NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for 1,3-BUTADIENE

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NTP Report on Carcinogens Listing for 1,3-Butadiene

Carcinogenicity

1,3-Butadiene is *known to be a human carcinogen* based on studies in humans which have consistently found excess mortality from lymphatic and hematopoietic cancers associated with occupational exposure to butadiene, studies in experimental animals which have shown that 1,3-butadiene induces benign and malignant neoplasms at multiple tissue sites in multiple species, and supporting mechanistic data. In 1991, an IARC expert panel concluded that 1,3-butadiene is probably carcinogenic to humans based on sufficient evidence for its carcinogenicity in experimental animals and limited evidence for its carcinogenicity in humans (IARC, 1992). The available human carcinogenicity data at that time included 1) a cohort study showing excess risk for lymphosarcoma and reticulosarcoma in workers who manufactured 1,3-butadiene monomer, 2) a significantly increased risk for leukemia among production workers in a study of styrene-butadiene rubber workers in eight plants in the United States and Canada, and 3) a large excess of leukemia that was associated with exposure to 1,3-butadiene and not to styrene in a case-control study within the cohort of styrene-butadiene rubber workers. Since that evaluation by IARC, newer data have confirmed and strengthened the previous evidence of a causal relationship between exposure to 1,3-butadiene and human cancer risk. Ward et al. (1996) found an excess of lymphosarcoma and reticulosarcoma among 1,3-butadiene production workers in a previously unstudied chemical plant. Matanoski et al. (1993) reported that the standardized mortality ratio for leukemia was 1.8 times higher than that of the U.S. population for long-term workers hired before 1960, who had worked in three of the eight previously studied styrene-butadiene rubber plants, and a second case-control study of the lymphopoietic cancers among styrene-butadiene rubber workers (new set of controls per case) confirmed the strong association and significant dose-response effect between increasing 1,3-butadiene exposure score and increasing risk for leukemia. Finally, a follow-up study of styrene-butadiene rubber workers concluded that exposure to 1,3-butadiene in the synthetic rubber industry produces a dose-related increase in the occurrence of leukemia (Macaluso et al., 1996; Delzell et al., 1996).

Experimental studies in laboratory animals demonstrated that 1,3-butadiene is carcinogenic to mice and rats at multiple organ sites. Sites of tumor induction in mice included the hematopoietic system, heart (hemangiosarcomas), lung, forestomach, harderian gland, preputial gland, liver, mammary gland, ovary, and kidney (Huff et al., 1985; Melnick et al., 1990; NTP, 1984). Sites of tumor induction in rats included the pancreas, testis, thyroid gland, mammary gland, uterus, and Zymbal gland (Owen et al., 1987).

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

1,3-Butadiene is metabolized to mutagenic and carcinogenic expoxides (epoxybutene and diepoxybutane) in all mammalian species studied, including humans. Mouse, rat, and human liver microsomes have been shown to oxidize 1,3-butadiene to epoxybutene (Csanády et al., 1992) and further oxidize the monoepoxide to diepoxybutane (Seaton et al., 1995). These metabolites form N^7-alkylguanine adducts. These adducts have been detected in liver DNA of mice exposed to 1,3-butadiene and identified in the urine of a worker exposed to 1,3-butadiene. Activated K-*ras* genes and inactivated tumor suppresser genes observed in 1,3-butadiene-induced tumors in mice are analogous to genetic alterations frequently observed in a wide variety of human cancers. Dose-related increases in *hprt* mutations have been observed in lymphocytes isolated from mice exposed to 1,3-butadiene or its epoxide metabolites and in occupationally exposed workers. The mutational spectra for 1,3-butadiene and its epoxide metabolites at the *hprt* locus in mouse lymphocytes are similar to the mutational spectrum of ethylene oxide, an alkylating agent that was recently classified by IARC as carcinogenic to humans. The mechanism of tumor induction by 1,3-butadiene in rodents and humans appears to be due to its metabolism to DNA-reactive intermediates resulting in genetic alterations in protooncogenes and/or tumor suppressor genes.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded; or

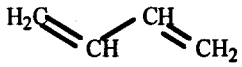
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

1,3-Butadiene
[106-99-0]



1.1 Chemical Identification

1,3-Butadiene (C_4H_6, mol. wt. = 54.09) is also called

Biethylene
Bivinyl
Butadiene
Buta-1,3-diene
trans-Butadiene
α,γ-Butadiene
Butadiene, inhibited
Divinyl
Erythrene
Pyrrolylene
Vinylethylene

