/1	6/1	2/1	--

[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 observed.

Source: Hackett et al., 1987a.

Table 5-10. 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 5-11. 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 5-12. 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

Table 5-12. Malformations and variations in CD-1 mice exposed to 1,3-butadiene by inhalation (continued)

Parameter	Concentration (ppm)			
	0	40	200	1,000
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.

at the two higher concentrations for females. Placental weights showed an effect similar to that of fetal body weights (Table 5-11). Malformations occurred sporadically and at low frequencies in all exposure groups (Table 5-12). 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. 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 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 showed 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 gross malformations in the mouse fetus, the dose-related increases in supernumerary ribs and reduced ossifications, particularly of the sternebrae, may indicate delayed or altered development and should be cause for concern.

5.2.4. Anderson et al., 1993

The acute and subchronic effects of inhalation exposure to 1,3-butadiene in male mice on fetal abnormalities were examined. For acute exposures, male CD-1 mice were exposed to 0, 1,250, and 6,250 ppm 1,3-butadiene for 6 h; 5 days later each male was mated to two females. Males used for subchronic exposures were treated with 0, 12.5, or 1,250 ppm 6 h/day, 5 days/week for 10 weeks. Following mating in both experiments, one female was killed on gd 17 and the other was allowed to litter. The female killed on gd 17 was examined for number of live fetuses, number and type of malformations in the fetuses, and number of postimplantation deaths. No treatment-related abnormalities were observed in offspring of males treated on the acute exposure protocol; one fetus from one control litter had a gastroschisis (fissure of abdominal cavity), and one fetus from each of the two low-dose litters was a runt (body weight $\leq$67% of mean litter weight). Following subchronic exposure of males to 1,3-butadiene, 7 of 306 fetuses sired by males exposed to 12.5 ppm ($p\leq0.05$) and 3 of 406 fetuses sired by males exposed to

1,250 ppm were affected compared with 0 of 278 fetuses sired by control males. Abnormalities in low-dose fetuses included four exencephalies, two runts (≤70% of mean litter weight), and one fetus with blood in the amniotic sac. At the high-dose level, one hydrocephaly and two runts (≤75% of mean litter weight) were observed. The authors calculated statistical significance on a fetal incidence basis rather than on a litter incidence basis. Because litter incidence rates were not included in the data, it is not possible to discern whether the affected fetuses were only from one or two litters or whether a high percentage of litters sired by exposed males were affected. Therefore, this study is inadequate to assess the developmental toxicity of 1,3-butadiene following exposure of males prior to mating.

5.3. STRUCTURE-ACTIVITY RELATIONSHIPS

Data on structure-activity relationship are summarized in Table 5-13.

5.3.1. NTP, 1986

The 1,3-butadiene dimer, 4-vinylcyclohexene, and its diepoxide, 4-vinyl-1-cyclohexene diepoxide, have been tested in long-term toxicological and carcinogenicity studies in rats and mice. The NTP study (1986) on 4-vinylcyclohexene used male and female F344 rats and male and female B6C3F$_1$ mice dosed by gavage with 0, 200, or 400 mg/kg 4-vinylcyclohexene in corn oil 5 days/week for 105 weeks. No nonneoplastic lesions attributed to exposure to 4-vinylcyclohexene were observed in the reproductive organs of male or female mice or rats, and hence data are not presented in Table 5-13. The incidences of granulosa cell neoplasms, mixed benign tumors, granulosa cell hyperplasia, and tubular cell hyperplasia were increased in female mice. Tubular cell hyperplasia is a proliferative lesion originating in the germinal epithelium; the hyperplastic cells invade the ovarian stroma forming tubular structures. The granulosa cell hyperplasia is a morphological continuum with granulosa cell neoplasms and tubular hyperplasia with mixed benign tumors and therefore should not be included with nonneoplastic lesions. The authors noted that female mice treated with 1,200 mg/kg 4-vinylcyclohexene for 5 days/week for 13 weeks had reduced numbers of primary and mature Graafian follicles whether they survived until termination (5/10) or died before termination (5/10).

In another NTP study (1989), male and female F344 rats and B6C3F$_1$ mice were treated topically with 4-vinyl-1-cyclohexene diepoxide 5 days/week for 13 weeks and 2 years. No nonneoplastic lesions occurred in reproductive organs of rats. Female mice treated for 13 weeks, however, showed evidence of diffuse ovarian atrophy in 10/10 animals that received 10 mg/mouse (highest dose) and in 4/10 receiving 5 mg/mouse. Uterine atrophy was observed in 2/10 animals that received 10 mg/kg. In the 2-year study, ovarian atrophy occurred in almost

Table 5-13. Reproductive and developmental toxicity of chemicals structurally similar to 1,3-butadiene

Chemical	Species	Dose	Effects	LOAEL	Reference
4-vinyl-1-cyclohexene	Male and female $B6C3F_1$ mice	2.5, 5, 10 mg/kg topically 5 days/week for 13 or 105 weeks	Females 13 weeks: ≥5 mg/kg: ovarian atrophy; 10 mg/kg: uterine atrophy 105 weeks: ≥2.5 mg/kg: ovarian atrophy Males 105 weeks: ≥5 mg/kg: inflammation of epididymis	5 mg/kg for 13 weeks 2.5 mg/kg for 105 weeks 5 mg/kg for 105 weeks	NTP 1989
Butadiene monoepoxide	Female $B6C3F_1$ mice	0.005, 0.02, 0.09, 0.36, 1.43 mmol/kg intraperitoneally once daily for 30 days	1.43 mmol/kg: reduced ovarian and uterine weights; decreased follicular counts	1.43 mmol/kg	Doerr et al., 1995, 1996
	Female Sprague-Dawley rats		none		Doerr et al., 1996
Butadiene diepoxide	Female $B6C3F_1$ mice	0.002, 0.009, 0.036, 0.14, 0.29 mmol/kg intraperitoneally once daily for 30 days	≥0.14 mmol/kg: decreased ovarian and uterine weights; decreased follicular counts	0.14 mmol/kg	Doerr et al., 1995, 1996
	Female Sprague-Dawley rats		≥0.14 mmol/kg: decreased ovarian weight 0.29 mmol/kg: decreased uterine weights	0.14 mmol/kg	Doerr et al., 1996
Isoprene	Male $B6C3F_1$ mice	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	7,000 ppm	Melnick et al., 1994
	Male F344 rats		13 weeks: none 26 weeks: 7,000 ppm: hyperplasia of interstitial cells	7,000 ppm for 26 weeks	Melnick et al., 1994

all groups 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 such as that in animals exposed to 1,3-butadiene was characterized by a complete absence of follicles and corpora lutea. Tubular hyperplasia occurred at a high incidence in all dose groups (35/49, 38/49, and 34/50, respectively, compared with 5/50 for controls). In male mice, subacute inflammation of the epididymis occurred in 0/50, 6/50, and 13/49, respectively, compared with 0/50 for controls.

5.3.2. Melnick et al., 1994

Differences in susceptibility between rats and mice were seen in inhalation studies with isoprene, the 2-methyl analogue of 1,3-butadiene. 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 weeks 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 also 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).

5.3.3. Doerr et al., 1996

This study tested the ovarian toxicity of the mono- and diepoxide metabolites of 1,3-butadiene in mice and rats. Butadiene monoepoxide (0.005, 0.02, 0.09, 0.36, or 1.43 mmol/kg), butadiene diepoxide (0.002, 0.009, 0.036, 0.14, or 0.29 mmol/kg), or vehicle (sesame oil) was administered intraperitoneally once daily to female $B6C3F_1$ mice and Sprague-Dawley rats for 30 days. Following day 30, animals were sacrificed by CO_2 inhalation, the ovaries and uteri were weighed, and the ovaries processed for histologic examination of preantral follicles. At the high dose, the monoepoxide resulted in reduced ovarian and uterine weights ($p \leq 0.05$) and decreased follicular counts in mice; rats, however, were unaffected. The diepoxide resulted in decreased ovarian weights ($p \leq 0.05$) in mice and rats at ≥ 0.14 mmol/kg and decreased uterine weights ($p \leq 0.05$) in mice at ≥ 0.14 mmol/kg and in rats at 0.29 mmol/kg. Because organ weights were given in a histogram, the absolute differences were not available; all significant reductions appeared to be approximately $\leq 50\%$ of the control values. The ED_{50} value was defined as the effective dose that reduces the follicular number to 50% of control. In mice, ED_{50} values for the monoepoxide were 0.29 and 0.40 mmol/kg and for the diepoxide were 0.1 and 0.14 mmol/kg for

small and growing follicles, respectively. However, in rats, only 32% of the follicular population was depleted at the highest diepoxide concentration. Therefore, mice were more sensitive than rats to the ovotoxic effects of the mono- and diepoxides of 1,3-butadiene, and the diepoxide was the more potent ovotoxicant in both species.

Doerr et al. (1995) also studied the ovarian toxicity of 4-vinylcyclohexene and several related olefins, including butadiene mono- and diepoxide. Mice were administered 1.43 mmol/kg of the monoepoxide or 0.14 mmol/kg of the diepoxide once daily for 30 days. Following day 30, the mice were killed and the ovaries removed and sectioned for histologic examination. Mean follicle counts in mice treated with the monoepoxide were depleted 98% and 71% for small and growing follicles, respectively, compared with controls. In mice treated with the diepoxide, follicle counts were depleted 85% and 63% for small and growing follicles, respectively, compared with controls. Structural analogs of vinylcyclohexene that contain only a single unsaturated site (vinylcyclohexane, ethylcyclohexene, cyclohexene) and their monoepoxide metabolites were not ovotoxic to mice. On the other hand, butadiene monoepoxide, butadiene diepoxide, and isoprene were ovotoxic. The study showed a relationship between chemical reactivity, as assessed by nicotinamide alkylation, and ovotoxicity with vinyldiepoxide and butadiene diepoxide that was 3.5 to 10 times more reactive than their monoepoxide precursors and other structurally related monoepoxides. It can be concluded that those compounds that are metabolized to a diepoxide or are a diepoxide are ovotoxic.

5.4. SUMMARY AND CONCLUSIONS

Evidence has been presented showing that 1,3-butadiene induces reproductive and developmental effects in rodents. Although the studies conducted by Carpenter et al. (1944) examined reproductive toxicity in four different species (rat, guinea pig, rabbit, and dog), the experimental protocol and the results obtained are inadequate for evaluating reproductive toxicity. 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) suggest that mice are much more sensitive than rats to the reproductive effects of 1,3-butadiene. Reproductive toxicity was not observed in either male or female rats exposed intermittently to 1,3-butadiene at concentrations up to 8,000 ppm for 2 years. However, ovarian atrophy was observed in female mice exposed to 6.25 to 625 ppm 1,3-butadiene.

Ovarian atrophy occurred in 39% of 49 mice at 6.25 ppm (the lowest concentration tested) only after exposure for 2 years, a time at which this condition is expected to appear in aged animals due to normal senescence mechanisms; however, it occurred in a significantly greater number of mice exposed to 1,3-butadiene than in control animals. Furthermore, ovarian atrophy was observed as early as 9 months after exposure to 200 and 625 ppm and 15 months

after exposure to 62.5 ppm. 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.

Similar ovarian lesions have been observed in mice after exposure to the 1,3-butadiene dimer, 4-vinylcyclohexene, administered by gavage for 13 weeks at 1,200 mg/kg for 5 days/week (NTP, 1986) or its diepoxide, 4-vinyl-1-cyclohexene diepoxide, administered by topical application for 13 weeks or 2 years (NTP, 1989). Rats administered 4-vinylcyclohexene or 4-vinyl-1-cyclohexene diepoxide did not develop ovarian lesions, thus showing a species-specific response similar to that after exposure to 1,3-butadiene.

Ovarian lesions induced by 1,3-butadiene, 4-vinylcyclohexene, or 4-vinyl-1-cyclohexene diepoxide are 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. Furthermore, Maronpot (1987) compared the ovarian toxicity and carcinogenicity of eight chemicals tested by NTP and concluded that the occurrence of ovarian lesions in a 90-day study may also indicate that ovarian neoplasia would be induced upon continued treatment.

Uterine atrophy is probably due to the indirect action of 1,3-butadiene metabolites and the consequent interruption of ovarian sex steroid stimulation of the uterus. Oocyte toxicity and destruction of the follicular and subsequent luteal components of the ovary result in reduced steroidogenesis by the ovary. It is well known that ovarian estrogens and progestins have a uterotropic function in laboratory rodents and humans.

Testicular atrophy, as reflected by reduced testis weight following 1,3-butadiene exposure for 9 and 15 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 results at very low concentrations (6.25 ppm) of 1,3-butadiene compared with that seen in males after 2 years of exposure. The sperm-head morphology study showed that male mice are affected at concentrations $\geq$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. As observed for other effects of 1,3-butadiene, the reproductive organs of male rats are more resistant than those of female mice exposed to 1,3-butadiene. This resistance in males may be attributed, in part, to the blood-testis barrier. No homologous anatomical barrier has been demonstrated in the Graafian follicle (Crisp, 1992). No adverse reproductive effects have been observed in male rats at concentrations up to 8,000 ppm (Owen et al., 1987; Owen and Glaister, 1990).

Several studies indicate that 1,3-butadiene affects spermatozoa and spermatids as determined by postimplantation deaths during the first 3 weeks after exposure. The data from Hackett et al. (1988b) is equivocal because of a lack of the dose-response relationship, but the studies by Anderson et al. (1993) and Adler et al. (1994) confirm the hypothesis. Further evidence that 1,3-butadiene is a germ cell mutagen was presented in a comparison of the latter two studies (Adler and Anderson, 1994). In the first experiment, the percentage of dominant lethality observed following 10 weeks of exposure of males to 1,250 ppm was 28.1%. During the acute exposure experiment, the sum of dominant lethality over the 3 weeks of mating was 23.1%. The results are in close agreement despite differences in protocols such as exposure regimen, strains of mice, and mating scheme. It appears that 1,3-butadiene affects spermatozoa and spermatids because the effects observed after 10 weeks are representative of the last 3 weeks of treatment and increasing the length of exposure did not add to the response.

The mechanism by which 1,3-butadiene induces ovarian lesions is not known, but it is unlikely that 1,3-butadiene is a direct-acting reproductive toxicant. Direct-acting compounds act as hormonal agonists or antagonists or chemically reactive compounds (such as alkylating agents), which directly interfere with hormone-receptor interactions or interact with macromolecules (Maronpot, 1987; Mattison et al., 1990). More likely, 1,3-butadiene is an indirect-acting reproductive toxicant. Indirect-acting toxicants require metabolic activation to exert their toxic effects, which then may proceed via mechanisms similar to those of direct-acting compounds, or they may interfere with endocrine homeostasis (Mattison et al., 1990).

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. No gross malformations were produced. The pattern for developmental effects induced by 1,3-butadiene was similar to that of 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 in rats (IISRP, 1982; Hackett et al., 1987a) and 40 ppm for mice (Hackett et al., 1987b). While the IISRP (1982) study showed increased frequencies for bipartite thoracic centers and minor skeletal defects combined at 200 ppm in rats, the response is 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 more recent 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). These independent observations

strengthen the evidence that a decreased maternal weight gain during early development might contribute to subtle adverse effects (1) undetected by insensitive indices in the studies or (2) at a later point in time in the F_1 or subsequent generations. An 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 and postimplantation loss was observed at 12.5 ppm in the Anderson et al. (1993) study, the lowest concentrations tested.

Reproductive and developmental toxicity studies show species specificity for exposure to 1,3-butadiene in rats and mice. Like other toxicological effects induced by 1,3-butadiene, mice are more sensitive than rats to the induction of reproductive and developmental effects. Pharmacokinetic studies show that uptake of 1,3-butadiene is about four times greater in mice than in rats at concentrations up to 1,000 ppm (Dahl et al., 1990) and about two times greater at 8,000 ppm (Dahl et al., 1991). These data indicate that the availability of 1,3-butadiene is greater in mice than in rats at comparable exposure concentrations. 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 10-fold 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. Because male animals were used, subcutaneous fat, which had similar levels of radioactivity as mammary tissue, probably contaminated the samples. The ovary, uterus, and testis, which are targets for 1,3-butadiene, were not analyzed by Bond et al. (1987). In a recent in vitro study, Sharer et al. (1992) showed that microsomes from the testes of rats and mice are ineffective in forming butadiene monoxide, but the cytosol fraction was very effective in forming glutathione conjugates. Therefore, it is unlikely that toxic effects on the testes are due to metabolites formed within the testes but rather are due to metabolites formed elsewhere, indicating that 1,3-butadiene is an indirect-acting reproductive toxicant in males.

Species-specific differences have also have been observed for the formation of metabolites. The monoxide hydrolase (detoxification) pathway is favored in rat microsomes, whereas the monooxygenase pathway is favored in mouse microsomes. Although these data do not fully explain the species differences, they show that the basis of the difference may be related to the greater availability of 1,3-butadiene in mice, the greater production of toxic intermediates, and a lower capacity for detoxification of these intermediates.

No data are available regarding the reproductive or developmental effects of the metabolites of 1,3-butadiene, 1,2-epoxybutene, and diepoxybutane. Mice are more sensitive than rats to the ovotoxic effects of the mono- and diepoxides of 1,3-butadiene, and the diepoxide is the

more potent ovotoxicant in both species. Data regarding the 1,3-butadiene dimer 4-vinylcyclohexene and its diepoxide provide evidence that 4-vinylcyclohexene induces ovarian and uterine atrophy after treatment by gavage for 13 weeks with 10 mg/kg 5 days/week and that 4-vinyl-1-cyclohexene diepoxide (2.5 to 10 mg/mouse) induces ovarian atrophy and tubular hyperplasia in mice after topical treatment for 2 years. Subacute inflammation of the epididymis is seen in male mice receiving 4-vinyl-1-cyclohexene (5 or 10 mg/mouse) topically for 2 years. Ovarian neoplasms are induced in mice by 4-vinylcyclohexene and its diepoxide. However, neither 4-vinylcyclohexene nor its diepoxide induce either neoplastic or nonneoplastic lesions in the ovaries of rats.

In conclusion, the animal data show that there is a potential reproductive hazard to humans upon exposure to 1,3-butadiene, with women being more sensitive than men. The quantitative aspects of this assessment will require application of pharmacokinetic parameters because humans may be less sensitive than mice (Chapters 3, 8). 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 (Section 9.3).

6. TOXICITY IN ANIMALS

This chapter updates the evaluation of animal studies published from 1985 through January 1997. The study by Owen et al. (1987) is not evaluated here because it was reviewed previously in U.S. EPA (1985) as the NTP study (1984), which was subsequently published by Owen et al. (1987).

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 1,250 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

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 hours/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 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 6-1, Figures 6-1 and 6-2) primarily due to 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; the method of 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 ≥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 ≥62.5 ppm and in female mice exposed to ≥200 ppm at 9 months. Mean cell volume was significantly increased in male mice exposed to 625 ppm and in females exposed to ≥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 ≥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.

Table 6-1. 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 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.

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 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 mice exposed to 1,3-butadiene for 103 weeks, nonneoplastic effects were observed in the bone marrow, liver, testes, ovary, heart, upper respiratory tract, and various other organs.

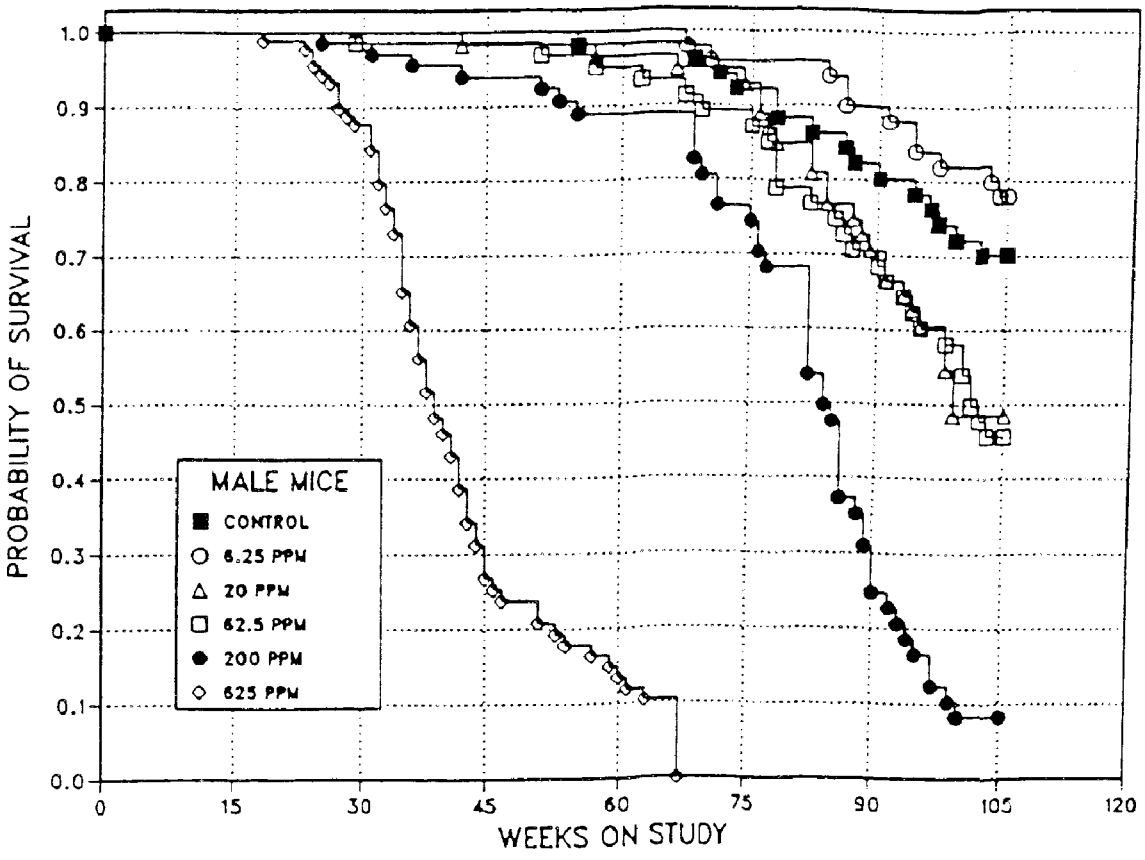


Figure 6-1. Kaplan-Meier survival curves for male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.
Source: NTP, 1993.

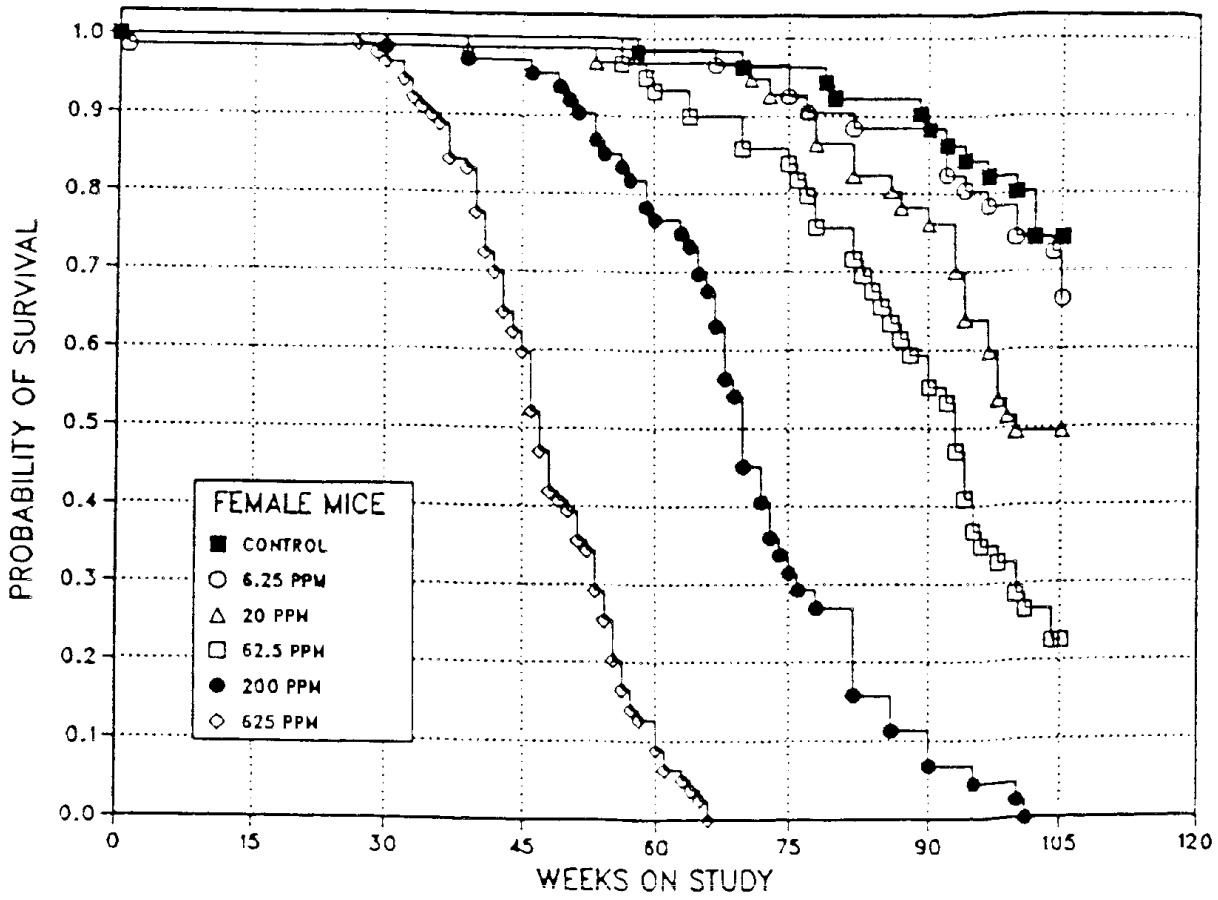


Figure 6-2. Kaplan-Meier survival curves for female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 103 weeks.
Source: NTP, 1993.

Effects in reproductive organs are discussed in Chapter 5. Organ weights, hematological indices, and serum chemistry were not evaluated at 103 weeks.

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 $\geq$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 6-2). 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 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 6-3).

Table 6-2. Incidence of hyperplasia in male and female B6C3F1 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.

Table 6-3. 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

The first NTP mouse inhalation study of 1,3-butadiene was terminated early due to 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 and 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, b, 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.

6.3.1. 2-Year Study (NTP, 1993)

The details of the study design are described in Section 6.2. 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. Alternate 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 6-4 and 6-5. 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 6-6 and 6-7.

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

Table 6-4. 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
	Hepatocellular carcinoma	11/50 (22%) 26.0% *p*=0.036[d]	16/50 (32%) 36.6% *p*=0.142	16/50 (32%) 44.8% *p*=0.389	17/48 (35%) 58.3% *p*=0.088	26/48 (54%) 100.0% *p*<0.001	1/72 (2%) 50.0% *p*=0.347

Table 6-4. 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 (cont.)	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 6-5. 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 6-5. 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 (cont.)	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 6-6. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months

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	½	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.
[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 6-7. Incidence of primary neoplasms in female B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation for 9 months and 15 months

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	½
Forestomach	Squamous cell papilloma or carcinoma	9 months						
		15 months				1/10	2/10	½
Liver	Hepatocellular adenoma or carcinoma	9 months						
		15 months	1/10	1/10	0/10	1/10	3/10	½
Ovary	Benign or malignant granulosa cell tumor	9 months					1/10	
		15 months				1/10	4/10	½
Mammary gland	Adenoacanthoma, adenocarcinoma, carcinoma, or malignant mixed tumor	9 months						
		15 months					2/10	½
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.
[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.

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 in 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 due to 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 (Section 3.2 of this chapter) to adenomas, carcinomas, and adenocarcinomas. Histologically, the alveolar/bronchiolar adenomas exhibited distortion of the alveolar structure due to 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 6-4 and 6-5). 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 6-4 and 6-5), 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-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 hours/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, 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.

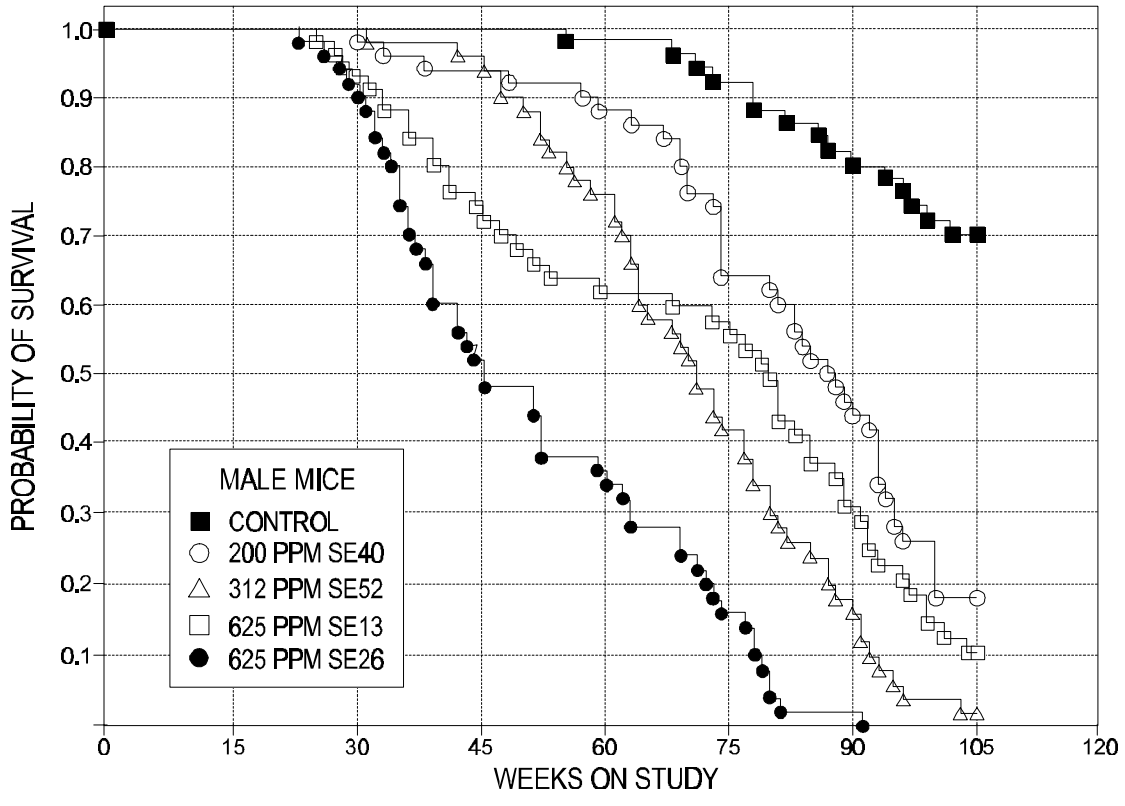


Figure 6-3. Kaplan-Meier survival curves for male mice in the stop-exposure inhalation study of 1,3-butadiene.
Source: NTP, 1993.

Table 6-8. 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 6-8. 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					
	Alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma	21/50 (42%) 54.9% --	36/50 (72%) 100.0% $p<0.001$[c]	28/50 (56%) 100.0% $p<0.001$	32/50 (64%) 100.0% $p<0.001$	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]	19/49 (39%) 91.1% $p=0.042$	19/50 (38%) 100.0% $p=0.045$	11/50 (22%) 100.0% $p=0.284$
	Hepatocellular carcinoma	11/50 (22%) 26.0% --	14/49 (29%) 50.3% $p=0.530$[d]	14/49 (29%) 90.9% $p=0.142$	10/50 (20%) 74.6% $p=0.453$	4/50 (8%) 50.5% $p=0.393$
	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 6-8. 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					
	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

Table 6-8. Incidence of primary neoplasms in male B6C3F$_1$ mice exposed to 1,3-butadiene by inhalation in the stop-exposure study (continued)

[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, 1993a.

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.

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 due to 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 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 6-8.

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.

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 the 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.

6.3.3. 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 (the lowest exposure concentration used) 1,3-butadiene 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 histopathological evaluations were performed and mortality and tumor incidences were analyzed statistically using multiple methods.

6.3.4. 1-Year Study (Irons et al., 1989; Irons, 1990)

To elucidate the mechanism of murine leukemogenesis, Irons and coworkers 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. RELATED COMPOUNDS

The draft report on the toxicology and carcinogenicity of 4-vinyl-1-cyclohexene, a dimer of 1,3-butadiene, was reviewed in U.S. EPA (1985). The final 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 based on increased ovarian neoplasms and equivocal evidence in male mice based on marginal increases of malignant lymphomas and alveolar/bronchiolar adenomas. 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.

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). In addition, 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 in 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.

6.5. DISCUSSION AND CONCLUSIONS

Previous long-term inhalation studies have shown that 1,3-butadiene is carcinogenic in rats and mice, inducing tumors at multiple organ sites (NTP, 1984; Owen et al., 1987). Results of the 2-year inhalation study (NTP, 1993) presented in this report confirmed the carcinogenicity for 1,3-butadiene in male and female B6C3F$_1$ mice as demonstrated in an earlier study (NTP, 1984).

Of particular interest in this 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 showed that the concentration is a greater contributing factor in the development of lymphocytic lymphomas than the 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 reveal 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).

Results of previous carcinogenicity studies reviewed in U.S. EPA (1985) have shown different effects of exposure to 1,3-butadiene in rats and mice, with mice being more sensitive to

the induction of carcinogenic effects than rats. The carcinogenic activity in Sprague-Dawley rats exposed to 1000 or 8000 ppm 1,3-butadiene was largely limited to endocrine tissues or hormonal responsive tissues, such as pancreas, Leydig cells of the testis, uterus, Zymbal 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 granuloma cell neoplasms of the ovary as well as malignant lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, squamous cell neoplasms of the forestomach, and hepatocellular neoplasms. The reason for the species difference is not known, but may in part be due to differences in toxicokinetics.

Toxicokinetic studies have shown species-related quantitative and qualitative differences in the metabolism and disposition of inhaled 1,3-butadiene that may, in part, account for the observed species variability in the toxicity (Chapter 3). 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. 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 (Csanáday and Bond, 1991).

Investigations by Irons and coworkers (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 in 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 only in 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 by 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 of 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 January 1997. 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 are not reviewed in this evaluation. Lemen et al. (1990) did not present any results, while 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 uncovered 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 (i.e., 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 were accrued for the follow-up period. There were 603 deaths from 1943 through 1979; death certificates were obtained for 579 (96%) individuals. 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.

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 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. The rationale for this comparison was based on the assumption that wartime exposures may have been higher than in postwar periods.

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[1] (i.e., non-Hodgkin's lymphoma, 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 (NSMR = 187, p>0.05, O = 6), Hodgkin's disease (NSMR = 197, p>0.05, O = 1), and residual lymphohematopoietic cancers (NSMR = 282, p>0.05, O = 4). An excess of kidney cancer (NSMR = 254, p>0.05) 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 (NSMR = 167, p>0.05, O = 10), Hodgkin's disease (NSMR = 130, p>0.05, O = 1), leukemia (NSMR = 201, p>0.05, O = 5), and residual lymphohematopoietic cancers (NSMR = 150, p>0.05, O = 4).

For those in the low-exposure group (Group I), excess mortality was seen for the same cancers (excluding Hodgkin's disease): all lymphohematopoietic cancers (NSMR = 128, p>0.05, O = 3), leukemia (NSMR = 105, p>0.05, O = 1), and residual lymphohematopoietic cancers (NSMR = 190, p>0.05, O = 2). 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 reticulosarcoma (ICD code 200), both the latency as well as number of years employed

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

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 their 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 is conducted in a butadiene (monomer) production facility in a cohort where confounding exposure from styrene is 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 malignancy are concentrated in workers employed for less than 10 years, 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*C*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 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 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 (6%) individuals. Of 2,582[2] employees in the cohort, 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). Prewar and postwar subcohort analyses demonstrated a statistically significant increase among the prewar subcohort for the same cause-specific deaths (SMR = 269, 95% CI = 108-555, O = 7), while an excess in the postwar subcohort was not statistically significant (SMR = 155, 95% CI = 17-558, O = 2).

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 routine exposure category (SMR = 561, 95% CI = 181-1,310, O = 5). Among workers who were ever employed in nonroutine exposure category, the excess was observed for all lymphohematopoietic cancers (SMR = 141, 95% CI = 70-253, O = 11) due to an increase in leukemia (SMR = 185, 95% CI = 68-403, O = 6). The lymphosarcoma in this group was slightly increased (SMR = 126, 95% CI = 14-454, O = 2).

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 30+ year and 0 to 9 year groups, the SMRs were 3,333 (O = 2) and 1,333 (O = 4), 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

[2]It was not explained in the paper how the cohort was reduced to 2,582 from 2,586.

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.

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: (1) 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). (2) 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. (3) The nonroutine exposure group (included skilled maintenance workers such as pipefitters, tinsmiths, instrument and electrical workers, and insulators, N = 865). (4) The routine exposure group (included process, lab, storage, and transport workers, N = 1056). Although 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 not explained in the paper. For this study, the investigators reviewed the results of the IH data and information obtained from the 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 the analysis of this study also. All the analytical methods included use of white male death rates of the U.S. population (since 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 only statistically significant elevated SMR observed was for lymphosarcoma and reticulosarcoma for workers employed for less than 5 years (SMR = 286, 95% CI = 104-622, O = 6). Again, this increase probably came entirely from the prewar employees (SMR = 254, 95% CI = 102-523, O = 7). The analysis by exposure group showed an increase for the same cause in the routine exposure group (SMR = 452, 95% CI = 165-984, O = 6). The analysis by latency and duration of employment yielded the largest increase in 0 to 9 years latency for the individuals employed for less than 5 years (prewar individuals?). The SMR was 3,333 based on two observed cases. No statistical test results were presented for this analysis.

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 (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 2,795 individuals, 999 were still alive, 574 were lost to follow-up (28 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 also remained the same for this follow-up.

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 up 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) non-Hodgkin's lymphoma, (4) multiple myeloma, and (5) leukemias.

Three different models were used for the survival analysis, i.e., 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 earlier two follow-up studies of this cohort (Divine, 1990; Divine et al., 1993). The survival analyses failed to show any significant increase in the risk ratios, in any cause-specific cancer, by any of the three methods.

Although the investigators have done a good job of estimating the exposure and have conducted various analyses, the increase observed in the prewar subcohort for lympho-reticulosarcoma, when exposures were probably the highest, still persists. Upon completion of this study, this cohort has 52 years of follow-up but has failed to show any increase in leukemias which were observed in SBR production workers.

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 employment in the 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 comprised 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 National Death Index (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. 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. Thus, despite the absence of any positive results, this study fails to provide any negative evidence towards 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 the 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 (during World War II) were selected for the study (i.e., 364 individuals). The vital status was determined through December 31, 1990, using the National Death Index. 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, which was based on four cases (SMR = 577, 95% CI = 157-148). A county-based comparison also resulted in 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).

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.

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 job coded for butadiene exposure. 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 prewar subcohort.

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.

Table 7-1. Epidemiologic studies of the health effects of exposure to 1,3-butadiene **C**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 **C**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 as earlier studies Survival analysis failed to show any SS excess in any cause-specific cancer	52 years follow-up Exposure estimation useful Major limitation is no exposure estimation available in prewar subcohort, which has the SS lymphosarcoma excess

Table 7-1. Epidemiologic studies of the health effects of exposure to 1,3-butadiene **C**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 has worked in monomer production during World War II Follow-up through December 31, 1990 U.S. population Kanawha County population	Jobs where only 1,3-butadiene exposure 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 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.

Because the findings of the published study are essentially the same, it will not be reviewed again.

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) also 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 National Death Index 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 the local health departments. The total cohort was reduced from 13,920 to 13,422 in this study. Of 12,113 workers for whom the 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.[3] 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 by 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. The 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.

[3]The percentages, which are quoted from the paper, add up to 101. This is due to the rounding of the numbers by the authors of the paper.

Statistically significant lower SMRs for all causes of deaths (81) and for all cancers (85) were virtually the same as in earlier studies. The SMRs for all causes of deaths 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 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 non-Hodgkin's lymphoma 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 non-Hodgkin's lymphoma 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 the 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., the 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 the 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 the 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 non-Hodgkin's lymphomas; 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 non-Hodgkin's lymphoma 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 were 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 that 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, 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 a 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 lymphoma 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 non-Hodgkin's lymphoma or multiple myeloma included together with potentially different etiologies or correlated exposure data. Thus, investigators suggest 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 were not substantiated by monitoring data. As correctly pointed out by them, 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, which is an important methodologic limitation of the study given the fact that lymphohematopoietic cancer recording on death certificates is unreliable (Percy et al., 1981). 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 by the investigators to verify the findings of their 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 the 55 records reviewed, two cases had been incorrectly coded on the death certificates as lymphohematopoietic cancers. Records were obtained 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 found by these investigators.

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 seven plants studied by JHU, one located in Texas that had a starting time of 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 the 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). The lymphosarcoma SMR for this subcohort was 102 based on 4 cases, while the SMR for other lymphopoietic cancer was 106 based on 17 cases. Neither of these excesses was statistically significant. The 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 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).

When this subcohort was further restricted to >10 years of employment and >20 years since hire, the 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. Furthermore, in this restricted subcohort, the SMRs for leukemia were 209 (95% CI = 100-385) and 228 (95% CI = 135-160) for the workers from plants with the solution polymerization process and workers from plants without such a process, 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 the 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).

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 the 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. There were only two leukemia cases who had exposure to styrene but none 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 general population as well as 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. 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 due to this elimination. 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. The job-exposure matrices (JEMs) were computed for 1,3-butadiene, styrene, and benzene, which were linked to work histories of each employee.

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 a good agreement among the methods of sampling, while the comparison of IH data indicated overestimations of 1,3-butadiene exposure.

For each job, 8-h time-weighted average (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 risk ratios (RR) 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 using the Mantel-Haenszel method and 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 0-64 ppm, 0-7.7 ppm, and <1 ppm, respectively, the median exposures being <2 ppm for 1,3-butadiene and 0.5-1.1 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 was 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 as compared with all decedents and three times higher as compared with all the other employees. The exposure to styrene was present in 90% of leukemia decedents, with median cumulative exposure in them being 22.4 ppm-years, two times and three times higher as compared with all the decedents and all other employees, respectively. Benzene exposure was found to be less frequent among leukemia decedents as compared with all the 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, 1-19, 20-79, 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 0.1-19, 20-79, 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.

There was neither any positive or negative interaction 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 as 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.

Studies in polymer production workers are summarized in Table 7-2.

7.3. SUMMARY AND DISCUSSION

1,3-Butadiene has been shown to be both mutagenic as well as carcinogenic in animals and humans. Data in animals, particularly in mice, show that butadiene is a multisite carcinogen

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene **C**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 and 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	One of the strengths is 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 **C**polymer production (continued)

Authors	Population studied	1,3-Butadiene exposure assessment	Results	Strengths and limitations
Matanoski et al. (1989) Santos-Burgoa et al. (1992) (continued)		A log transformation of the scores was used in the analyses Analyses were done: - by ever/never exposed - by high- vs. low-exposure - both matched (conditional) and unmatched (unconditional)	SS OR = 7.61 for leukemia **$** 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 **C**polymer production (continued)

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 conducted 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 the 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 **C**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

Table 7-2. Epidemiologic studies of the health effects of exposure to 1,3-butadiene **C**polymer production (continued)

SS = Statistically significant.
SMR = Standard mortality ratio.
IH = Industrial hygiene.
RR = Risk ratios.
OR = Odds ratio.
LHC = Lymphohematopoietic cancers.

even at the lowest dose of 6.25 ppm (NTP, 1993). Occupational populations are exposed to butadiene in the production/recovery of butadiene monomer and production of resins and plastics. Exposure to this colorless, odorless gas is entirely via inhalation due to its extremely volatile nature. The general population is exposed to butadiene in ambient air, the major sources of its release in ambient air being automotive exhaust and cigarette smoke. Its potential to cause cancer in humans has become an important public health issue.

Butadiene becomes diluted in ambient air and is eliminated by photooxidation. Thus it is difficult to study the health effects of exposure to butadiene in the general population. Since exposure to butadiene is ubiquitous in the general population, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are exposed to 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 standard mortality ratios observed in occupational cohorts, computed using the general population as the reference group, are underestimations of real risk.

7.3.1. Monomer Production

To evaluate the carcinogenicity of 1,3-butadiene, cohorts from monomer and polymer production were studied by several investigators. The largest cohort of monomer production workers was initially studied by Downs et al. (1987) and had three follow-ups by Divine (1990), Divine et al. (1993), and Divine and Hartman (1996). The cohort included 2,586 workers initially and had 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. They 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, the investigators (Divine and Hartman, 1996) estimated the potential exposure to butadiene for each employee by their work histories (in 1-year segments), using job categories and calender time periods. Cumulative exposures were obtained by summing the scores of all the years of employment.