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless	Budavari (1996)
Physical State	Gas	Budavari (1996)
Melting Point, °C	-108.97	Budavari (1996)
Boiling Point, °C at 760 mm Hg	-4.5	Budavari (1996)
Specific Gravity, at 20 °C/4 °C	0.621	Lewis (1992)
Vapor Density	1.87	Lewis (1992)
Vapor Pressure, mm Hg at 21 °C	1840	Lewis (1992)
Odor	Mild aromatic odor	Budavari (1996)
Solubility:		
Water	Sparingly soluble in water	Budavari (1996)
Organic Solvents	Slightly soluble in methanol and ethanol Soluble in diethyl ether, benzene, and carbon tetrachloride Very soluble in acetone	Budavari (1996), HSDB (1997)
Partition Coefficients:		
Log octanol/water	1.99	HSDB (1997)
Factor for converting air concentrations in ppm to mg/m^3	2.212	

1,3-Butadiene is a dangerous fire hazard when exposed to heat, flame, or powerful oxidizers (HSDB, 1997; Lewis, 1992). When exposed to air, it will also form explosive peroxides that are sensitive to shock or heating above 27 °C. 1,3-Butadiene will explode upon contact with aluminum tetrahydroborate.

1.3 Shipping and Packaging

Because 1,3-butadiene is a highly volatile gas at room temperature, it is transported to consumers as a liquefied gas under pressure (Morrow, 1990). Means of transportation include pipeline, barge, tank car, and tank truck. During transportation, 1,3-butadiene contains an antioxidant inhibitor such as *tert*-butylcatechol, hydroquinone, or di-*n*-butylamine (Kirshenbaum, 1985).

2.0 HUMAN EXPOSURE

2.1 Use

Seventy-five percent of 1,3-butadiene produced is used in synthetic rubber manufacture (Morrow, 1990). In 1986, 95% of 1,3-butadiene produced in the United States was used for the production of styrene-butadiene rubber (SBR) (32.7%), polybutadiene rubber (22.3 %), adiponitrile (12.5%), styrene-butadiene latex (9.9%), chloroprene (6.6%), acrylonitrile-butadiene-styrene (ABS) resins (4.4%), nitrile rubber (2.7%), and other uses, including export (3.9%). The major end-use products for most of these copolymers are tires (84% of SBR and 75% of the polybutadiene in North America) and nylon products (adiponitrile) (Kirschner, 1996).

2.2 Production

1,3-Butadiene is isolated by distillation or extraction from crude butadiene which is a byproduct of ethylene production. In 1996, 3.8 billion pounds (lb) (1.7 million metric tons or Mg) of 1,3-butadiene was produced, making it the 36th largest chemical product in the United States (Chemical and Engineering News, 1997). According to Chemical Market Associates Inc. (Rubber and Plastics News, 1997), global 1,3-butadiene consumption is anticipated to increase by 4.1% annually. Projected global production of 1,3-butadiene for the year 2001 is 18.5 billion pounds (8.4 million Mg), with North American production being 5.5 billion pounds (2.5 million Mg).

2.3 Exposure

2.3.1 Environmental Exposure

2.3.1.1 Air

Manufacturing, transporting, or using 1,3-butadiene are among the major anthropogenic sources of 1,3-butadiene releases to the environment (ATSDR, 1992; Eastern Research Group, USA, 1996).

Sources of 1,3-butadiene emissions include facilities producing 1,3-butadiene (8 in Texas and 2 in Louisiana in 1993 with capacity of 1,958,000 tons/yr [1,776,000 Mg/yr]), styrene-butadiene copolymer (40% of 1,3-butadiene consumption), polybutadiene, neoprene, acrylonitrile-butadiene-styrene (ABS) copolymer, nitrile elastomer, and adiponitrile. Lesser emissions are discharged by facilities producing styrene-butadiene-vinylpyridine (SBV) latex,

butadiene-vinylpyridine latex, tetrahydrophthalic anhydride, Captan®, Captafol®, 1,4-hexadiene, dodecanoic acid, butadiene dimers, methyl methacrylate-[acrylonitrile]-butadiene-styrene resins, ethylidene norbornene, butadiene-furfural cotrimer, sulfolane, and 1,3-butadiene cylinders. Because 1,3-butadiene is an impurity at 6 ppm in vinyl chloride monomer, emissions of 210 µg 1,3-butadiene per kilogram poly(vinyl chloride) (PVC) have been estimated for PVC production (Eastern Research Group, USA, 1996).