The findings of all four investigations were essentially the same even after 52 years of follow-up. There were deficits 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 0-9 years. This excess was seen in the prewar subcohort in all three follow-up studies (SMR = 269, and SMR = 254 in both Divine, 1990, and in Divine et al., 1993; Divine and Hartman, 1996). No information on exposure levels was

available for this period, but it was believed that the exposures were high during the prewar 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 and with latency of 0-9 years. It should be noted that after 52 years of follow-up, no elevated mortality was observed for leukemia, which was the main finding in 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 butadiene during World War II (Ward et al., 1995, 1996c). The exposure to butadiene was assumed based on job categories, and no adjustments for confounding by other chemicals were done. As observed in the Divine Studies (1990, 1993, 1996), a statistically significant excess for lymphosarcoma (SMR = 577) also was observed in this cohort.

A third cohort of 614 workers exposed to monomer was studied by Cowles et al. (1994) and the study failed to show any excess mortality or morbidity. Due to several methodologic limitations, this study failed to provide any negative evidence towards the causal association between exposure to butadiene and occurrence of lymphosarcoma that was observed in the other two cohorts.

7.3.2. Polymer Production

A further follow-up and reanalysis of a large SBR polymer production workers' cohort (Matanoski and Schwartz, 1987) was conducted by Matanoski et al. (1989, 1990). This follow-up added 3 years to the earlier study. The findings of this follow-up were essentially the same as the earlier study. The only statistically significant excesses were found 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 in 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 in these individuals using the job records and levels of exposures to 1,3-butadiene and styrene associated with those jobs independently 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 was also observed for increasing risk of leukemia with increasing exposure levels of butadiene.

Because the findings of the nested case-control study were questioned by Acquavella (1989) and Cole et al. (1993), as they were in disagreement with the base cohort study, Matanoski et al. (1993) reevaluated the analysis of the nested case-control study by choosing a new set of three controls per case. The investigators also verified the cause of death by obtaining the hospital records. The findings of the new analysis were similar to the earlier analysis.

Furthermore, they estimated the exposures to the cohort based on measurements provided by seven rubber plants, IISRP, and NIOSH. In an analysis of the subcohort from three plants who had the geometric means of exposure, statistically significant excesses were observed for all lymphohematopoietic cancers (SMR = 163) as well as for leukemia and aleukemia (SMR = 181).

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 1970's and 1980's 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 blacks (SMR = 227). The excesses were also 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.

7.3.3. Relevant Methodologic Issues and Discussion

Throughout this chapter, various methodologic issues including strengths and limitations are discussed. The major concerns are lack of exposure information and short follow-up periods in earlier studies, small cohort size, lack of data on confounding variables, and lack of latency analysis in one study. Furthermore, death certificates were used by all the investigators, which could lead to misclassification bias. Validation of diagnosis of lymphohematopoeitic cancer was not done in any of the studies except in Matanoski et al. (1993). This is a methodologic concern given the fact that lymphohematopoeitic cancer recording on death certificates is unreliable (Percy et al., 1981).

Lack of exposure information is another major limitation in Cowles et al. (1994) and Ward et al. (1995, 1996c). Cowles et al. (1994) made no attempt to even do job classification. This 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) also did not attempt any exposure estimation. This cohort also was very small but was restricted to workers who had worked in the 1,3-butadiene production period (during World War II). 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. 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 attempted the exposure estimation in their last follow-up (Divine and Hartman, 1996) and found that except for an excess observed for lymphosarcoma and reticulosarcoma in the prewar subcohort, there were no excesses in any cause-specific cancer mortality. However, investigators did not have any information on work histories or levels of 1,3-butadiene exposure during the prewar period, which made exposure estimation in the prewar workers impossible. Even after 52 years of follow-up and extensive analyses, this cohort has not observed any excess in mortality from leukemia that was observed in SBR workers. Nonetheless, the finding of excess mortality from lymphosarcoma and reticulosarcoma is consistent with findings of Meinhardt et al. (1982) and Ward et al. (1995, 1996c). In addition, the excess of lymphosarcoma and reticulosarcoma in short-term workers but not in long-term workers was consistent with the similar findings 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 the 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 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. The Cole et al. (1993) calculations resulted in approximately 104 leukemia cases if relative odds of 7.6 were applicable to 60% of the cohort that was exposed to 1,3-butadiene and an additional 9.2 expected leukemias for the remaining 40% cohort that was not exposed, resulting in an observed 113 leukemias for the cohort as against 22 leukemias actually observed in the cohort study. Variability in both the prevalence of exposure and the relative odds were 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 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 were 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 towards the status of cases and controls, thus the distribution of misclassification should be the same in cases and controls.

Although the controversy about the cohort and case-control study is still not resolved, the nested case-control study was the first one to demonstrate a strong association between exposure to 1,3-butadiene and occurrence of leukemias.

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.

Based on these monomer and polymer production worker cohorts, it is obvious that an increased number of lymphohematopoietic cancers is observed in these populations. A clear difference is becoming apparent though. Increased lymphosarcomas develop in workers exposed to 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 polymer (Matanoski et al., 1990, 1993; Santos-Burgoa et al., 1992; Delzell et al., 1996; Macaluso et al., 1996). Furthermore, the lymphosarcomas were observed in the monomer workers, who were probably exposed to higher levels of 1,3-butadiene for shorter periods of time (wartime workers) and not in long-term workers with low levels of exposures. A confirmation of this observation comes from the stop-exposure studies conducted by Melnick et al. (1990a). They observed that at a similar total exposure, the incidence of lymphoma was greater among mice exposed to higher concentrations of butadiene for a shorter period of time (625 ppm for 26 weeks) than among mice exposed to a lower concentration for a longer period of time (312 ppm for 52 weeks). Consequently, this suggests that it is the concentration of 1,3-butadiene rather than the duration of exposure that is important in the occurrence of lymphomas. There is a null relationship between exposure to 1,3-butadiene monomer and occurrence of leukemias that is observed in polymer workers. This may be due to very low exposures to 1,3-butadiene in monomer production workers or exposure to a necessary co/modifying factor or a confounding factor in SBR production workers. Data are currently lacking to confirm or refute any of these possibilities. The findings of Delzell et al. (1996) and Macaluso et al. (1996) are inconsistent with confounding by exposure to other chemicals. The findings of excess leukemias in SBR production workers are consistent with a causal association with exposure to 1,3 butadiene.

7.3.4. 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 proves 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, it provides 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 risk of lymphohematopoietic cancers were assessed resulted in the following:

- Temporality . There is temporality of exposure to 1,3-butadiene prior to the occurrence of lymphosarcoma in monomer workers and leukemias in SBR workers.

- Strength of association . Strength of association between exposure and the occurrence of lymphosarcoma in the prewar period ranged from 154% to 477% higher risk among workers exposed to 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 SBR in ever-hourly workers as compared with the general population (Delzell et al., 1996). Internal comparison of 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 lymphosarcoma (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 plants. Excesses for both lymphosarcoma as well as leukemia were observed by McMichael et al. (1974, 1976) and Meinhardt et al. (1982).

- Specificity . All monomer studies showed an increased risk of lymphosarcoma while SBR studies showed an increased risk of leukemia. Overall, they show increased risks of lymphohematopoietic system cancer among populations exposed to 1,3-butadiene. 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).

- 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 a very small number of people were exposed to high levels of 1,3-butadiene for a shorter period of time who showed the occurrence of lymphosarcoma. They could not be further stratified to evaluate the dose response.

- 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 conclusion, some of the causality criteria apply to monomer workers and occurrence of lymphosarcoma while all the criteria apply well for leukemia among SBR workers. Based on strength of association, dose-response relationship, specificity of cancer (leukemia**C**specific cell type is not known at this time), and biological plausibility, there is sufficient evidence to consider 1,3-butadiene a known human carcinogen.

8. PHARMACOKINETIC MODELING

8.1. 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 (Hattis and Wasson, 1987; Hallenbeck, 1992; Kohn and Melnick, 1993; Johanson and Filser, 1993; Evelo et al., 1993; Medinsky et al., 1994). 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 are 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 probably 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 six 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. Each of these PBPK models assumes, for simplicity, that the transfer of 1,3-butadiene to tissues is blood flow-limited, that each tissue compartment is "well mixed," and that tissue concentrations are in equilibrium with the venous blood concentration leaving the tissue.

8.2. PBPK MODELS FOR 1,3-BUTADIENE

8.2.1. Hattis and Wasson (1987)

The first PBPK model for 1,3-butadiene was that 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 K_M and preliminary V_{max} 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 partition coefficients 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 8-1.

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. Hattis and Wasson concluded that differences in pharmacokinetics fail to account for differences in carcinogenesis between mice and rats and that, with respect to risk assessment, uncertainties in the PBPK modeling are trivial compared with the differences in apparent sensitivities between these species.

Table 8-1. Parameter values used in the Hattis and Wasson (1987) PBPK model

Parameter	Rat	Mouse	Human
Alveolar ventilation (L/min)	0.15	0.0233	11.38[a] 4.8
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
Blood:air partition coefficient[c]	---------- 0.35 ----------		
P_f	---------- 118.2 ----------		
P_m	---------- 5.26 ----------		
P_{lvr}	---------- 5.4 ----------		
V_{max} (mol/min)	1.47E-6[d]	1.87E-7[d]	8.0E-5[e]
K_M (mol/L)	---------- 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.

The Hattis and Wasson (1987) model is not discussed further here because it has been superseded by new data and other modeling efforts.

8.2.2. 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 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.

8.2.3. 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 in advance from published methodologies, all of the mouse, rat, and human parameter estimates were from the literature; none of them were adjusted to obtain a fit to experimental data. The parameter values used by Kohn and Melnick (1993) are summarized in Table 8-2. 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

Table 8-2. 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	

Table 8-2. Parameter values used in the Kohn and Melnick (1993) PBPK model (continued)

Parameter	Mouse	Rat	Human
Biochemical parameters[d]			
Liver V cyt1 (nmol/h/mg)	155.4	35.4	70.8
Liver Km cyt1 (mM)	0.002	0.00375	0.00514
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
Liver micro prot (mg/L)	11,600	16,800	14,500
Liver cyto prot (mg/L)	82,800	108,000	58,000
Lung V cyt1 (nmol/h/mg)	138.6	9.6	9
Lung Km cyt1 (mM)	0.00501	0.00775	0.002
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
Lung micro prot (mg/L)	3,000	3,000	3,000
Lung cyto prot (mg/L)	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).

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.

vitro data of Csanády et al. (1992). The lung values were also assumed to apply to the viscera compartment. Csanády et al. detected DEB formation only in mouse liver preparations. Therefore, Kohn and Melnick (1993) included this reaction 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 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, monoepoxide 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 are currently available. 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 recently 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).

8.2.4. 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**C**lungs and arterial blood, muscle and vessel-rich tissues, fat, and liver**C**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 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 8-3.

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 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

Table 8-3. Parameter values used in the Johanson and Filser (1993) PBPK model

Parameter		Mouse	Rat
Physiological data			
Body weight (g)	Standard animal	25	250
	Simulations	27.5	157.5-217.5[a]
Alveolar ventilation (mL/min)	Standard animal	15	70.2
	Simulations	proportional to $bw^{2/3}$	
Cardiac output (mL/min)	Standard animal	17	83
	Simulations	proportional to $bw^{2/3}$	
Blood flows (% of cardiac output)	Muscle and VRG	66	66
	Fat	9	9
	Liver	25	25
Compartment volumes (% of body weight)	Lung and arterial	1	1
	Muscle and VRG	75	80
	Fat	10	7
	Liver	5.5	4
Partition coefficients [b]			
1,3-Butadiene	Lung and arterial, muscle and VRG, liver	0.25	
	Fat	7.23	
	Blood	3.03	
1,2-Epoxy-3-butene	Lung and arterial, muscle and VRG, liver	0.706	
	Fat	1.89	
	Blood	83.4	

Table 8-3. 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; assumed same for all species.
[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^b = (body weight)^b.

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 1,000 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. Johanson and Filser are reportedly working on a corresponding PBPK model for humans, but it has not yet been published.

8.2.5. 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 Loesser (1985) for the mouse and for 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 8-4.

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 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.

Table 8-4. 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	6.14	19.17
Fat	2.34	6.52
Muscle	3.81	11.13
Vessel-rich tissue	10.75	33.60
Bronchial lung area	1.79	5.514
Alveolar lung area	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		
$V_{max,total}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	465	200
$V_{max,liver}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	318	171
$V_{max,bronchial}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	77	13
$V_{max,alveolar}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	70	16
K_M (μM)	8	5

[a]Same for all species.
[b]Mean value of kidney:blood and brain:blood.

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 the 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.

8.2.6. Medinsky et al. (1994)

The most recent PBPK model published for butadiene is the model of Medinsky et al. (1994) for 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**C**liver, lung, fat, slowly perfused tissue group, rapidly perfused tissue group, and blood**C**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 8-5.

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 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 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

Table 8-5. Parameter values used in the Medinsky et al. (1993) 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:blood	0.617	1.10
Muscle: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:blood	1.09	1.54
Muscle: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 8-5. Parameter values used in the Medinsky et al. (1993) PBPK model (continued)

Parameter	Rat	Mouse
Rate constants for oxidative metabolism of 1,3-butadiene [d]		
Liver V_{max} (µmol/kg/hr)	62	338
K_M (µmol/L)	3.75	2.00
Lung V_{max} (µmol/kg/hr)		
K_M (µmol/L)	1.01	21.6
	7.75	5.01
Rate constants for EB metabolism in the liver [d]		
Oxidation V_{max} (µmol/kg/hr)		26
K_M (µmol/L)		15.6
Hydrolysis V_{max} (µmol/kg/hr)	260	754
K_M (µmol/L)	260	1590
glutathione conjugation K (L/kg/hr)	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.

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 affect 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 they 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).

8.3. SUMMARY

Pharmacokinetic modeling of 1,3-butadiene has not yet elucidated the reasons for the interspecies differences in carcinogenic response between mice and rats. It appears that either the PBPK models are not sufficiently sophisticated to adequately model the relevant pharmacokinetics (e.g., the models may need to incorporate the production and disposition of DEB) or a pharmacodynamic component(s) (e.g., DNA susceptibility or repair) is required to accurately correlate dose to response.

Furthermore, uncertainties in the existing PBPK models and data make them unreliable for use in risk assessment. Serious uncertainties exist pertaining to the model structures, parameter values, and validation. For example, there are discrepancies among the models and

data as to the importance of extrahepatic and extrapulmonary metabolism, competitive interaction between 1,3-butadiene and EB for oxidative metabolism, and glutathione depletion, and none of the models fully describe the kinetics for DEB.

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, measurements of V_{max} and K_M for the oxidation of 1,3-butadiene to EB in mouse, rat, and human liver microsomes by Csanády et al. (1992) and by Duescher and Elfarra (1994) differ by up to 80-fold, and Seaton et al. (1995) measured reaction rates for the oxidation of EB to DEB by rat and human liver microsomes that Csanády et al. were unable to detect (Chapter 3). Use of the in vitro metabolic data of Csanády et al. (1992) in the 1,3-butadiene PBPK models appears to result in an underprediction of total metabolism. Such underprediction could result from (1) an inability of the in vitro data to reflect the in vivo metabolic potency, (2) inaccuracies in the measurement of metabolic reaction rates or microsomal protein content in the tissues, or (3) a deficiency in the models such that they do not fully characterize 1,3-butadiene metabolism (e.g., by not including metabolism in other tissues). This 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. There is also a paucity of human in vitro data for extension of the PBPK models to humans. The few measurements that have been made on a few metabolic parameters show a high amount of variability.

Another area of uncertainty is that of model validation. The existing models have been subjected to a very limited validation, mostly by comparison of simulation results with chamber uptake data. 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 suggests that the chamber data are relatively insensitive to various features of the models and might be of limited use for model validation. For the PBPK models to be more reliable, they should be validated against tissue concentration data for various metabolites in various tissues. More recently, 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. The results of simulations using the Medinsky et al. (1994) model suggest that the model does not conform adequately to the tissue concentration data. Any PBPK model for 1,3-butadiene would require more rigorous validation before it could be considered reliable for use in risk assessment.

8.4. CONCLUSIONS

As discussed above, the existing PBPK models and data cannot 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 (1) evaluation of the kinetics of DEB in rodents as well as in humans, (2) investigation of the validity of the in vitro metabolic data for extrapolating to in vivo exposure, (3) clarification of the values of various physiological parameters such as the ventilation rate, (4) better characterization of the distribution of values for the human metabolic rates, and (5) more measurement of tissue concentrations of metabolites for model validation. It is possible that more information on the specific mechanisms of action is required to explain interspecies differences in the various target tissues.

In any event, 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 risk from animals to humans. Any attempt to extrapolate the risk in rodents to humans, given the dramatic and unresolved interspecies differences between the mouse and rat, would involve far greater uncertainties than basing a 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.

9. QUANTITATIVE RISK ASSESSMENT FOR 1,3-BUTADIENE

9.1. EPIDEMIOLOGICALLY BASED CANCER RISK ASSESSMENT

9.1.1. Exposure-Response Modeling

In general, it is preferable to use high-quality epidemiologic data when they are available over toxicologic data for quantitative risk assessment purposes. In the past, available epidemiologic data on 1,3-butadiene have been inadequate for quantitative risk assessment, and previous risk assessments relied primarily on models based on the NTP mouse bioassay studies (reviewed in Chapter 1).

The recently reported findings by Delzell et al. (1995) from a retrospective cohort mortality study of synthetic production workers exposed to 1,3-butadiene (reviewed in Chapter 7) present an opportunity to perform a quantitative 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 peak*years) by linking the JEM with the study subject's 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, 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) Multiplicative: $RR = e^{\beta X}$

(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: $RR = 1 + \beta_1 X^{½}$

The Poisson regression analyses revealed a positive exposure-response relationship between cumulative exposure to 1,3-Butadiene or styrene and leukemia mortality. 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. 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 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 (i.e., see Table 63 in Delzell et al., 1995).

The results that were obtained by the investigators from fitting the alternative relative rate models described above are summarized in Table 9-1. These results are from models that simultaneously evaluated the effects of 1,3-butadiene and styrene exposure. The regression parameter for 1,3-butadiene cumulative exposure was found to be statistically significantly greater than 0 ($p<0.05$) in all of the models evaluated, whereas a nonsignificant and weaker relationship was observed for styrene.

The power and square root models were found to provide the best fit to the data based on comparison of the model deviances. However, the differences in deviances between the various

Table 9-1. Results from exposure-response models of continuous cumulative exposure to 1,3-butadiene and styrene using alternative structural forms reported by Delzell et al. [a]

Structural model form	1,3-Butadiene (ppm-years)			Styrene (ppm-years)		
	Model deviance	β estimate (S.E.) [b]	LRT p-value [c]	Model deviance	β Estimate (S.E.) [b]	LRT p-value [c]
Multiplicative: $RR = e^{\beta X}$	486.0	0.0041 (0.0019)	0.04	485.9	0.0052 (0.0053)	0.34
Linear: $RR = 1 + \beta X$	486.0	0.0068 (0.0050)	0.04	485.7	0.0079 (0.0088)	0.30
Power: $RR = e^{\beta[\ln(1 + X)]}$	485.6	0.2028 (0.0972)	0.03	485.2	0.1494 (0.1183)	0.21
Square root: $RR = 1 + \beta_1 X^{1/2}$	485.6	0.1293 (0.1024)	0.03	485.4	0.0968 (0.1090)	0.23

[a] Adapted from Table 67 in Delzell et al. (1995). Results presented are adjusted for age, calendar year, years since hire, race, and exposure to 1,3-butadiene or styrene.
[b] S.E. is the standard error for the exposure parameter estimates.
[c] LRT, likelihood ratio test for the exposure effect (1,3-butadiene or styrene).

models are slight. The authors expressed a preference for the square root model as the best model based on its goodness of fit and its simplicity. This model was refined into a "final model" by omitting styrene and race because the effect of these variables on the estimated parameter for 1,3-butadiene exposure was considered to have been minimal. In addition, certain age, calendar year, and years since hire categories were collapsed for the final model for similar reasons. The final model is summarized in Table 9-2. The relationship between cumulative 1,3-butadiene exposure and leukemia mortality was highly statistically significant in this model (p=0.002).

9.1.2. Prediction of Lifetime Excess Risk of Leukemia

The relative rate models presented in the report by Delzell et al., which are summarized in Tables 9-1 and 9-2, were used as a basis for predicting the lifetime excess risk of leukemia mortality for varying levels of continuous environmental exposures to 1,3-butadiene. These lifetime risk estimates were made using the relative rate estimates and an actuarial program that

Table 9-2. Results from "final" square root exposure-response model of continuous cumulative exposure to 1,3-butadiene reported by Delzell et al. [a]

Variable	βeta		Likelihood ratio test	
	Estimate	S.E. [b]	χ^2 (d.f.) [c]	p-value
Loglinear terms				
Constant	-10.02	0.47		
Age:			13.2 (2)	0.001
40-69	0			
70-79	0.89	0.33		
80+	1.71	0.48		
Calendar year:			3.85(1)	0.050
1950-89	0			
1990-91	0.72	0.34		
Years since hire:			7.64 (1)	0.006
10-19	0			
20+	1.09	0.44		
Linear term				
(1,3-butadiene ppm-years)$^{0.5}$	0.17	0.10	9.41 (1)	0.002

[a] This table is an adaptation of Table 68 in Delzell et al. (1995).
[b] S.E. is the standard error of the parameter estimate.
[c] Chi-square (χ^2) and degrees of freedom (d.f.) based on the likelihood ratio statistic.

takes into account the effects of 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. Exposures to 1,3-butadiene were assumed to be continuous for the entire lifetime, and the risks were computed up to age 85. 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 another

[1]This program is an adaptation of the approach that was previously used in BEIR IV. Health Risks of Radon and Other Internally Deposited Alpha Emitters. National Academy Press, Washington, DC, 1988, pp. 131-134.

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 lifetime risk of leukemia associated with varying levels of environmental exposure to 1,3-butadiene based on the alternative model forms are illustrated in Figures 9-1 to 9-5. Estimates of risks and exposure levels corresponding to levels of risk of potential regulatory interest are presented in Tables 9-3 and 9-4. These estimates appear to vary by several orders of magnitude depending on the model used. For example, at the 1 in a million risk level, the 95% upper confidence intervals for 1,3-butadiene exposure range from 0.1 ppb (parts per billion) (based on the multiplicative model) to 1 e-6 ppb (based on the final square root model).

Consistent with the proposed EPA cancer guidelines, these results were also used to estimate the exposure level (EC_p; "effective concentration") and 95% lower confidence intervals (LEC_p) associated with varying levels of risk (p) ranging from 0.1 to 10%, which are summarized in Table 9-5. Although the new EPA guidelines emphasize the derivation of exposure levels associated with a 10% 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 increase the lifetime risk of leukemia death by 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 a lower (e.g., 0.1%) risk level would seem to be a more reasonable choice in this circumstance. The analogous relative rates for increased risks of 1% or 0.1% are 2.7 and 1.17, respectively, which better correspond with the set of SMRs reported by Delzell et al. (1995). The exposure levels corresponding to a 1% risk level are illustrated in Figures 9-1 to 9-5. When a 1% risk level is used, the LEC_1 from these analyses ranges from 0.07 to 0.6 ppm based on the different relative rate models. Using the final model presented by Delzell et al. (1995) would yield an LEC_1 of 0.12 ppm.

[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.)

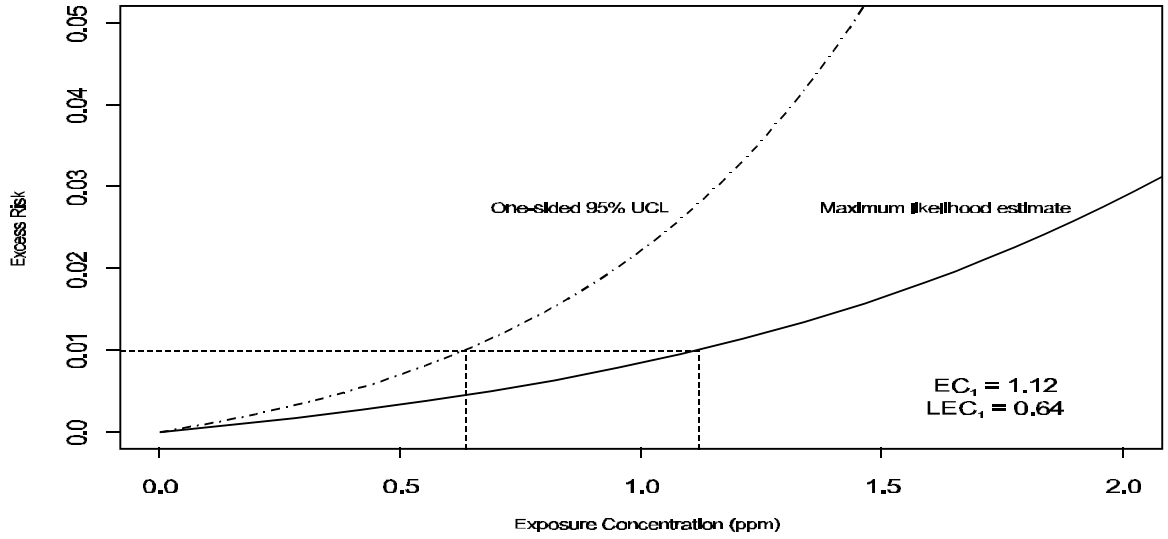
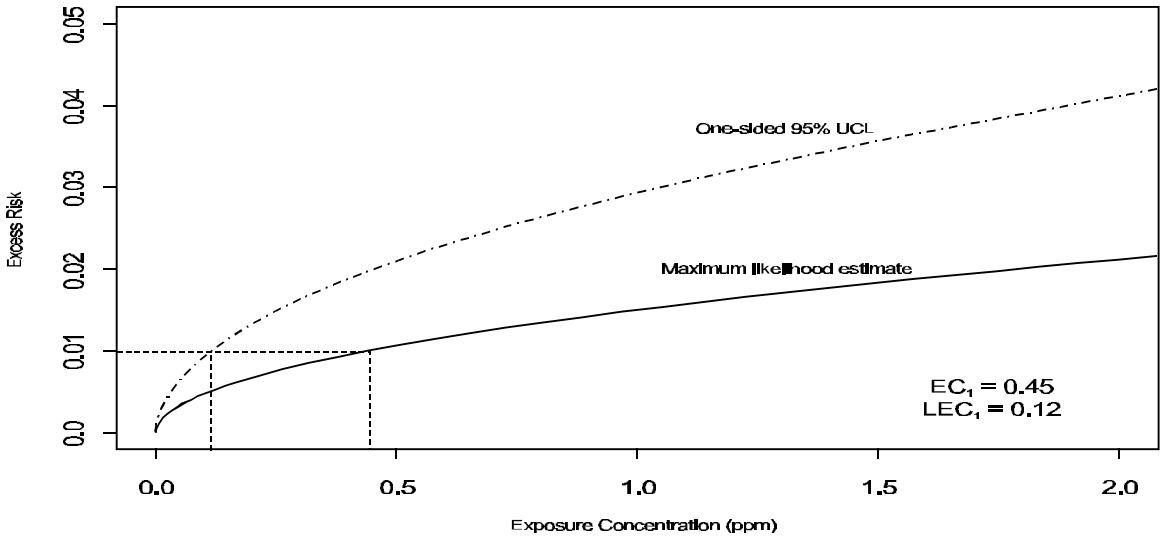


Figure 9-1. Excess risk and 95% upper confidence limit excess risk estimates based on the multiplicative model reported by Delzell et al., 1995.*
* Multiplicative model: $RR = e^{\beta x}$



Figur

e 9-2. Excess risk and 95% upper confidence limit excess risk estimates based on the power model reported by Delzell et al., 1995.*
* Power model: $RR = e^{\beta[1v(1+X)]}$

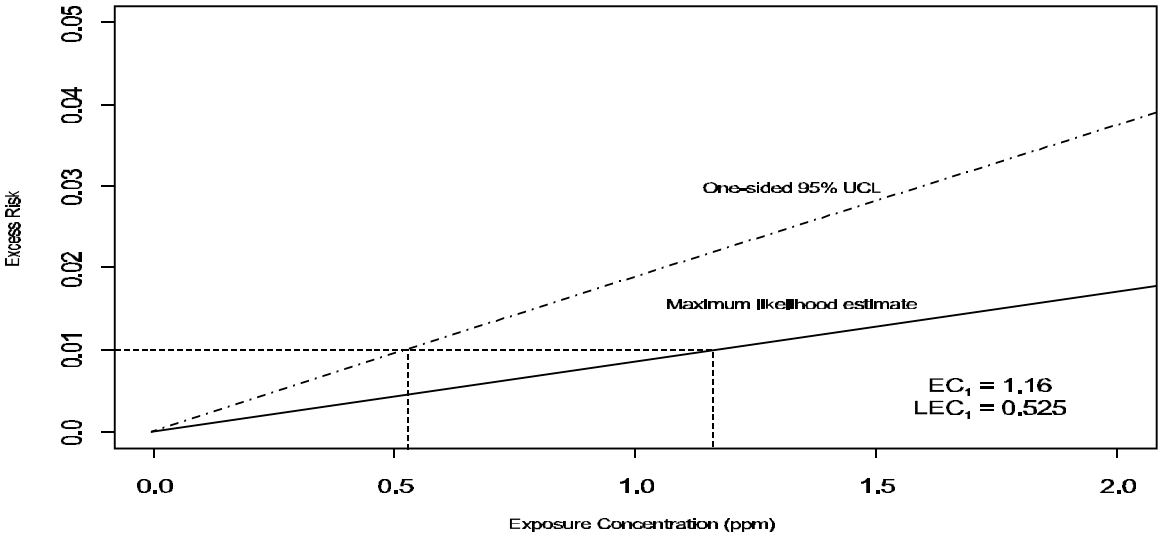


Figure 9-3. Excess risk and 95% upper confidence limit excess risk estimates based on the linear excess relative rate model reported by Delzell et al., 1995.*
* Linear excess model: $RR=1+\beta X$

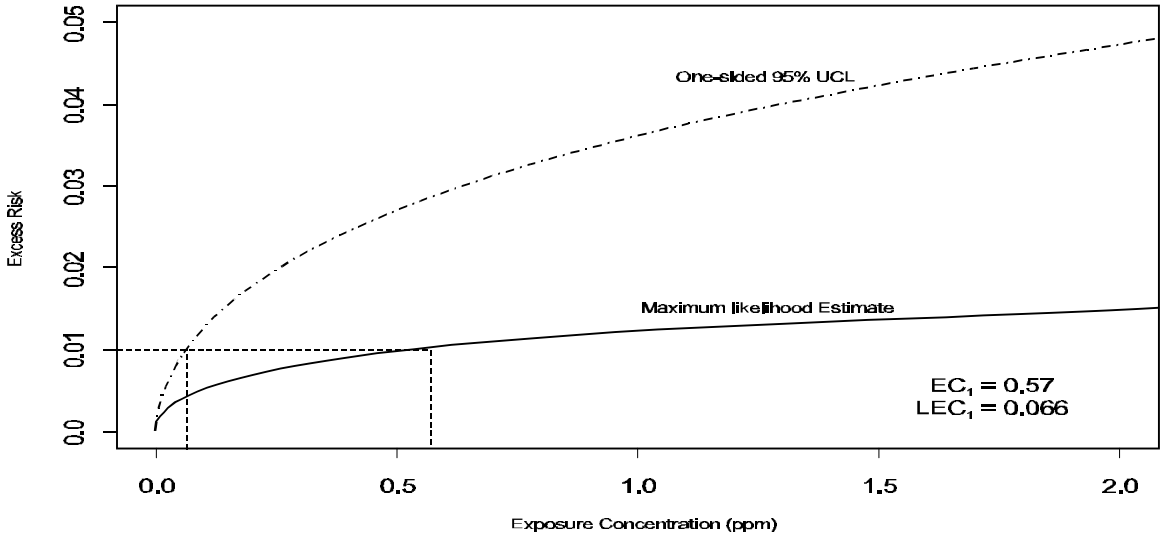


Figure 9-4. Excess risk and 95% upper confidence limit excess risk estimates based on the final square root model reported by Delzell et al., 1995.*
* Final square root model: $RR=1+\beta x^{½}$

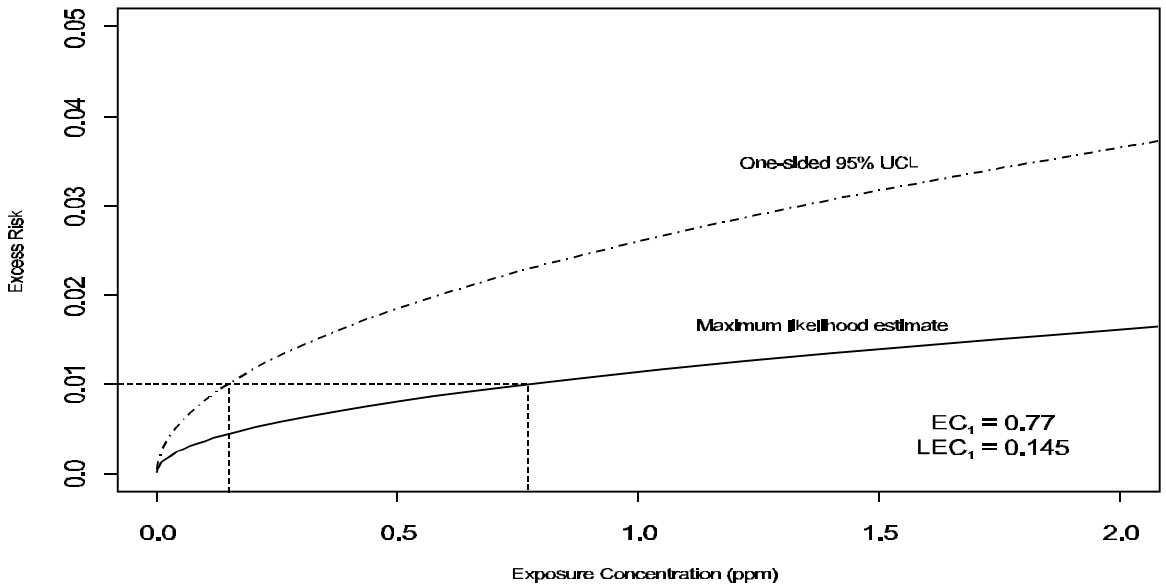


Figure 9-5. Excess risk and 95% upper confidence limit excess risk estimates based on the square root model reported by Delzell et al., 1995.*

* Square root model: $RR=1+\beta x^{1/2}$

Table 9-3. Maximum likelihood estimates (MLEs) of excess risk with one-sided 95% upper confidence limits (95% UCL) from several models reported by Delzell et al. (1995) for continuous lifetime exposures to varying concentrations of 1,3-butadiene

Model	Concentration (ppm)	MLE excess risk	95% UCL excess risk
Multiplicative: $RR = e^{\beta X}$	1.0E-04	5.2E-07	9.2E-07
	1.0E-03	5.2E-06	9.2E-06
	1.0E-02	5.3E-05	9.3E-05
Power: $RR = e^{\beta[\ln(1+X)]}$	1.0E-04	2.6E-05	4.6E-05
	1.0E-03	2.4E-04	4.4E-04
	1.0E-02	1.6E-03	3.1E-03
Linear: $RR = 1 + \beta X$	1.0E-04	8.7E-07	1.9E-06
	1.0E-03	8.7E-06	1.9E-05
	1.0E-02	8.7E-05	1.9E-04
Initial square root: $RR = 1 + \beta_1 X^{1/2}$	1.0E-04	1.1E-04	2.6E-04
	1.0E-03	3.6E-04	8.4E-04
	1.0E-02	1.1E-03	2.6E-03
Final square root: $RR = 1 + \beta_1 X^{1/2}$	1.0E-04	1.5E-04	3.0E-04
	1.0E-03	4.8E-04	9.4E-04
	1.0E-02	1.5E-03	3.0E-03

Ratios are also presented in Table 9-5 that were calculated by dividing the excess risk (p) by the corresponding LEC_p for each model. Each ratio is the slope of the line segment connecting the point (LEC_p, p) with the origin. Based on the LEC_1, these ratios vary by approximately one order of magnitude from 0.016 to 0.15. If these LEC_1-based ratios were used to calculate the concentration corresponding to a 1 in a million excess lifetime risk by linear interpolation[3], the values would range from 7 to 64 parts per trillion. The final model presented by Delzell et al. (1995) would yield a corresponding exposure level of 12 parts per trillion.

Table 9-4. MLEs of parts per million continuous exposure concentrations associated with varying excess risk levels with one-sided 95% lower confidence

[3] Linear interpolation between the origin and the point (LEC_p, p) is also referred to as "linear extrapolation."

limits (95% LCL) based on relative rate results of several models reported by Delzell et al. (1995) and U.S. population rates

Model	Excess risk	MLE (ppm)	95% LCL (ppm)
Multiplicative: $RR = e^{\beta X}$	1E-6	1.9E-4	1.1E-4
	1E-5	1.9E-3	1.1E-3
	1E-4	1.9E-2	1.1E-2
Power: $RR = e^{\beta[\ln(1+X)]}$	1E-6	3.9E-6	2.2E-6
	1E-5	3.9E-5	2.2E-5
	1E-4	4.0E-4	2.2E-4
Linear: $RR = 1 + \beta X$	1E-6	1.1E-4	0.52E-4
	1E-5	1.1E-3	0.52E-3
	1E-4	1.1E-2	0.52E-2
Initial square root: $RR = 1 + \beta_1 X^{1/2}$	1E-6	7.6E-9	1.4E-9
	1E-5	7.6E-7	1.4E-7
	1E-4	7.6E-5	1.4E-5
Final square root: $RR = 1 + \beta_1 X^{1/2}$	1E-6	4.4E-9	1.1E-9
	1E-5	4.4E-7	1.1E-7
	1E-4	4.4E-5	1.1E-5

9.1.3. Sources of Uncertainty

It is apparent from the results presented in Table 9-5 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 either of the 1% and 10% excess risk levels spanned approximately one order of magnitude, whereas the range for the 0.1% level spanned nearly two orders. In this instance, it seems more reasonable to utilize the results at the 1% risk level because this corresponds to exposures that are 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 9-1 fit the observed data nearly

Table 9-5. Maximum likelihood (EC_p) and 95% lower-bound (LEC_p) estimates of the continuous exposure concentrations associated with varying levels of excess risk (p)

Structural model form	Percentage excess risk (p)	1,3-Butadiene exposure levels (ppm)		Ratio [a]
		Maximum likelihood (EC_p)	Lower 95% bound (LEC_p)	
Multiplicative model: $RR = e^{\beta X}$	10	3.3	1.87	5.3 E-2
	1	1.12	0.64	1.6 E-2
	0.1	0.18	0.10	1.0 E-2
Power: $RR = e^{\beta[\ln(1+X)]}$	10	1000	15	6.7 E-3
	1	0.57	0.066	1.5 E-1
	0.1	0.0054	0.0025	4.0 E-1
Linear model: $RR = 1 + \beta X$	10	12.5	5.65	1.8 E-2
	1	1.16	0.525	1.9 E-2
	0.1	0.116	0.0525	1.9 E-2
Initial square root:	10	88	16.8	5.9 E-3
	1	0.77	0.145	6.9 E-2
	0.1	0.0076	0.00144	6.9 E-1
Final square root: $RR = 1 + \beta_1 X^{1/2}$	10	51	13.5	7.4 E-3
	1	0.45	0.12	8.3 E-2
	0.1	0.0044	0.0012	8.3 E-1

[a] The ratio is the excess risk (p/100%) divided by the one-sided lower 95% confidence limit on the exposure estimate (LEC_p).

as well. Moreover, for a given linear extrapolation, the ratios in Table 9-5 show that the sensitivity of the result to the choice of excess risk level varies considerably for these models, with the linear model being least sensitive and the two square root models being most sensitive. Of the two square root models, however, the final relative rate model could be advantageous to the other model if the omitted parameters for the effects of race and styrene exposure are unnecessary.

A major source of uncertainty in this analysis is the potential for 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, 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 for exposure misclassification is unknown, but preliminary analyses suggest that it may have dampened and possibly distorted the observed dose-response relationship (letter from Delzell and Macaluso to Aparna Koppikar, April 2, 1996).

Another concern about the study has been expressed regarding the assignment of peak exposures in the analysis, which was defined as average exposures equal to or greater than 100 ppm over 15 min. 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 to Aparna Koppikar, April 2, 1996). The models used in this risk assessment assume a constant dose-rate effect and do 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 who conducted the study by Delzell et al. (1995).

9.1.4. Summary and Conclusions

Risk estimates for environmental exposures are derived from an analysis by Delzell et al. (1995) of an occupational retrospective cohort mortality study of approximately 16,000 workers in six North American styrene-butadiene rubber manufacturing plants. The analysis of this study is based on follow-up during 1943-1991, with an average follow-up of 25 years and about 25% of the cohort deceased. While 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% C.I. = 1.04-1.91) for all ever-hourly men (Delzell et al., 1996). The consistency of this leukemia result with other findings from previous epidemiology studies with 1,3-butadiene plus other data led to the conclusion that this increase was due to 1,3-butadiene and to the decision to perform a quantitative risk assessment with this database.

While this cohort had been previously studied (Matanoski et al., 1987, 1988, 1989, 1990, 1994), the Delzell et al. update and analyses are especially 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 dose response. Exposure metrics included cumulative ppm-years and number of years with peak exposures of at least 100 ppm for at least 15 min. Additional individual worker exposure information on both styrene and benzene allowed analyses to adjust for these potential confounding exposures. The Delzell et al. (1995) report includes these analyses.

The Delzell et al. analysis used Poisson regression analysis with nine categories for cumulative exposure of 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. Relative rate models run within the Poisson analysis included the (1) multiplicative, (2) power, (3) linear, and (4) 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. While Delzell et al. selected the square root model as their final choice because of a slightly better likelihood fit, none of the models fit the data significantly better or worse than the others.

The quantitative risk analysis presented here uses the results of the Delzell et al. analyses, which include the styrene exposure variable as a covariate, to extrapolate risk from occupational work-time exposure to lifetime environmental continuous exposure. This is done by adjusting the 1,3-butadiene parameter estimates calculated by Delzell et al. to reflect continuous rather than work-time exposures and by using life table modeling techniques to convert the relative rate exposure-response relationship to a lifetime additional risk dose-response relationship. These techniques have been used before by EPA as well as other governmental 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 those currently proposed for revision (U.S. EPA, 1996). For the 1986 Guidelines, the risk estimates are calculated as a potency or slope factor derived from fitting a linear model (default case) to the observed data and applying the same model to lower exposure concentrations. For the proposed guidelines revisions, the risk estimates are obtained by first calculating a "point of departure" within the range of observation using any of the appropriate models and then extrapolating to 0 by means of a straight line. The LED_{10} (i.e., lower confidence limit on a dose associated with 10% extra risk) is proposed in the guidelines revisions as the standard point of departure; however, the LEC_{01} and EC_{01} are 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, because exposure levels are expressed as exposure concentrations rather than doses, and because the issue of whether to use LEDs or EDs in the final guidelines has not yet been resolved.

The results of the extrapolations using the four relative rate models are shown in the quantitative risk analysis and presented in Figures 9-1 to 9-4. They show that although in the observable risk range of 1%, the MLEs of required continuous exposure (EC_{01}) are close, varying 2.6-fold from 0.45 to 1.16 ppm, the LEC_{01} estimates range from 0.066 to 0.64, or about 10-fold. Furthermore, as the risk extrapolation decreases 10-fold to a 0.001 risk level, the ML exposure estimates for the various models diverge much more rapidly, a 45-fold range from 0.004 ppm to 0.18 ppm. At the 10^{-5} risk level, the exposure estimates diverge by nearly four orders of magnitude. Clearly, the final risk estimates based on the 1986 guidelines extrapolation procedures are highly dependent on the choice of model, but those of the proposed guidelines revisions, which extrapolate from the LEC_{01}, are less affected.

For the 1986 guidelines approach, the model of choice is the linear default. 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 the linear model is much less protective of public health, by nearly five orders of magnitude, than is the Delzell et al. square root model choice. For this approach, the maximum likelihood potency (slope) estimate is:

$$B = 8.7 \times 10^{-3} \text{ (ppm)}^{-1}.$$

For the suggested default approach under the proposed guidelines revisions, the EC_{01} level is chosen because that is within the observable response range of leukemia deaths. At the EC_{01} level, the different models provide dose estimates ranging from 0.45 ppm to 1.16 ppm and the 95% LCLs on dose ranging from 0.066 to 0.64. Without specific directions for choice from the proposed guidelines, potency estimates based on each of the models examined by Delzell et al. are presented in Table 9-6.

The cancer potency estimates using EC_{01}s as the point of departure range from 8.7×10^{-3}/ppm (linear model) to 0.022/ppm (final square root model). The square root model was the model preferred by Delzell et al. based on goodness of fit and simplicity; thus they chose that model for various refinements, resulting in the final square root model. The cancer potency estimates based on LEC_{01}s range from 0.016/ppm to 0.15/ppm, with the final square root model yielding 0.083/ppm while the linear model yields 0.019/ppm. Although the proposed Guidelines do not offer explicit guidance on choice of model, it may be appropriate in this particular case to use the final square root model to obtain the point of departure because this model benefits from the refinements performed by Delzell et al.