Of 184 facilities reporting on 1,3-butadiene emissions to the U.S. EPA for the 1995 Toxic Chemicals Release Inventory (TRI95, 1997), 175 reported a total of 2,913,561 lb (1,321,562 Mg) of 1,3-butadiene released to air [as calculated by the NLM Computer, July 17, 1997]. Thirty-five facilities reported total air emissions under 200 lb (0.091 Mg); 37 between 200 and 1000 lb (0.091 and 0.454 Mg); 49 between 1000 and 10,000 lb (0.454 and 4.54 Mg); 25 between 10,000 and 20,000 lb (4.54 and 9.07 Mg); 16 between 20,000 and 50,000 lb (9.07 and 22.7 Mg); and 13 between 50,000 and 490,000 lb (22.7 and 222.3 Mg). The 13 facilities reporting atmospheric releases of 1,3-butadiene in excess of 50,000 lb (22.7.3 Mg) were in three industrial categories. These were industrial organic chemicals, not elsewhere classified (SIC 2869), plastics materials and resins (SIC 2821), and synthetic rubber (SIC 2822). Of these 13 facilities, 8 were producers of 1,3-butadiene (6 in Texas and 2 in Louisiana), with a facility in Channelview, TX, releasing the greatest amount of 1,3-butadiene (490,000 lb; 222.3 Mg) to the atmosphere (TRI95, 1997). Total nonpoint air emissions [as calculated by the NLM Computer, August 12, 1997] were 1,437,468 lb (651.9 Mg) from the 169 facilities reporting nonpoint air emissions such as process venting and equipment leaks (TRI95, 1997). Thus, nonpoint or fugitive emissions represented 49% of total reported butadiene emissions in 1995.

A nationwide 1,3-butadiene inventory (including vehicle emissions and emissions from manufacturing and producing facilities) calculated annual butadiene emissions to air to be 102,000 Mg/yr for the year 1990 (Ligocki et al., 1994), considerably higher than the TRI 1990 reports of 2294 Mg/yr for industrial emissions. Calculations were based on butadiene emission factors for the various emission sources considered.

1,3-Butadiene is emitted from furnaces at secondary lead smelting facilities handling automotive lead-acid batteries that contain plastic battery separators or that have hard rubber casings. In 1992, petroleum refineries were the fourth largest emitters of 1,3-butadiene with 1,3-butadiene being released from blowdown vents; catalyst regeneration process vents; and miscellaneous vents at vacuum distillation, alkylation, and thermal cracking units (Eastern Research Group, USA, 1996).

Volatilization of 1,3-butadiene from wastewaters of styrene-1,3-butadiene copolymer production at publicly owned treatment works (POTW) has been calculated to be 21 tons/yr (19 Mg/yr) (Eastern Research Group, USA, 1996).

1,3-Butadiene is naturally formed as a byproduct of forest fires (HSDB, 1997). Emissions from wood burning in a wood-stove and small-scale model experiments showed that 1 to 2% by weight of total nonmethane hydrocarbons emitted were 1,3-butadiene (Barrefors and Peterson, 1995).

Incomplete combustion of a variety of fuels forms 1,3-butadiene as a product. 1,3-Butadiene comprises 0.5 to 2 % of the total organic gas emissions from most types of

combustion (Ligocki et al., 1994). It can also be found in exhaust emissions from motor vehicles as a product of incomplete combustion of gasoline and diesel oil and from the thermal breakdown of plastics (ATSDR, 1992; Eastern Research Group, USA, 1996).

California has run dispersion modeling from a typical freeway source and has estimated that gasoline-fueled vehicles emit 0.011g/mi (Cooper and Reisman, 1992). Ligocki et al. (1995a) calculated that onroad gasoline vehicle exhaust contained 0.59% 1,3-butadiene by weight. Diesel vehicle exhaust contained 1.55% 1,3-butadiene by weight.

Predicted trends of 1,3-butadiene emissions were determined for motor vehicles in 1990 (urban, 26,000 Mg/yr; rural, 9,400 Mg/yr; total 35,400 Mg/yr) (Ligocki et al., 1994).