Table 9-6. Cancer potency (unit risk) estimates based on linear extrapolation from the LEC_{01} or EC_{01} calculated from the models presented by Delzell et al.

Model	EC_{01} (ppm)	Potency estimate (ppm^{-1}) (i.e., $0.01/EC_{01}$)	LEC_{01} (ppm)	Potency estimate (ppm^{-1}) (i.e., $0.01/LEC_{01}$)
Multiplicative	1.12	8.9×10^{-3}	0.64	0.016
Power	0.57	0.018	0.066	0.15
Linear	1.16	8.7×10^{-3}	0.525	0.019
Initial square root	0.77	0.013	0.145	0.069
Final square root	0.45	0.022	0.12	0.083

As the estimates of choice, the MLEs of both the potency and EC_{01} are chosen. The main reason for this choice is that these estimates are based on human data from a large, well-conducted study. Although EPA has historically used upper-limit potency estimates for animal-to-human extrapolations, these upper limits derive their use more from computational instabilities of the MLEs in the quantitative risk models used. Human-to-human extrapolations typically use a simpler linear model form that does not have these instabilities. Furthermore, the human data inherently engender far less uncertainty in the risk estimates, so one may have more confidence in the use of MLEs from human data than from animal data.

9.2. CANCER RISK ESTIMATES BASED ON RODENT BIOASSAYS

9.2.1. Rat-Based Estimates

Cancer risk estimates based on the 1981 Hazelton 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.

9.2.2. 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 hours/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 [males]), heart (hemangiosarcomas), lung, forestomach, Harderian gland, liver, preputial gland (males), ovary (females), and mammary gland (females), when adjusted for intercurrent mortality (Melnick and Huff, 1993). 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.

9.2.2.1. 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 9-7). These sites were considered to be the most relevant 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

Table 9-7. 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, adenocanthonomas, 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.

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.

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 8). 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 9-7 using the LMS model. The multistage model has the form:

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where P(d) represents the lifetime risk (probability) of cancer at dose d, 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 the 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 9-7 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

$q_1^* = 0.10$

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), 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% 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.

MLE of risk for a dose of 1 ppm = 7.4×10^{-2}

MLE of risk for a dose of 1 ppb = 7.6×10^{-5}

MLE of dose for a risk of 0.10 (EC_{10}) = 1.4 ppm

95% UCL on dose for a risk of 0.10 (LEC_{10}) = 1.0 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 take into account 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.

9.2.2.2. 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 an 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; 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 dose groups for which it appeared that complete histopathological 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 (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 method of maximum likelihood.

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, while 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 (it was felt that there were insufficient data 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 9-8 (based on female mouse data) and 9-9 (male mice). For all tumor types except the heart hemangiosarcomas (both sexes) and the forestomach (male mice), the one-stage Weibull was the preferred model. For male mice, the heart hemangiosarcomas and forestomach tumors were best described by the two-stage model, while for female mouse heart hemangiosarcomas, a three-stage model was preferred.

Human unit cancer risk (or potency) estimate results (extra risk) are presented in Tables 9-10 (based on female mouse data) and 9-11 (male mice). 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

Table 9-8. Parameter estimates for multistage Weibull time-to-tumor model based on female mouse tumor incidence, w/o 625 ppm group

Tissue	Q0	Q1	Q2	Q3	Z
Lymphocytic lymphoma	6.23×10^{-10}	1.67×10^{-10}	-	-	3.92
Heart hemangiosarcoma	0	0	0	2.88×10^{-17}	6.10
Lung	5.83×10^{-9}	3.40×10^{-9}	-	-	3.69
Mammary	2.47×10^{-6}	5.42×10^{-5}	-	-	1.27
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
Ovary	7.83×10^{-9}	1.48×10^{-8}	-	-	3.05
Histiocytic sarcoma	3.68×10^{-14}	1.23×10^{-14}	-	-	6.03

Table 9-9. Parameter estimates for multistage Weibull time-to-tumor model based on male mouse tumor incidence, w/o 625 ppm group

Tissue	Q0	Q1	Q2	Z
Lymphocytic lymphoma	1.84×10^{-8}	1.28×10^{-9}	-	3.08
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
Histiocytic sarcoma	0.0	1.04×10^{-13}	-	5.50

Table 9-10. Human unit cancer risk estimates (extra risk, computed for risks of 10^{-6}) based on female mouse tumor incidences, w/o 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
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
Mammary	0.0321	0.0203	5.09	3.23	0.0310
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
Ovary	0.0358	0.0218	4.74	2.89	0.0346
Histiocytic sarcoma	0.1283	3.36×10^{-3}	30.8	0.806	0.1241

Table 9-11. Human unit cancer risk estimates (extra risk, computed for risks of 10^{-6}) based on male mouse tumor incidences, w/o 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}
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
Histiocytic sarcoma	0.02162	0.01394	7.42	4.78	0.02090

model is greater than the unit risk estimate of 0.10/ppm generated above from multiple female mouse tumor 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% UCL's for human risk at various exposure levels, allowing for the 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

$$95\%\ UCL = MLE + 1.645\sigma,$$

where the standard deviation 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 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 9-8 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 male mouse based- results (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 mice, the selected models for both the heart hemangiosarcomas and the forestomach tumors were nonlinear (Table 9-9); 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 9-12.

9.2.3. 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 sensitive species and sex (the female mouse). Histiocytic sarcomas were excluded because they introduced excessive variance into the upper bound while contributing 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 the mouse to humans. The two rodent species for which bioassay data were

Table 9-12. Unit potency estimates (extra risk) summed across 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

available**C**the mouse and the rat**C**varied significantly in their carcinogenic responses to 1,3-butadiene, in terms of both site specificity and degree 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 have been unable to explain the species differences in carcinogenic response (Chapter 8), and it is likely 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 (public health protective) for the 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, as 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 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 8). 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).

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 take into account the differences in mortality between the exposure groups in the mouse bioassay; however, it is unknown how well this model is predicting the low-dose extrapolated risks for 1,3-butadiene.

There are also uncertainties pertaining to the specific assumptions used in conducting these multistage Weibull time-to-tumor analyses. Some 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, as 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 3 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 (1) model selection (i.e., stage of Weibull model) from among models deemed to be comparable, (2) tumor context assumptions, and (3) 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. Realizing that different quantitative methodologies and assumptions were used to calculate the various risk estimates, recall that 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 best estimate (MLE) of human incremental lifetime unit cancer risk

extrapolated from the leukemias observed in occupational epidemiology studies was 9×10^{-3}/ppm.

9.3. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

9.3.1. Introduction

The reproductive and developmental effects of 1,3-butadiene are among the 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). In the first type of study, developmental toxicity of 1,3-butadiene was evaluated in studies in mice and rats that included 10-day exposures via inhalation at 0, 40, 200, and 1,000 ppm on gestation days (gd) 6-15 for 6 h/day (Hackett et al., 1987a, b). In rats, no effects were detected at any exposure level for developmental toxicity (200 ppm was the NOAEL for maternal toxicity), while reduced fetal weights were seen in mice at all exposure levels (Table 9-13). Thus, 40 ppm was considered a LOAEL for mice.

In the second type of study, male-mediated effects of 1,3-butadiene were evaluated in a dominant lethal study in which CD-1 mice were exposed to 0, 12.5, or 1,250 ppm for 6 h/day, 5 days/week, 10 weeks (Anderson et al., 1993, 1995). One group of females at each exposure level was killed on gd 17, while another was allowed to litter. At 12.5 ppm, the frequency of late deaths and congenital abnormalities on gd 17 were increased, while in litters allowed to deliver their pups, changes in implantation numbers, postimplantation loss, litter size, and weight at birth and at weaning were significantly different only at 1,250 ppm. In addition, body weights of F_1 males at all time points and of F_1 females at several time points between 8 and 71 weeks of age were significantly increased above controls at both 12.5 ppm and 1,250 ppm. Based on the data from animals killed on gd 17, there was no NOAEL for dominant lethal effects in the study (Table 9-14).

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 9-15). 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).

Table 9-13. Prenatal (developmental) toxicity study (Hackett et al., 1987b)

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

Fetal Weight Data

Exposure level	No. litters	Mean fetal weight/litter
0	18	1.341
40 ppm	19	1.282
200 ppm	21	1.126
1000 ppm	20	1.038

Table 9-14. Male-mediated developmental toxicity (Anderson et al., 1993, 1995)

Species/strain: CD-1 mice, adult males
Exposure time: 10 weeks
Exposure regimen: 6 h/day, 5 days/week
Exposure levels: 0, 12.5 ppm, 1250 ppm

Exposure level	Number exposed	No. implants (no. preg. females)	% Early and late deaths	% Live implants
0	25	12.09 (23)	4.68	94.6
12.5 ppm	25	12.75 (24)	7.52	92.2
1250 ppm	50	10.68 (38)	22.91	76.8
Exposure level	Mean litter size at birth (no. litters)	Mean no. implants (no. litters)	% Post-implantation loss	Mean litter size at weaning (no. litters)
0	12.22 (18)	12.81 (16)	4.88	12.17 (18)
12.5 ppm	11.14 (21)	12.35 (17)	9.05	10.95 (20)
1250 ppm	9.06 (33)	10.47 (32)	23.88	9.03 (33)

Table 9-15. NTP chronic study (1993)

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

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 9-15. NTP chronic study (1993) (continued)

Incidence Data - Uterine and Testicular 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)

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)

In conclusion, each of these three types of studies indicated the potential for 1,3-butadiene to affect reproduction and development in mice at low levels of exposure.

9.3.2. Fetal Weight Modeling

Fetal weight data (Table 9-13) 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 model allows for nesting of fetal data within litters and takes into account intralitter correlations and 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:

(1) a decrease below the 5th percentile of the control distribution, or

(2) a decrease below the 10th percentile.

The model was used to estimate: (a) the EC_{05}* and the LEC_{05}** associated with a 5% additional risk of obtaining a fetal weight below the 5th percentile of the controls, or (b) the EC_{10} and LEC_{10} associated with a 10% additional risk of obtaining a fetal weight below the 10th percentile of controls, based on Kavlock et al. (1995). 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 methods of maximum likelihood. In order to get an acceptable fit, an intercept parameter (d_0) was included in the model (sometimes referred to as a threshold parameter, i.e., the point at which the model can no longer distinguish from background). The parameter constraints were: $d_0 \geq 0$; $\gamma \geq 1$; $0 \leq \propto - \theta_1 s \leq 1$.

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, based on Kavlock et al. (1995):

(1) a 5% reduction in mean fetus weight/litter from the control mean,

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*The EC is the effective (exposure) concentration associated with a given level of risk, 5% in this case.

**The LEC is the lower confidence limit on the effective concentration associated with a given level of risk. The LEC is also known as the benchmark concentration.

(2) a reduction in mean fetus weight/litter to the 25th percentile of the control distribution, and

(3) a reduction in mean fetus weight/litter to 0.5 SD below the control mean.

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.

A 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. 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 standard deviation 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 corresponding to 10% additional risk corresponds to a change from the control mean of 0.61 SD. Since 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 approaches for fetal weight are shown in Table 9-16 and Figures 9-6 to 9-8. The log-logistic model resulted in an adequate fit of the data. Since the log-logistic model requires converting continuous data to quantal responses, the continuous power model was also applied, but did not give an adequate fit with all four exposure levels. When fit to 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. The log-logistic model accounts for intralitter correlation and litter size, but requires conversion of continuous data to quantal responses. Neither the continuous power nor the hybrid model are currently structured to account for intralitter correlation or litter size. The version of the hybrid model used here does not allow use of the standard deviation (σ) for individual dose groups, so the σ at dose d_0 was used for all dose groups. The continuous power and hybrid models take advantage of the power of modeling the continuous data, but the hybrid model expresses the EC and LEC as a quantal estimate of risk, allowing direct comparison with ECs and LECs for quantal endpoints. Given the various advantages and limitations of these models, the hybrid model is considered the preferred approach for modeling continuous data.

Table 9-16. Fetal weight modeling (LOAEL = 40 ppm)

Model	Response	Cutoff	EC	LEC	p-Value
Log-logistic (1-4)[a]	Individual fetal weight	5th percentile	EC_{05} = 46.85	LEC_{05} = 27.02	0.079
		10th percentile	EC_{10} = 49.69	LEC_{10} = 38.89	0.067
Continuous power (1-3)[a]	Mean fetal weight/litter	5% relative reduction	65.08	53.51	0.77
		25th percentile	45.10	36.66	
		0.5 SD absolute reduction	50.99	41.44	
Hybrid model[a] (1-4)	Mean fetal weight/litter	P_0 = 0.05	EC_{10} = 28.19	LEC_{10} = 13.67	0.08

[a]Exposure levels modeled in each case are shown in parentheses.

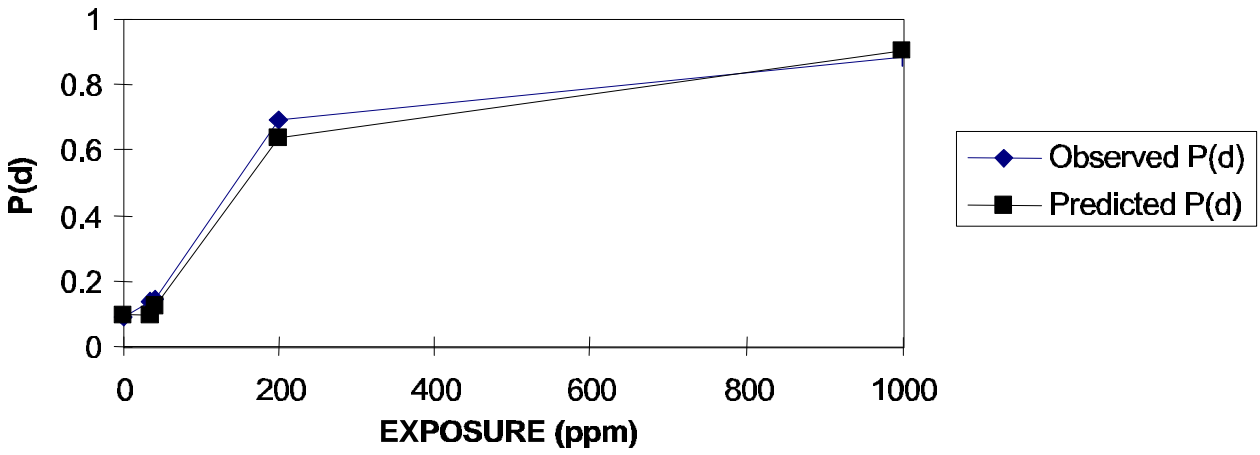
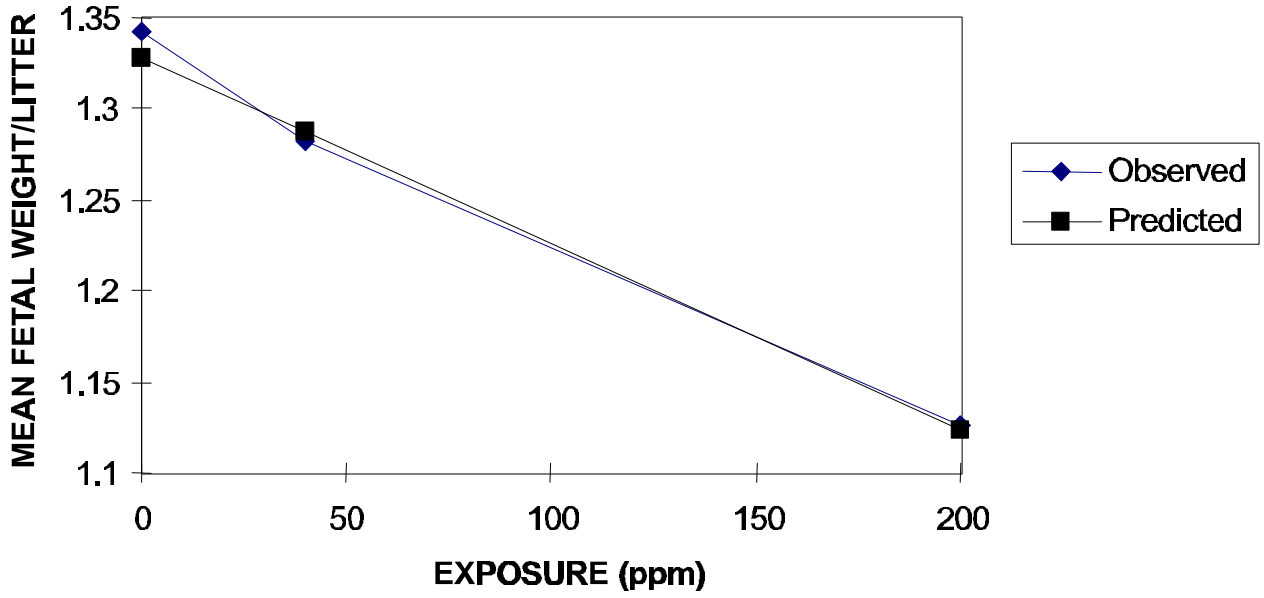


Figure 9-6. Observed versus predicted dose (exposure) probability P(d) of fetal weight reduction below the 10th percentile of controls using log-logistic model.

Figure 9-7. Observed versus predicted mean fetal weight per litter using continuous model.



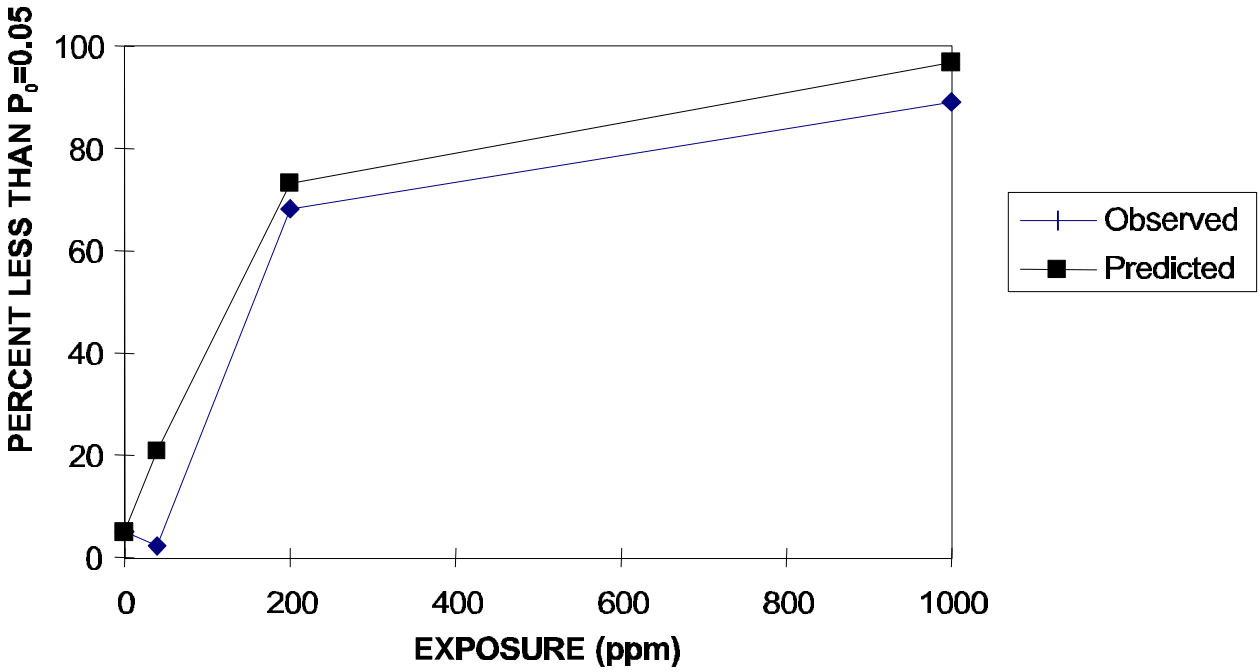


Figure 9-8. Observed versus predicted percent of mean fetal weights per litter less than the 5th percentile of controls ($P_0 = 0.05$) using hybrid model.

9.3.3. Male-Mediated Developmental Toxicity Modeling

Several endpoints from animals killed at gd 17 and after birth were modeled using a log-linear model:

$$y(d) = \alpha + \beta x [\ln(1 + d)]$$

This model was used because of the wide spacing of doses and the lack of linearity in the dose-response relationship. The data were limited in that only two exposure levels in addition to controls were used, and the exposure levels differed by two orders of magnitude.

Although a statistically significant effect was noted at 12.5 ppm and 1,250 ppm for the incidence of late deaths in the original paper (Anderson et al., 1993), the response in late deaths at the higher exposure level was lower than at 12.5 ppm, probably because there were so many early deaths at the higher level. For the same reason, the incidence of congenital abnormalities was higher at 12.5 ppm than at 1,250 ppm. When early and late deaths were combined, a consistently increasing response with increasing exposure level was seen. When combined, the incidence in the controls was increased from 0 to 13 (4.68% of total implants), while in the 12.5 ppm group the incidence increased from 7 (2.29%) to 23 (7.52%).

Unfortunately, fetal weights were not reported in the prenatal portion of the dominant lethal study, and only total litter weights (which are confounded by the number of live pups) were reported in the postnatal portion of the study. When mean pup weight per litter was calculated, there was no difference among F_1 controls and treated offspring, and in some cases, a slight increase was seen (data not shown). This is interesting in light of the fact that treated F_1 male and female weights were increased above controls at 8 through 71 weeks of age. No modeling of these data was conducted.

Table 9-17 shows the results of modeling the dominant lethal data. The ECs and LECs for both 5% and 10% responses are shown. The log-linear model gave a good fit for all the data except for the number of implants in the prenatal study (see Figures 9-9 to 9-15; note that "dose" refers to 24-h adjusted exposure). This apparently was due to the fact that the number of implants was somewhat higher in the 12.5 ppm group than in controls or the 1,250 ppm group. Given that these data are from fetuses or pups within litters, it is likely that an EC_{05} and LEC_{05} can be estimated from the data with some degree of reliability. Also, based on the studies of Allen et al. (1994a and b), the LEC_{05} (BMC_{05}) for such endpoints was similar to the NOAEL on average. Although certain endpoints not modeled here (late fetal deaths and congenital malformations) were statistically increased in both the 12.5 ppm and 1,250 ppm exposure

Table 9-17. ECs and LECs for male-mediated developmental toxicity [a]

	Prenatal data			Postnatal data			
Estimate	No. implants	Early and late deaths	Live implants	No. implants	Post-implantation loss	Litter size at birth	Litter size at weaning
EC_{05}	0.21	3.4	3.5	0.12	3.2	0.1	0.1
LEC_{05}	0.12	2.4	2.4	0.08	2.2	0.07	0.07
EC_{10}	0.47	18	19	0.26	16	0.20	0.20
LEC_{10}	0.26	10	11	0.17	9.0	0.15	0.15
p-Value	0.12	0.66	0.99	0.95	0.99	0.54	0.45
NOAEL	220 ppm	2.2 ppm	2.2 ppm	220 ppm	2.2 ppm	2.2 ppm	2.2 ppm

[a]Exposures were adjusted to 24-h daily exposures (e.g., $12.5\left(\frac{6}{24}\right)\left(\frac{5}{7}\right) = 2.2$ ppm).

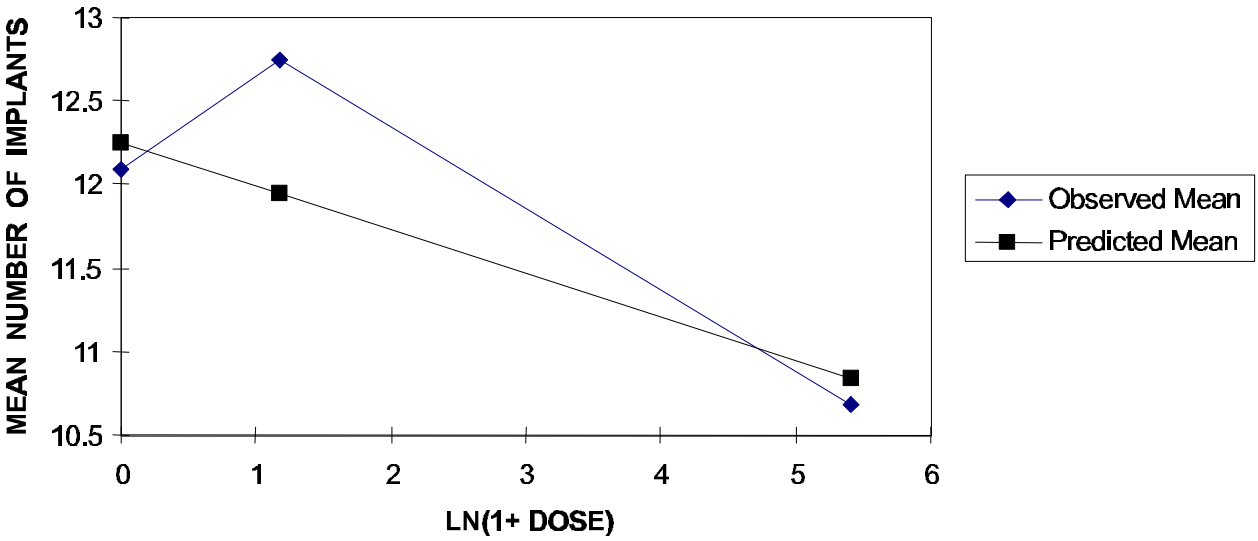
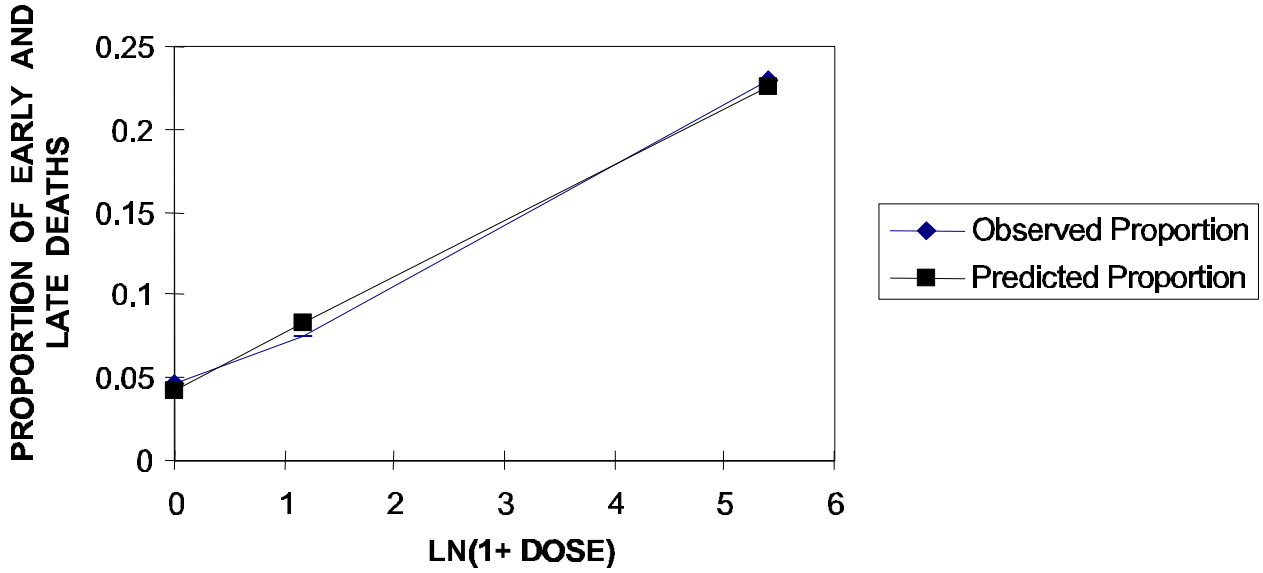


Figure 9-9. Observed versus predicted mean number of implants (prenatal) using log-linear model.

Figure 9-10. Observed versus predicted proportion of early and late deaths per implantation (prenatal) using log-linear model .



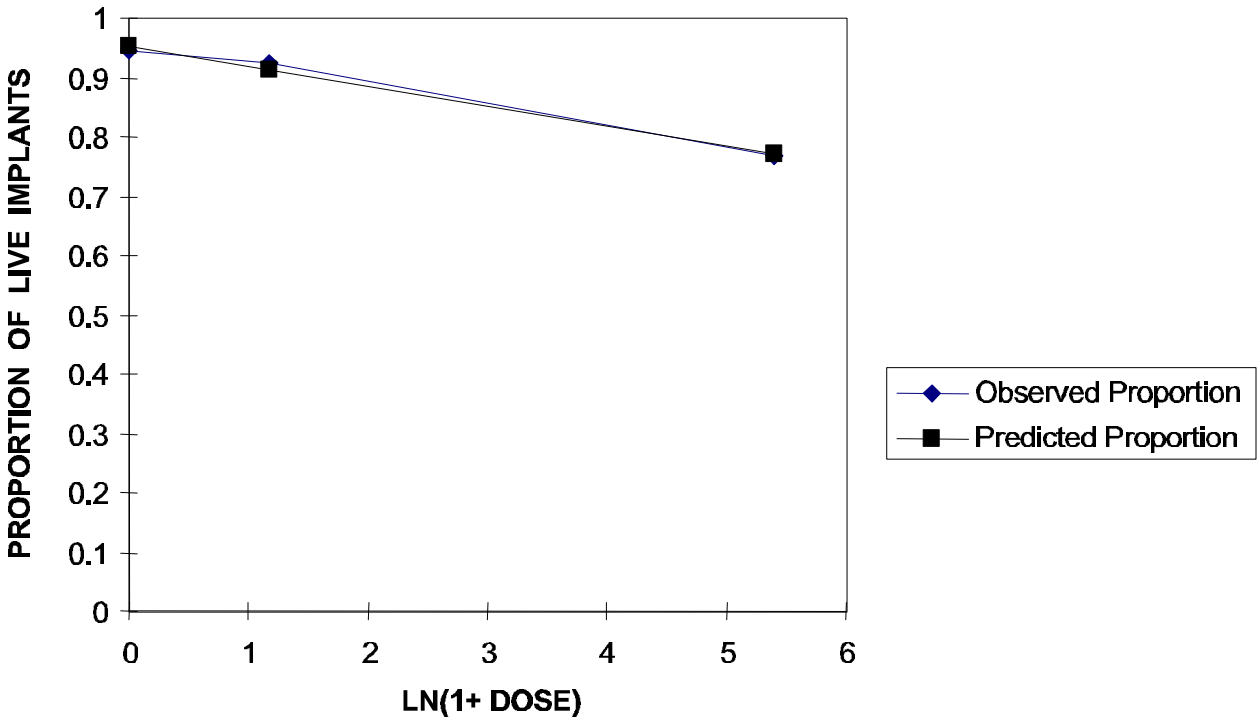
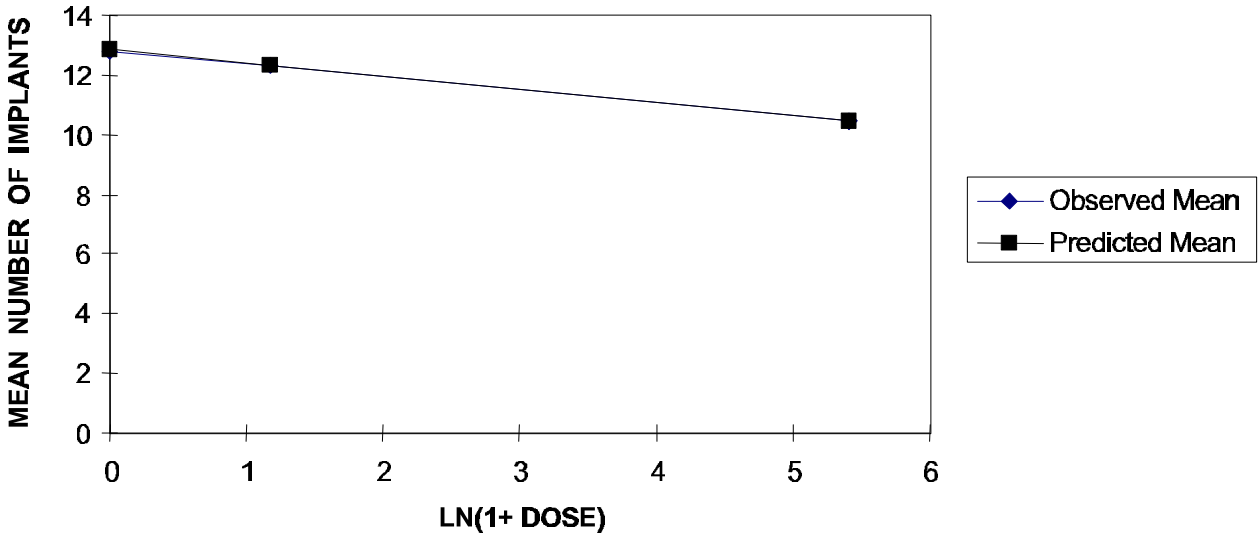


Figure 9-11. Observed versus predicted proportion of live implants (prenatal) using log-linear model.

Figure 9-12. Observed versus predicted mean number of implants (postnatal) using log-linear model.



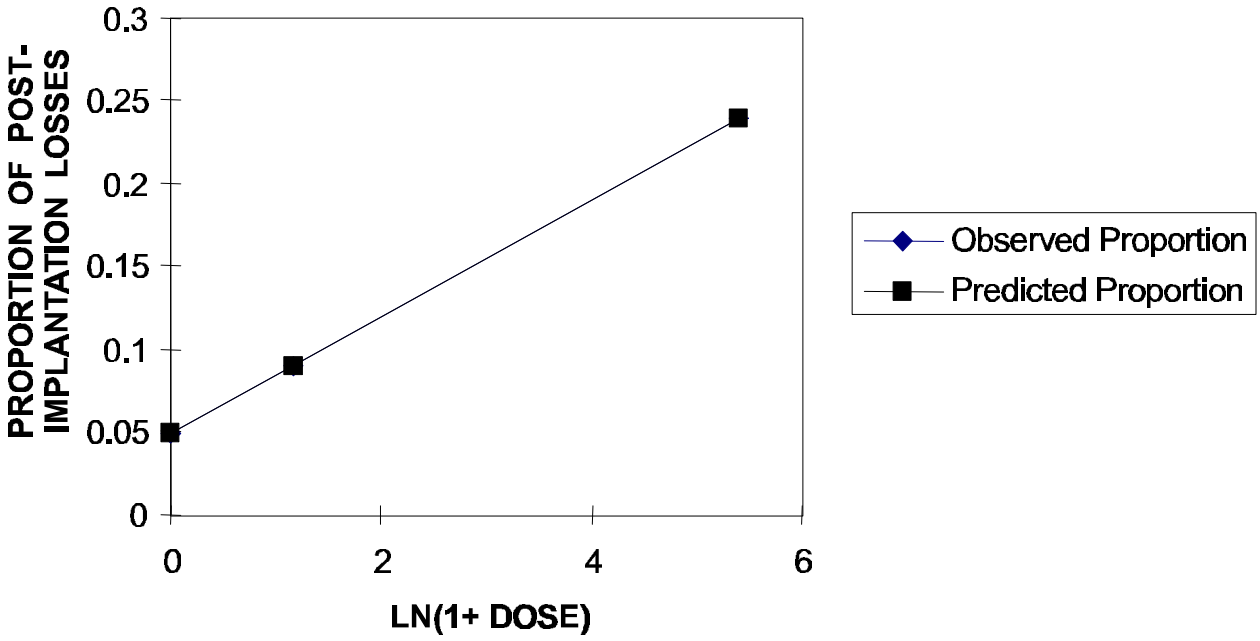
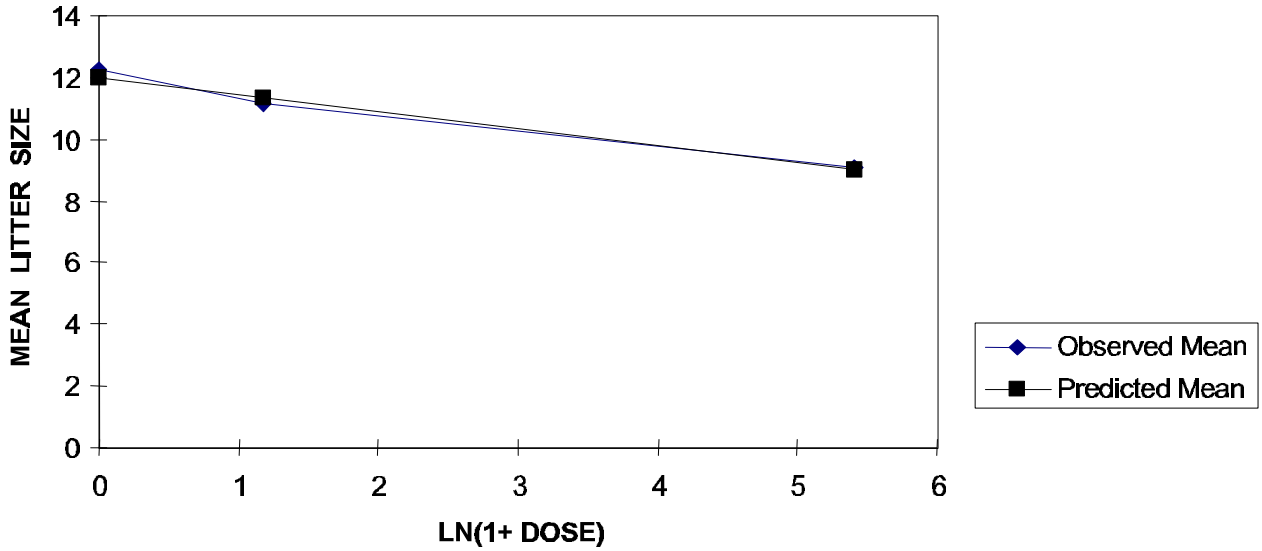


Figure 9-13. Observed versus predicted proportion of post-implantation losses (postnatal) using log-linear model.

Figure 9-14. Observed versus predicted mean litter size at birth using log-linear model.



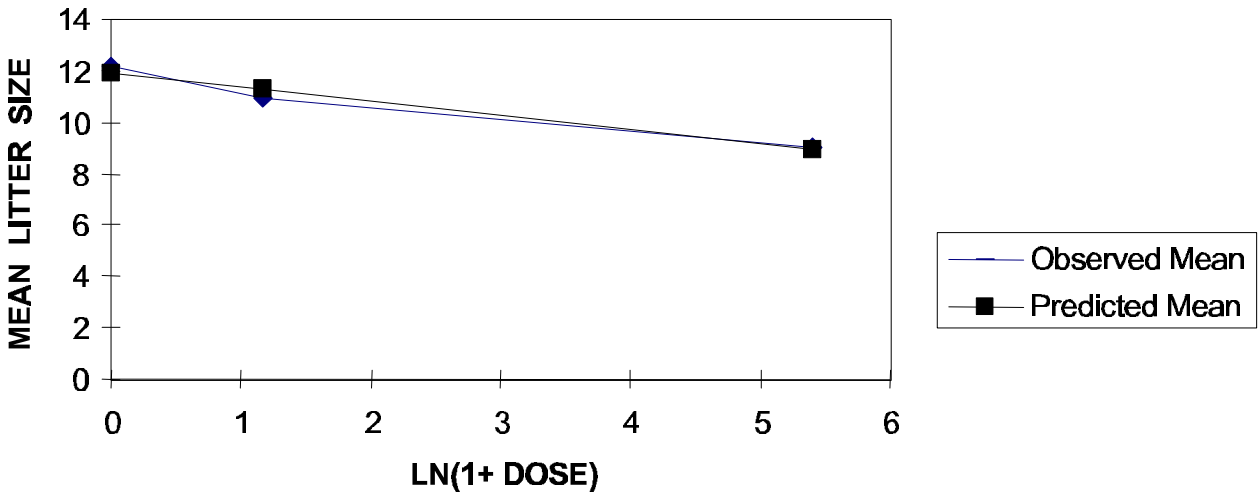


Figure 9-15. Observed versus predicted mean litter size at weaning using log-linear model.

groups, no other endpoints showed a statistically significant increase at 12.5 ppm by pairwise comparison. However, there was a trend toward an increase in the incidence of early and late fetal deaths and percent postimplantation loss, and a decrease in percent live implants and litter size at birth and at weaning in the 12.5 ppm exposure group. Given the overall effect seen on development in this study, the NOAEL for most endpoints was considered to be much closer to 12.5 ppm than to 1,250 ppm. Since litter size at birth and at weaning showed the lowest ECs and LECs, these endpoints will be used for calculation of an RfC.

9.3.4. Ovarian, Uterine, and Testicular Atrophy Modeling

The quantal Wiebull 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_2S 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. 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 9-15. 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 that are 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 9-18 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 9-16; 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.

Table 9-18. ECs and LECs for ovarian, uterine, and testicular atrophy using the quantal Weibull and log-logistic models [a]

Endpoint	Model	NOAEL/LOAEL	EC_{10}	LEC_{10}	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., $6.25 \left(\frac{6}{24}\right)\left(\frac{5}{7}\right) = 1.1$ ppm)
[b]Exposure levels included in the model.
[c]Extra risk. All other values are estimates of additional risk.

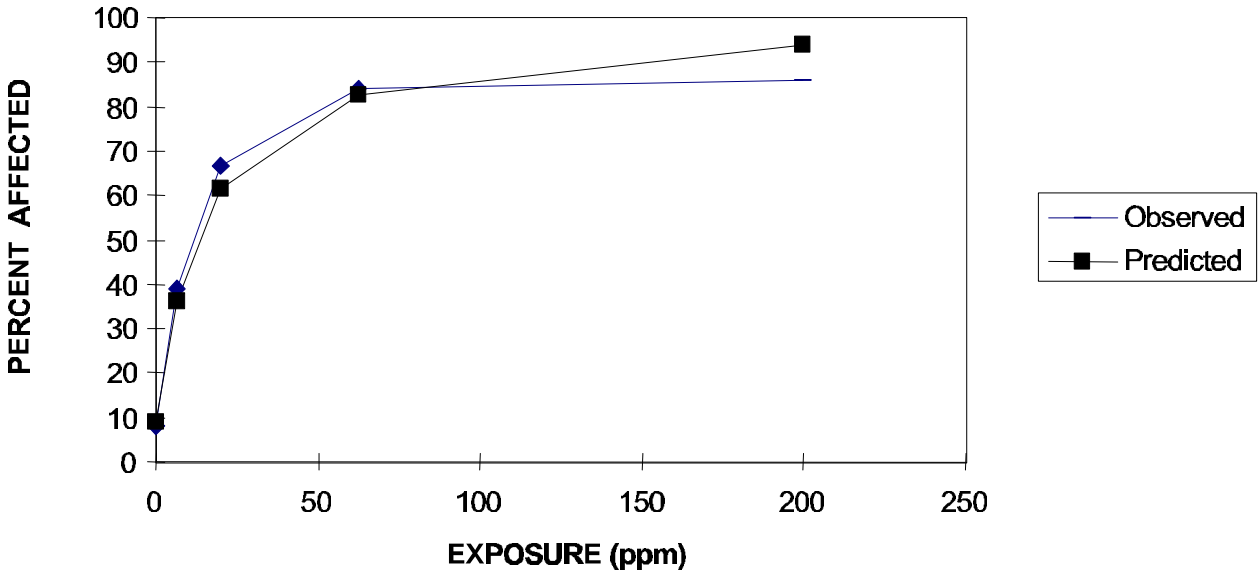


Figure 9-16. Ovarian atrophy (groups 1-5) using log-logistic model.

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 9-18 and Figures 9-17 and 9-18; 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 (Allen et al., 1994b) that, for quantal responses, the LEC_{10} is near or below the range of detectable responses. Also, the Proposed Guidelines for Carcinogen Risk Assessment (EPA, 1996) propose use of an LED_{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.

Although some 9- and 12-month interim sacrifice data were available for ovarian, uterine, and testicular atrophy (Table 9-15), 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. To account for the variability in time of death, time-to-response analyses were done using the multistage Weibull model as discussed in Section 9.2.2.2. Exposures were adjusted to the equivalent

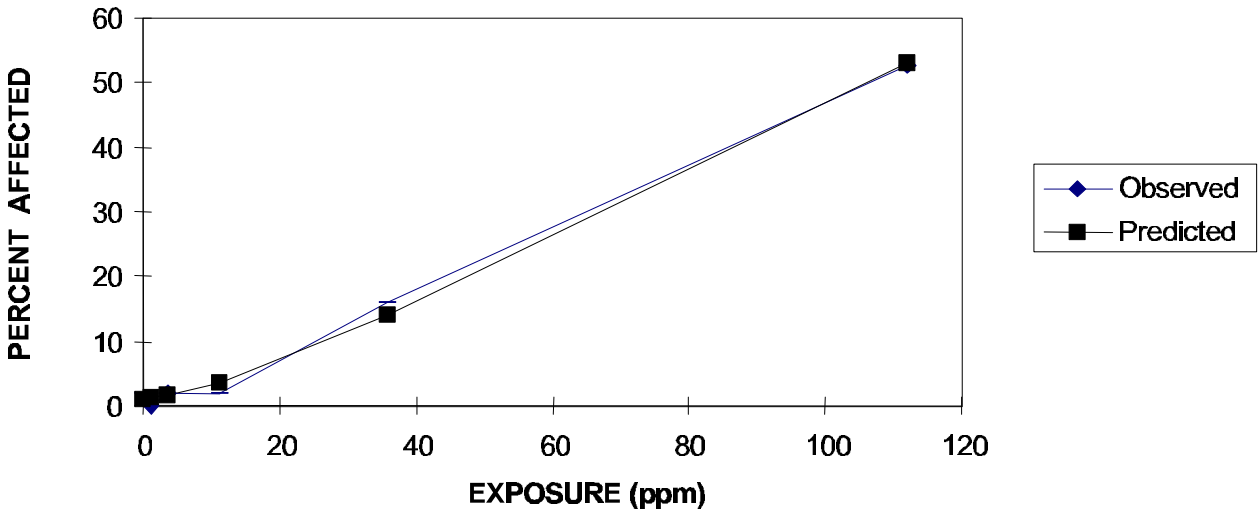
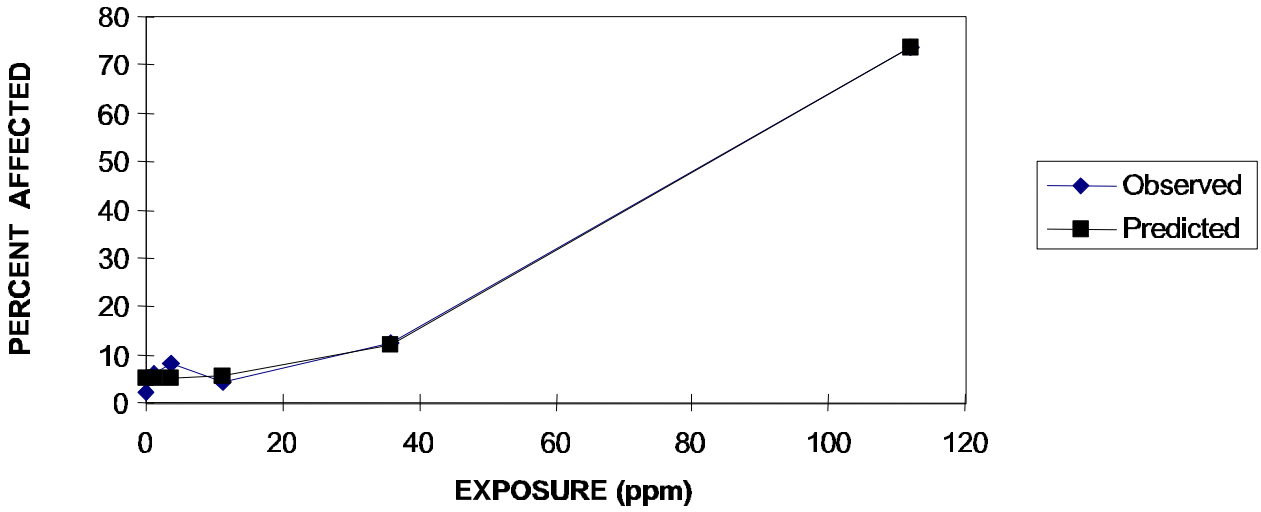


Figure 9-17. Uterine atrophy (groups 1-6) using quantal Weibull model.

Figure 9-18. Testicular atrophy (groups 1-6) using quantal Weibull model .



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 9-19.

Results of the Weibull time-to-response model are shown in Table 9-20. 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, while 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 an RfC.

The time-to-response model also allows for the calculation of risks at ages less than full lifetime. Thus, if one is concerned about ovarian or uterine atrophy primarily during a woman's reproductive years, one can calculate corresponding EC_{10}s and LEC_{10}s. Assuming reproductive capabilities until 45 years of age yields EC_{10} = 1.3 ppm and LEC_{10} = 1.1 ppm for ovarian atrophy (625 ppm dose group excluded) and EC_{10} = 31 ppm and LEC_{10} = 22 ppm for uterine atrophy (625 ppm group included).

Table 9-19. Parameters for Weibull time-to-response model used to model reproductive effects observed in mice based on ppm butadiene exposure [1]

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

[1]Each response was considered to be incidental with induction time, T0=0. See Section 9.2.2.2 on time-to-tumor modeling of the mouse carcinogenicity data for a discussion of the Weibull model structure and selection.

Table 9-20. 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	Based on ppm butadiene exposure	
		EC_{10}	LEC_{10}
Ovarian atrophy	no	0.497	0.382
	yes	0.473	0.369
Uterine atrophy	no	18.8	12.0
	yes	24.0	15.6
Testicular atrophy	no	44.3	15.9
	yes	6.54	5.39

9.3.5. 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)**C**fetal weight reduction
2. Subchronic exposure (10 weeks)**C**male-mediated developmental toxicity
3. Chronic exposure**C**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, except for two LECs calculated using the continuous power model, which were near this value. Since the hybrid modeling approach is considered the preferred method for modeling continuous data, the LEC_{10} of 13.7 ppm from this model will be used for calculating the reference concentration for developmental toxicity for short-term exposure (RfC_{DT}).

Results of the analysis for male-mediated developmental toxicity following 10 weeks of exposure gave ECs and LECs much lower than those from the 10-day exposures based on fetal weight. Therefore, the LEC_{10} for the dominant lethal study will be used to calculate an RfC for a subchronic exposure scenario.

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 5- to 6-fold below the LOAEL of 6.25 ppm. When the time-to-response model was applied to account for interim sacrifice data and early mortality, an LEC_{10} of 0.38 ppm (extra risk) was calculated, a value similar to that using the log-logistic model.

Ovarian atrophy has been shown to be related to the amount of the diepoxide metabolite in the tissue (Doerr et al., 1996). Uterine atrophy may be secondary to ovarian atrophy, and thus may also be related to the amount of diepoxide metabolite formation. Modeling of the ovarian atrophy and uterine atrophy data was considered based on internal dose of the diepoxide metabolite. However, an adequate pharmacokinetic model was not available to estimate levels of the diepoxide metabolite (Chapter 8).

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.

9.4. REFERENCE CONCENTRATIONS FOR REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

9.4.1. Introduction

As discussed in Chapter 5 and Section 9.3, a variety of reproductive and developmental effects have been observed in mice and rats exposed to 1,3-butadiene by inhalation. (There are no human reproductive or developmental data available for 1,3-butadiene.) In this section, sample reference concentrations (RfCs) are calculated for the most sensitive reproductive and developmental endpoints, i.e., 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 RfC will be based on the endpoint resulting in the lowest calculated RfC level. This RfC will be solely an RfC for reproductive and developmental effects (R/D RfC) and not a true RfC because other noncancer endpoints were not considered.

9.4.2. Calculation of RfCs

The most sensitive developmental effect was decreased fetal weight in the mouse. The most sensitive reproductive effects observed in subchronic exposure studies were decreased litter size at birth and at weaning in dominant lethal studies of mice (i.e., male mice are exposed to 1,3-butadiene and effects on litters are measured after mating to unexposed females). These effects are highly correlated and both yielded the same modeled effective dose results (Table 9-17). Litter size at birth reflects both decreased implants and increased fetal deaths, while litter size at weaning also reflects neonatal deaths. Dominant lethal effects in humans would likely be manifested as spontaneous abortions, miscarriages, stillbirths, or very early deaths. 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 9-21 summarizes the EC_{10} (i.e., the exposure concentration resulting in a 10% increase in risk based on modeling the exposure-response data in the observable range), the LEC_{10} (i.e., the 95% lower confidence limit on the exposure concentration estimated to result in a 10% 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 9-21 also provides sample calculations of RfCs using the NOAEL (or LOAEL) as well as the LEC_{10} as "points of departure." Uncertainty factors are then applied to the "point of departure" to calculate the RfC.

Typically, a factor of 10 is used for interspecies uncertainty when the "point of departure" is based on nonhuman data; however, when ppm equivalence across species is assumed as was done here, a factor of 3 is used instead. Thus, in Table 9-21, an interspecies uncertainty factor of 3 was used for all endpoints except ovarian atrophy. For ovarian atrophy, there is convincing evidence that the diepoxide metabolite (1,2:3,4-diepoxybutane, DEB) is required to elicit the effect and, while the differences cannot be quantified without an adequate physiologically based pharmacokinetic (PBPK) model, it is expected that humans produce lower concentrations of DEB than mice, based on differences in metabolic rates. Thus, an uncertainty factor of 1.5 was used for ovarian

atrophy to account for differences between mice and humans in the amount of DEB produced, yet allow that humans may be more sensitive to DEB.

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 9-21.

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

Table 9-21. Points of departure and RfC calculations for reproductive and developmental effects of 1,3-butadiene

Effect	NOAEL (or LOAEL) (ppm)	EC_{10} (ppm)	LEC_{10} (ppm)	Interspecies uncertainty factor	Intraspecies uncertainty factor	Acute/ subchronic-to-chronic uncertainty factor	LOAEL-to-NOAEL uncertainty factor	Risk reduction factor [a]	RfC based on NOAEL (ppm)	RfC based on LEC_{10} (ppm)
Decreased fetal weight		28	14	3	10	1[b]		3		0.14
	40 (LOAEL) (10 d, 6h/d, GD 6-15)			3	10	1[b]	10		0.13	
Decreased litter size at birth (or at weaning) (dominant lethal effect)		0.20[c]	0.15[c]	3	10	3		3		0.0005
	2.2 (LOAEL) (10 week, adjusted to 24 h/d)			3	10	3	10		0.002	
Ovarian atrophy		0.50[d]	0.38[d]	1.5[e]	10	1		3		0.008
	1.1 (LOAEL) (2 year, adjusted to 24 h/d)			1.5[e]	10	1	10		0.007	
Testicular atrophy		6.5[d]	5.4[d]	3	10	1		3		0.05
	36 (2 year, adjusted to 24 h/d)			3	10	1	1		1.2	

[a]To decrease risk to below what would be a detectable level, analogous to the LOAEL-to-NOAEL uncertainty factor.
[b]Although from acute study, only exposure during gestation is assumed to be relevant to fetal weight.
cAdjusted to 24-h daily exposure.
[d]Adjusted to chronic continuous exposure.
[e]There is strong evidence that ovarian atrophy is caused specifically by the metabolite 1,2:3,4-diepoxybutane, and humans are thought to produce less of this metabolite than mice, although their relative sensitivity to the metabolite is unknown (see text).

gestation are relevant. Although dominant lethal effects appear to occur with exposure during a specific time period of spermatogenesis (i.e., only certain stages of developing sperm appear susceptible), chronic exposure might result in continuous induction of these effects, so a factor of 3 was used.

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 typical "point of departure" corresponds to a 10% increased response level, which is explicitly not a no-effect level. In this risk assessment, a risk reduction factor of 3 was used to extrapolate to a level below which no detectable effects would be expected, analogous to the LOAEL-to-NOAEL uncertainty factor. Final guidance on this methodology is still being developed by EPA.

In addition to the sample RfCs presented in Table 9-21 for lifetime 1,3-butadiene exposure, two RfCs were calculated for subchronic exposure. An RfC_{DT} of 0.14 ppm for developmental toxicity from short-term exposures was calculated for decreased fetal weight, using the same factors depicted in Table 9-21. This RfC_{DT} is identical to the sample RfC calculated for decreased fetal weight because no subchronic-to-chronic uncertainty factor was used in that calculation. Finally, an RfC for subchronic exposure was calculated for the decreased litter size endpoints from the subchronic dominant lethal study. Using the LEC_{10} of 0.15 ppm and uncertainty factors of 3 for interspecies extrapolation, 10 for intraspecies variability, and 3 for risk reduction (analogous to the LOAEL-to-NOAEL uncertainty factor), as described above, yields an R/D RfC for subchronic exposure of 0.0015 ppm.

9.4.3. Discussion

The EC_{10}s in Table 9-21 suggest that the dominant lethal (male-mediated) effect is the most sensitive reproductive/developmental endpoint (i.e., the "critical" endpoint), and thus should be the basis for the final R/D RfC. The dominant lethal effect also yields the lowest sample RfC of 0.5 ppb. To arrive at the final R/D RfC, a further uncertainty factor of 3 is used to account for the lack of comprehensive reproductive testing, especially the absence of a multigenerational study. This final calculation yields an R/D RfC of 0.15 ppb.

There are substantial uncertainties in estimating low-exposure human risks for reproductive and developmental effects observed in animals exposed to high concentrations of an agent. It is generally believed that there is a nonlinear 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, so RfCs are calculated for noncancer effects rather than exposure-based risk estimates. The major uncertainties considered in deriving an RfC include the extrapolation of effects observed in animals to humans (interspecies extrapolation), the potential existence of sensitive human subpopulations resulting from human (intraspecies) variability, and various deficiencies in the database. These areas of uncertainty are addressed to some extent by the uncertainty factors. Other methodological uncertainties arise in the determination of the "point of departure" and in the selection of the relevant exposure metric for equating 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 the 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 failed to attain statistical significance. 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 LEC_{10} is being considered as the default "point of departure" to take into account statistical variability around the EC_{10} 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_{10}s, as shown for quantal models by Allen et al. (1994b).

As shown in Table 9-21, these two approaches yielded nearly identical RfCs for decreased fetal weight and for ovarian atrophy. For the dominant lethal effect of decreased litter size, the RfCs were similar, with that from the NOAEL/LOAEL approach four-fold higher than that from the benchmark concentration approach. For testicular atrophy, on the other hand, the NOAEL-based RfC is over 20 times higher than the LEC_{10}-based 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. This had the effect of increasing the effective percent affected in the

midrange of the exposure-response curve, which otherwise is fairly flat. This assessment advances the use of the benchmark dose/concentration approach.

Uncertainties also exist in the choice of exposure metric. Ideally, NOAEL or LOAELs and LEC_{10}s (or EC_{10}s) 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, the 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 time frame for exposure averaging is unclear. Typically, daily exposures resulting in nondevelopmental effects have been adjusted to an equivalent 24-h exposure, while exposures resulting in developmental effects have not been adjusted (U.S. EPA IRIS online database, 1997). Consistent with this approach, 1,3-butadiene exposures resulting in dominant lethal effects have been adjusted to a 24-h exposure, whereas exposure levels from fetal weight studies have not been adjusted. The exposure concentrations for these subchronic and acute effects have not been adjusted to reflect total duration of exposure because the critical time frames are unknown. Thus, for example, a 1-day exposure is treated equivalently to a 10-week exposure to the same daily level. Also, for developmental effects, a 4-h exposure to 50 ppm would be treated equivalently to an 8-h exposure to 50 ppm.

Finally, there are uncertainties in the uncertainty factors used to derive the RfC from the "point of departure." These factors are largely arbitrary. In particular, the shape of the exposure-response curve below the observable range is unknown, and it is uncertain that the NOAEL or the LOAEL/10 or the $LEC_{10}/3$ actually represent no-effect levels, independent of the application of the interspecies and intraspecies uncertainty factors.

9.4.4. Conclusions

In conclusion, an R/D RfC of 0.15 ppb was calculated for the critical endpoint of the dominant lethal effect of decreased litter size at birth (or at weaning), 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 reproductive or developmental effects during a lifetime.

In addition, an RfC_{DT} of 0.14 ppm for developmental toxicity from short-term exposures was calculated based on fetal weight data for mice, and an R/D RfC for subchronic exposure of 0.0015 ppm was obtained based on the dominant lethal results in mice. Each of these RfCs was calculated using benchmark concentration methodology.

9.5. CONCLUSIONS ON QUANTITATIVE RISK ESTIMATES

In this chapter, a lifetime extra unit cancer risk (MLE) of 9×10^{-3} 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. Using this cancer potency estimate, the chronic exposure level resulting in an increased cancer risk of 10^{-6} can be estimated as follows: $(10^{-6})/(9 \times 10^{-3}/\text{ppm}) = 1 \times 10^{-4}$ ppm = 0.1 ppb. The 95% UCL on the unit cancer risk was 2×10^{-2} per ppm.

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 epidemiological 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 doses and reference concentrations were calculated for an assortment of reproductive and developmental effects observed in mice exposed to 1,3-butadiene. An R/D RfC of 0.15 ppb was obtained for the critical effect of decreased litter size at birth (or at weaning) observed in dominant lethal studies of mice, using a benchmark concentration approach to obtain the "point of departure." This R/D RfC is presumed to be a chronic exposure level without "appreciable risk" of reproductive or developmental effects. Although other noncancer effects were not examined, the reproductive endpoints were quite sensitive, and it is likely that the R/D RfC is protective against other noncancer effects as well.

Finally, an RfC_{DT} of 0.1 ppm for developmental toxicity from short-term exposures was calculated from mouse fetal weight data, and an R/D RfC for subchronic exposures of 0.0015 ppm was derived from the dominant lethal results in mice, each using benchmark concentration methodology.

10. WEIGHT OF EVIDENCE

10.1. EVALUATION

1,3-Butadiene is a colorless, odorless chemical that exists in ambient air in gaseous form. This extremely volatile chemical is very slightly soluble in water and is not found in soil and food. Thus, exposure to 1,3-butadiene is mainly via inhalation. Increased mortality from leukemias and lymphomas was observed among male workers occupationally exposed to 1,3-butadiene in polymer and monomer production, respectively. No information is available in females. The data from one Canadian and seven U.S. polymer production plants show that exposure to 1,3-butadiene is causally associated with occurrence of leukemias (cell type is not known at this time).

Two lifetime inhalation studies in mice and one lifetime inhalation study in rats found occurrence of malignant tumors in multiple sites in both mice and rats. Increased occurrence of lymphomas in a 1-year inhalation study in Swiss mice indicated that the presence of retrovirus was not an essential factor for the development of 1,3-butadiene-induced lymphomas.

Once inhaled, 1,3-butadiene is distributed throughout the body. The relative distribution of 1,3-butadiene in different organs is unknown at this time. 1,3-butadiene is metabolized by oxidation to a monoepoxide, diepoxide, and epoxy diol. Which metabolite(s) is responsible for the causation of cancer is still uncertain. Differences in measured concentration levels of these metabolites in mice and rats do not provide an explanation for the differences observed in malignancies in these two species. All three of these metabolites have been shown to be mutagenic in vivo and in vitro.

10.2. CONCLUSION

Based on the overall evidence from human, animal, and mutagenicity studies, 1,3-butadiene is concluded to be a known human carcinogen.

11. RISK CHARACTERIZATION

11.1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) first published a health assessment of 1,3-butadiene in 1985. The 1985 assessment concluded that 1,3-butadiene was a possible 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 effort was to review the new information and determine if any changes were needed to the earlier conclusions.

This reassessment is intended to serve as a source document for risk assessors inside and outside the Agency. Its development, however, was prompted primarily by a request from EPA's Office of Mobile Sources (OMS) to support decision making regarding the Air Toxic Rule's Section 202 (l) (2) of the Clean Air Act Amendment. The scope of the document has been limited to address only the health effects specifically requested by OMS: carcinogenicity, mutagenicity, and reproductive/developmental toxicity. Similarly, a detailed exposure assessment was not requested and not conducted. For background purposes, however, some exposure information has been included.

The major findings of this report are as follows. First, sufficient evidence exists to consider 1,3-butadiene a known human carcinogen. The evidence for this includes findings in epidemiologic studies as well as clear evidence that 1,3-butadiene is an animal carcinogen and is metabolized into genotoxic metabolites by experimental animals and humans.

Second, based on linear modeling of human data, the best estimate of lifetime extra cancer risk from continuous 1,3-butadiene exposure is about 9×10^{-3}/ppm, or 9×10^{-6}/ppb. In other words, it is estimated that 9 persons in 1 million exposed to 1 ppb 1,3-butadiene continuously for their lifetimes would develop cancer as a result of their exposure. Lower cumulative exposures are expected to result in risks that are proportionately lower.

Third, although there are no human data on reproductive or developmental effects, a variety of such effects have been observed in mice and rats exposed to 1,3-butadiene. A reproductive/developmental reference concentration (RfC) of 0.05 ppb was calculated based on the critical reproductive effect of reduced litter sizes, reflecting increased prenatal mortality, observed among the offspring of male mice exposed to 1,3-butadiene.

Fourth, there are insufficient data to determine if children or other special subpopulations are differentially affected by exposure to 1,3-butadiene. Heavy smokers are likely to be more heavily exposed than the general population.

This chapter will briefly summarize and integrate the critical data and analyses on which these findings are based and discuss the strengths and weaknesses of those data and the resulting confidence in the findings. 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.

11.2. EXPOSURE OVERVIEW

Approximately 3 billion pounds of 1,3-butadiene are produced annually in the United States. 1,3-Butadiene is used primarily in the manufacture of styrene-butadiene rubber, plastics, and thermoplastic resins. Environmental releases occur from process vents during these operations. 1,3-Butadiene is not a component of gasoline or diesel fuel, but is formed as a by-product of incomplete combustion. Mobile sources, including both on-road and nonroad engines, are estimated to account for 79% of all 1,3-butadiene emissions (EPA, 1992). 1,3-Butadiene emissions from vehicles are reduced by catalytic converters; total emissions may decline as older cars without converters are removed from service.

The compound is highly volatile and slightly soluble in water. Thus, environmental releases result primarily in emissions to the atmosphere. In the atmosphere, 1,3-butadiene undergoes rapid destruction by photoinitiated reactions, and 50% of it is removed in approximately 6 hours (U.S. DHHS, 1992). Although it is degraded rapidly in the atmosphere, 1,3-butadiene is almost always present at low concentrations in urban and suburban areas. Because of this, the general population is exposed to some levels via inhalation. 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) (Lofroth et al., 1989; Brunnemann et al., 1990).

11.3. CANCER HAZARD ASSESSMENT

11.3.1. Human Evidence

Sufficient evidence exists to consider 1,3-butadiene a known human carcinogen.

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 supportive of a causal relationship between an exposure and a health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually proves 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, it provides credible evidence for causality. The following discussion addresses the strengths and limitations of the epidemiologic studies of workers occupationally exposed to 1,3-butadiene, from which the human evidence is derived, and then summarizes how adequately the causality criteria apply.

The conclusion of "sufficient evidence" of human carcinogenicity is based on more than 10 epidemiologic studies examining five different groups of workers. These studies are summarized in Table 11-1.

The strongest evidence comes from the follow-up study of a cohort of 15,000 synthetic rubber workers (UAB cohort) conducted by Delzell et al. (1996) and Macaluso et al. (1996) and reported in two components. The cohort was derived from seven U.S. plants and one Canadian plant. The follow-up was from 1943 to 1994. Investigators estimated the exposures to 1,3-butadiene, styrene, and benzene for each worker (Macaluso et al., 1996). Quantitative exposures were calculated and limited validation of exposure estimates were attempted by various means. Cumulative and peak exposures were calculated for each worker. Comparison with the U.S. population resulted in significant excesses for leukemia in ever-hourly workers (43% higher than general population) and its subcohort of blacks (127%) (Delzell et al., 1996). Significant excesses were also found in the ever-hourly subcohort for year of death (87% for 1985+), year of hire (100% for 1950-59), age at death (79% for <55 years), and for more than 10 years employment and more than 20 years since hire (92% for whites and 336% for blacks). Laboratory workers, maintenance workers, and polymerization workers also showed higher risks of 331%, 165%, and 151%, respectively. All these analyses were conducted adjusting for styrene and benzene. When internal comparison was carried out using the estimated ppm-years
exposure data, risk ratios increased with increasing exposures. These findings demonstrate specificity and strength of association. A fairly consistent association between exposure to butadiene and occurrence of leukemia across the plants was also found. Furthermore, the trend test for increasing risk of leukemia with increasing exposure to 1,3-butadiene was statistically significant (dose response).

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

Table 11-1. Summary of 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 each worker	Excess mortality due to leukemia; 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, and 1993 Santos-Burgoa et al., 1992	Cohort studies using qualitative exposures; case-control study using estimated quantitative exposures for each case and control	Excess mortality due to lumpho- hematopoietic cancers; 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 studies using qualitative exposures, last study made quantitative exposure estimates	Excess mortality due to lymphosarcoma in prewar workers
3 U.S. monomer production plants (Union Carbide cohort)	364, 1940-1990	Ward et al., 1995 and 1996a	Cohort study using qualitative exposures	Excess mortality due to lymphosarcoma in World War II workers
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 mortality or morbidity

[a]Six U.S. plants and one Canadian plant were common in Johns Hopkins University (JHU) and University of Alabama, Birmingham (UAB) studies.

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 misclassification 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* to health effects evaluation. Second, the excess mortality observed for leukemia was based on death certificates and was not verified by medical records. This may have resulted in some misclassification of leukemias. Third, histologic typing of leukemia was also not available. Thus, currently it is not known whether a single cell type or more than one cell type is associated with the exposure to 1,3-butadiene.

A large cohort of synthetic rubber workers (JHU cohort)[1], assembled from one Canadian and seven U.S. plants, was also studied by Matanoski and Schwartz (1987) and then followed up by Matanoski et al. (1989, 1990). The follow-up included a nested case-control study (Santos-Burgoa et al., 1992). Approximately 13,500 individuals were followed from 1943 to 1985. A significant excess of lymphohematopoietic cancer was observed in the cohort study. The nested case-control study from this cohort, comprising 59 cases of lymphohematopoietic cancers and 193 matched controls, found significantly increased relative odds for leukemia. Increases of 7 times in the high-exposure group and of 4 times in the low-exposure group were observed in the ever/never exposed analysis, of 9 times in the matched analysis, and of 8 times in the conditional analysis (specificity and strength of association). Exposures to 1,3-butadiene and styrene were estimated for each case and control using job records and levels of exposures to 1,3-butadiene and styrene associated with those jobs, independently of the case or control status. A significant trend of increasing risk of leukemia with increasing exposure level of 1,3-butadiene was also observed (dose response).

The findings of excess leukemia risk in the nested case-control study were questioned by Acquavella (1989) and Cole et al. (1993), as these findings were inconsistent with the absence of excess leukemia risk in the base cohort study. Thus, Matanoski et al. (1993) reevaluated the original nested case-control study by choosing a new set of three controls per case. The investigators also verified the cause of death by obtaining the hospital records (25 out of 26 were correctly recorded on the death certificates). The findings of the new analysis were similar to those of the earlier analysis. Although the controversy about the cohort and case-control study is still not resolved, the nested case-control study demonstrates a strong association between exposure to 1,3-butadiene and occurrence of leukemias.

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[1] One Canadian plant and six U.S. plants were common in the JHU and UAB studies.

The main strengths of the JHU cohort study are as follows. First, this was the first large cohort study of polymer production workers. Second, adjustments for confounding exposures were conducted. Third, analyses by duration of employment and for latency were carried out. Fourth, the nested case-control study was well conducted and well analyzed, with quantitative estimation of exposures for each case and control as well as verification of leukemia.

Limitations of the JHU cohort study included the exclusion of more than 50% of the population because of the lack of work histories, work start date, and exposure data. In addition, 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, the estimated exposures were crude and not substantiated by air monitoring data. Exposure misclassification may have occurred based on the estimated exposures 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.

Three different cohorts of monomer production workers were studied. The largest cohort of approximately 2,800 workers in a Texaco plant followed from 1943 to 1994 by several investigators (Downs et al., 1987; Divine, 1990; Divine et al., 1993; Divine and Hartman, 1996). All the investigations essentially found lower than expected mortality from all causes and total cancers as compared to the general population. The only significant excess mortality observed was for lymphosarcoma in the prewar subcohort of workers who had worked for less than 10 years and had a latency of 0-9 years; 154% to 169% higher than the general population. Even though exposures were estimated in the last follow-up, no information about exposure levels was available for the prewar period; however, it is believed that exposures were high.

The major strengths of this study are, first, it is the largest 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.

The main limitation was lack of exposure information in the earlier follow-ups. Furthermore, although the investigators estimated the exposures for each individual in their last follow-up, no information was available on work histories or levels of 1,3-butadiene exposure during the prewar period, which made exposure estimation in the prewar workers impossible.

A small cohort of 364 individuals who had potential exposure to 1,3-butadiene at three Union Carbide plants during World War II was studied by Ward et al. (1995, 1996a). This investigation also found a statistically significant excess for lymphosarcoma by 477%, which was based on four cases (specificity and strength of association). The observation of excess lymphosarcoma was consistent with the finding in the Texaco cohort study. The main limitations

of this study are that the cohort was small and that exposures were assumed based on job categories. 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. This study failed to show any increased mortality or morbidity. Due to several methodologic limitations such as lack of exposure information, short follow-up, and lack of information on confounders, this study failed to provide any negative evidence toward the causal association between exposure to 1,3-butadiene monomer and occurrence of lymphosarcoma that was observed in the other two studies.

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). This is a methodologic concern, given the fact that lymphohematopoietic cancer recording on death certificates is unreliable (Percy et al., 1981).

Based on these monomer and polymer production workers' cohorts, it is obvious that an increased number of lymphohematopoietic cancers is observed in these populations. A clear difference is becoming apparent, though. Increased lymphosarcomas develop in monomer workers, whereas excess leukemias occur in polymer workers. Furthermore, the lymphosarcomas observed in the monomer workers were among wartime workers, who were probably exposed to higher levels of 1,3-butadiene for shorter periods of time and not in long-term workers with low levels of exposure. A similar observation comes from the stop-exposure studies conducted by Melnick et al. (1990c). They observed that for a given total exposure, the incidence of lymphoma was greater among mice exposed to higher concentrations of butadiene for a shorter period of time (625 ppm for 26 weeks) than among mice exposed to a lower concentration for a longer period of time (312 ppm for 52 weeks). Consequently, this suggests that it may be the concentration of 1,3-butadiene rather than the duration of exposure that is important in the occurrence of lymphomas. There is a null relationship between exposure to 1,3-butadiene monomer and occurrence of leukemias, which are observed in polymer workers. This may be due to the exposure patterns for 1,3-butadiene in monomer production workers or to the absence of exposure to a necessary co/modifying factor or a confounding factor that occurs in polymer production workers. Data are currently lacking to confirm or refute any of these possibilities. The findings of the UAB study, which investigated styrene and benzene exposures as well, suggest that the observed associations of leukemia with 1,3-butadiene exposure are not due to

confounding by exposure to other chemicals. The findings of excess leukemias in polymer production workers are consistent with a causal association with exposure to 1,3-butadiene.

Table 11-2 shows the application of the causality criteria to the studies discussed above.

As these criteria are well satisfied, it is concluded that there is sufficient evidence to consider 1,3-butadiene a known human carcinogen.

11.3.2. Animal Data

***1,3-Butadiene is an animal carcinogen*.**

Chronic bioassay studies provide unequivocal evidence that 1,3-butadiene is a multisite carcinogen in both rats and mice. 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 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).

Table 11-2. Epidemiologic causality criteria

Criteria	Monomer plant workers	Polymer plant workers
Temporality: exposure occurred prior to effect	Yes	Yes
Specificity of cancer	Lymphosarcoma	Leukemia (specific cell type[s] not known at this time)
Strength of association	154% to 477% higher mortality from lymphosarcoma than general population	7 to 9 times higher relative odds for leukemia (nested case-control study)[a]; 151% to 331% higher mortality from leukemia than general population
Consistency	2 of 3 studies agree	Fairly consistent across the plants
Dose-response relationship	Cannot be demonstrated due to lack of quantitative exposure data	Yes
Biological plausibility	Yes	Yes

[a] Relative odds is the ratio of the frequency of exposure to 1,3-butadiene in cases to the frequency of exposure to 1,3-butadiene in controls, where both the cases and controls are from the same occupational cohort.

11.3.3. Other Supportive Data

1,3-Butadiene is metabolized into 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). The enzymes responsible for the metabolic activation of 1,3-butadiene to these epoxide metabolites exist in humans as well as 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 3,4-epoxy-1,2-butanediol has been observed in vitro using tissues from mice, rats, and humans. Activation rates for 1,3-butadiene are typically higher in the mouse than in the rat, reflected by higher tissue concentrations of EB and DEB in the mouse versus the rat. Activation rates in humans exhibit a high degree of variability and appear to span the range between mice and rats.

Among the genotoxic effects of 1,3-butadiene is an N^7-alkylguanine adduct that has been observed in the liver DNA of exposed mice and in the urine of an exposed worker. Similarly, increased frequencies of *hprt* mutations have been observed in the lymphocytes of mice and rats exposed to 1,3-butadiene and in lymphocytes of occupationally exposed workers. Even though these mutations may not be directly related to tumor development, they provide in vivo evidence of similarities in the disposition and genotoxic action of 1,3-butadiene between mice and humans.

11.3.4. Cancer Characterization

1,3-Butadiene is a known human carcinogen.

This characterization is supported by the three findings discussed above: (1) epidemiologic studies showing increased leukemias 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 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986), 1,3-butadiene would be classified as a "Group A"—Human Carcinogen. It is characterized as a "Known Human Carcinogen" according to EPA's 1996 *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996).

11.4. QUANTITATIVE RISK ESTIMATION FOR CANCER

Lifetime extra cancer risk is estimated to be about 9×10^{-3} per ppm continuous 1,3-butadiene exposure, based on human data.

The Delzell et al. (1995) retrospective cohort study of more than 15,000 male styrene-butadiene rubber production workers provides high-quality epidemiologic data for estimating the human cancer risk from 1,3-butadiene exposure. In the Delzell et al. 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. 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 reported by Delzell et al. was used to calculate a maximum likelihood estimate (MLE) of 8.7×10^{-3}/ppm (or 9×10^{-3}/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 0.02/ppm. There were insufficient exposure-response data to calculate a lymphoma risk estimate from the monomer cohorts.

Alternatively, interpreting the proposed new carcinogen risk assessment guidelines (U.S. EPA, 1996), linear extrapolation from the LEC_{01} or the EC_{01} (i.e., the 95% lower confidence limit or MLE, respectively, 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 epidemiology data. The models presented by Delzell et al. yield LEC_{01} and EC_{01} values ranging from 0.066 to 0.64 ppm and from 0.45 to 1.16 ppm, respectively. The corresponding cancer potency estimates range from 0.016/ppm to 0.15/ppm (based on the LEC_{01}) and from 8.7×10^{-3}/ppm to 0.022/ppm (based on the EC_{01}). The square root model provided the best fit to the data and was chosen by Delzell et al. for further refinements. Thus, their final square root model might be the appropriate model to select for determination of the ultimate "point of departure" for linear extrapolation. Based on this model, a cancer potency estimate of 0.08/ppm is obtained from the LEC_{01} of 0.12 ppm, and a potency estimate of 0.02/ppm is obtained from the EC_{01} of 0.45 ppm. These unit risk estimates are roughly two- and fourfold higher, respectively, than the MLE and upper bound estimates calculated using the linear model described above.

For comparison purposes, human unit cancer risk estimates based on extrapolation from the results of lifetime animal inhalation studies are summarized in Table 11-3. These potency estimates are 95% upper confidence limits on unit 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. Table 11-3 also includes unit risk estimates based only on the

Table 11-3. 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; linearized multistage model.
[b] Based on 1993 NTP study; Weibull multistage time-to-tumor model.

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.

In both rodent species, females are apparently more sensitive than males, as evidenced by the higher risk estimates. The "best estimate" (i.e., MLE from the linear model) of 8.7×10^{-3}/ppm for extra cancer risk from the human (male) leukemia data exceeds the upper bound estimates based on the male rat data and on the male mouse data for lymphocytic lymphomas, and is 25 times lower than the upper bound estimate based on all male mouse tumors.

Human health risk estimates based on extrapolation from high-quality epidemiologic results are preferable to those based on rodent data because they avoid the uncertainties inherent in extrapolating across species and, typically, the human exposures in epidemiologic studies are closer to anticipated environmental exposures than the high exposures used in animal studies, thus reducing the extent of low-dose extrapolation. In the case of 1,3-butadiene, while the rat exposures were far in excess of human exposures, the lowest EXPOSURE in the 1993 NTP mouse study (4.7 ppm, 8 h 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 either 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.

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 for low-dose extrapolation. Although Delzell et al. expressed preference for the square root model based on its goodness of fit, the four exposure-response models that they investigated were virtually indistinguishable on statistical grounds, and because the specific mechanisms of 1,3-butadiene carcinogenesis are unknown, there is no biological basis for choosing one model over another. Even though the models give similar results in the observable range, they deviate substantially at lower exposures. For example, at a lifetime continuous exposure of 1 ppb, the preferred model of Delzell et al. yields a cancer potency estimate almost two orders of magnitude higher than that obtained by the linear model. However, there was no apparent biological reason to depart from a default assumption of linearity, so the linear model was used in this risk assessment.

Fourth, it is uncertain which potential modifying or confounding factors should be included in the model. The linear model of Delzell et al., which was used in this risk assessment, adjusted for age, calendar year, years since hire, race, and exposure to styrene. However, these investigators dropped styrene and race from their preferred square root model to obtain their final model. Furthermore, there may be other relevant factors that weren't included in the models at all.

Fifth, there are uncertainties in the parameter estimates used in the models. The study of Delzell et al. 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.

In addition, there are important concerns raised by comparison with the rodent data. First, the rodent studies suggest that 1,3-butadiene is a multi-site carcinogen. 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. Delzell et al. did observe a slight increase in lung cancer among maintenance workers. The reported excess cancer risk estimate, 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.

Despite these uncertainties, confidence in the excess cancer risk estimate of 9 × 10^{-3}/ppm is relatively high. First, the estimate is based 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 9×10^{-3}/ppm, the chronic (70 year) exposure level resulting in an increased cancer risk of 10^{-6} (i.e., one in a million) can be estimated as follows: $(10^{-6})/(9 \times 10^{-3}/\text{ppm}) = 1 \times 10^{-4}\text{ppm} = 0.1$ ppb.

11.5. SUMMARY OF REPRODUCTIVE/DEVELOPMENTAL EFFECTS

A variety of reproductive and developmental effects have been observed in mice and rats exposed to 1,3-butadiene by inhalation. There are no human data on reproductive or developmental effects.

The most sensitive 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 no NOAEL for this effect. Generally, however, it is thought that there is an exposure threshold, and while effects on fetal growth in humans cannot be ruled out, they are not expected to occur from low environmental exposures to 1,3-butadiene. No developmental toxicity was observed in rats.

The most sensitive reproductive endpoints observed in subchronic exposure studies were litter size at birth and at weaning in dominant lethal studies of mice (i.e., male mice are exposed to 1,3-butadiene and effects on litters are measured after mating to unexposed females). Litter size at birth reflects both decreased implants and increased fetal deaths, while litter size at weaning also reflects neonatal deaths. Dominant lethal effects in humans would likely be manifested as spontaneous abortions, miscarriages, stillbirths, or very early deaths. The dominant lethal responses are believed to represent a genotoxic effect; however, a large number of sperm would have to be affected to result in any meaningful increase in risk, because the chances of any single sperm both having a critical mutation and fertilizing an egg are minuscule. Thus, dominant lethal effects are not expected in humans exposed to low environmental exposures, although the possibility of such effects or of transmissible genetic mutations cannot be ruled out.

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 and likely has an exposure threshold. 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), although an exposure threshold is assumed for this endpoint as well. Uterine atrophy was also observed in the highest exposure groups: however, this is thought 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. It is further expected, based on metabolic data, that humans would produce lower concentrations of this metabolite than do mice. Thus, it is likely that humans are less sensitive to 1,3-butadiene-induced ovarian atrophy than are mice. No reproductive effects were reported in the 2-year rat study. In conclusion, ovarian atrophy is not expected in humans from environmental exposures to 1,3-butadiene; although, the effect cannot be ruled out.

11.6. QUANTITATIVE ESTIMATION (RfC) FOR REPRODUCTIVE/ DEVELOPMENTAL EFFECTS

An RfC for reproductive and developmental effects of 0.15 ppb was obtained for the critical effect of decreased litter size at birth (or at weaning), based on subchronic dominant lethal studies in the mouse.

A reference concentration (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," i.e., 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}. In this assessment, the RfC is only for reproductive and developmental effects (R/D RfC), because other noncancer effects were not considered. Of the 1,3-butadiene reproductive/developmental effects, the critical effect was decreased litter size at birth or at weaning (both of these effects yielded the same EC_{10}), as observed in dominant lethal studies of male mice. An R/D RfC was calculated based on the LEC_{10}, which was calculated using benchmark concentration methodology, and uncertainty factors for interspecies extrapolation (3), intraspecies variability (10), extrapolation from subchronic study to chronic exposure (3), the absence of multigenerational studies (3), and "risk reduction" to extrapolate to a level at which no detectable effects are expected (analogous to the LOAEL-to-NOAEL uncertainty factor) (3). The resulting R/D RfC is 0.15 ppb [0.15 ppm/(3×10×3×3×3)]. The actual risks at low exposure levels are unknown; the R/D RfC merely provides a bound on chronic exposure below which no "appreciable risk" of reproductive or developmental effects is expected.

Although other noncancer effects were not examined, the reproductive endpoints were quite sensitive, and it is likely that the R/D RfC is protective against other noncancer effects as well.

In addition, a RfC_{DT} of 0.1 ppm for developmental toxicity from short-term exposures was calculated from the mouse fetal weight data, and a R/D RfC for subchronic exposures of 0.0015 ppm was derived from the dominant lethal results in mice, each using benchmark concentration methodology to obtain the "point of departure" for applying uncertainty factors.

11.7. SPECIAL SUBPOPULATIONS

11.7.1. Sensitive Subpopulations

It is uncertain whether children or other subpopulations have greater susceptibility to exposure to 1,3-butadiene than the general population.

There is no information available on health effects 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.

Tobacco smoke contains 1,3-butadiene as well as other carcinogens, and there are a few studies suggesting 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 metabolic 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 butadiene metabolites in cultured human lymphocytes. However, the critical/rate-limiting mechanistic steps are unknown at present; thus, it is unknown whether or not there are actual human subpopulations that may have notably different susceptibility to 1,3-butadiene.

11.7.2. 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).

11.8. FUTURE RESEARCH NEEDS

Although 1,3-butadiene is classified as a known human carcinogen in this assessment, there are some data gaps in various areas which, if filled, will refine the assessment. The specific research needs are as follows:

Epidemiology

- The medical records for the leukemia cases in the studies by Delzell et al. and Macaluso et al. should be reviewed to verify the cell types of leukemias.
- Further follow-up of these studies is recommended because it will give an opportunity to observe whether any noncancer effects, such as cardiovascular, or any cancers with a longer latency period are associated with exposure to 1,3-butadiene.

- Studies in other polymer facilities around the world could also add to the human evidence of carcinogenicity.

- All epidemiologic studies to date have examined male cohorts. Some 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 reproductive/developmental study would help determine if female workers are at risk of reproductive effects or if exposed fetuses are at risk of developmental effects.

- A reproductive study of exposed males is also needed to examine potential dominant lethal effects in humans.

Toxicology

- 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. study may have had insufficient power to detect an effect.

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.

11.9. SUMMARY AND CONCLUSIONS

The purpose of this effort was to review the new information that has become available since EPA's 1985 health assessment of 1,3-butadiene 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 a *known human carcinogen*, based on three types of evidence: (1) epidemiologic studies showing increased leukemias in workers occupationally exposed to 1,3-butadiene (by inhalation), (2) 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.

The best estimate of human lifetime extra *cancer risk* from chronic exposure to 1,3-butadiene is 9×10^{-3} *per ppm* based 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. 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 0.1 ppb. The 95% upper bound on unit risk from the linear model is 0.02/ppm.

1,3-Butadiene also causes a variety of reproductive and developmental effects in mice and rats; no human data on these effects are available. The most sensitive effect was reduced litter size at birth and at weaning observed in studies in which exposed male mice were mated with unexposed females. In humans, such an effect might be manifested as an increased risk of spontaneous abortions, miscarriages, stillbirths, or very early deaths. Based on this critical effect of reduced litter size, a reference concentration (i.e., a chronic exposure level presumed to be "without appreciable risk") of 0.15 ppb for reproductive and developmental effects was calculated from the modeled benchmark concentration (LED_{10}) of 0.15 ppm. The actual risks at low exposure levels are unknown; this RfC merely provides a bound on chronic exposure below which no "appreciable risk" of reproductive or developmental effects is expected.

There are insufficient data from which to draw any conclusions on potentially sensitive subpopulations.

In summary, the primary changes in EPA's conclusions about the health effects of 1,3-butadiene from the 1985 document to this one are:

- The cancer classification has been changed from probable to known human carcinogen.

- The unit cancer risk estimate has been changed from 0.25/ppm (upper bound based on mouse data) to 0.009/ppm (best estimate based on linear modeling and extrapolation of human data).

- For the first time, an RFC (0.15 ppb) is calculated for reproductive/developmental effects.

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