Cigarette smoke is also an environmental source of 1,3-butadiene. Releases into the air in sidestream smoke have been variously estimated at 152 to 400 μg 1,3-butadiene per cigarette (Ligocki et al., 1995b). Calculations based on 400 μg/cigarette indicate that 1,3-butadiene concentrations in the homes of smokers would be increased by about 4 $\mu g/m^3$, and concentrations in air at workplaces allowing smoking would increase by 13 $\mu g/m^3$ (Wallace, 1991).

Certain cooking oils release 1,3-butadiene when heated. For example, 1,3-butadiene emissions were approximately 22-fold higher from heated unrefined Chinese rapeseed oil than from heated peanut oil. Of three fatty acids tested, heated linolenic acid produced the greatest amount of 1,3-butadiene. Although cooking oils in the United States are refined for purity, U.S. rapeseed oil (canola) also emitted 1,3-butadiene (Shields et al., 1995).

1,3-Butadiene air concentrations were measured in Raleigh, NC, in 1988. Measurements taken inside vehicles ranged from 4.2 to 17.2 $\mu g/m^3$ (1.9 - 7.8 ppb) [mean 3.3 ± 2.4 $\mu g/m^3$ (1.59 ± 1.1 ppb)] compared to 1.2 to 6.9 $\mu g/m^3$ (0.5 - 3.1 ppb) [mean 2.9 ± 1.0 $\mu g/m^3$ (1.3 ± 0.45 ppb)] measured outside the cars, and 1.3 $\mu g/m^3$ (0.59 ppb) measured on the urban sidewalks. Driving measurements represented urban, interstate, and rural highways and roads with urban values representing rush-hour traffic conditions (Chan et al., 1991).

Urban ambient air levels of 1,3-butadiene in Sweden were reported to be from 0.5 to 5 mg/m^3 (0.2 - 2.0 ppb) (Lofgren and Petersson, 1992). High concentrations of 1,3-butadiene were consistently observed in the proximity of exhaust pipes of gasoline-fueled vehicles.

The Urban Air Toxics Monitoring Program (UATMP) was developed in 1987 by the EPA. The 1990 program covering March 1990 through February 1991, collected 349 samples from 12 sites every 12 days for 24-hour periods. 1,3-Butadiene was identified in 106 of 349 samples (30.4 %). The range of concentrations was 0.03 to 142.66 ppb (0.07 to 315.3 $\mu g/m^3$) for samples identified as having a mean of 3.24 ppb (7.16 $\mu g/m^3$) of 1,3-butadiene. The mean concentration based on all samples was 0.98 ppb (2.17 $\mu g/m^3$) where zero was used for samples containing 1,3-butadiene at below the limit of detection (McAllister et al., 1991).

The Chemical Manufacturers Association (CMA) studied baseline VOC measurements in Washington, D. C., from March 12, 1990, to March 11, 1991. 1,3-Butadiene was detected in 26.79% of the samples collected for 24-hour periods, once every 6 days. Preliminary results indicated a mean 1,3-butadiene concentration of 0.13 ± 0.17 ppb (0.29 ± 0.38 $\mu g/m^3$). The maximum concentration observed was 0.83 ppb (1.8 $\mu g/m^3$). The mean was calculated using randomly generated values between zero and the detection limit for all samples in which

butadiene was below the limit of detection. Washington, D.C., was selected since it was one of the largest cities that did not contain large industrial air pollution sources (Hendler and Crow, 1992).

Outdoor 1,3-butadiene concentrations in six United States urban settings were in the range 0.3 - 1.6 $\mu g/m^3$ (0.14 - 0.72 ppbv) (Wallace, 1991). California's statewide population-weighted exposure to ambient (outdoor) airborne 1,3-butadiene was estimated to be an average of 0.37 ppb (0.82 $\mu g/m^3$). One-hour outdoor concentrations ranged to a high of 17.7 ppb (39.1 $\mu g/m^3$). Similar indoor concentrations were observed in taverns where heavy smoking conditions existed (Seiber, 1996).

2.3.1.2 Water

TRI95 (1997) reported releases of 1,3-butadiene totaling of 5,398 lb (2.45 Mg) to surface water in 1995 [as calculated by the NLM Computer, July 17, 1997].

2.3.1.3 Land

TRI95 (1997) reported a total of 277 lb (0.126 Mg) of 1,3-butadiene released to land in 1995 [as calculated by the NLM computer, July 17, 1997].

2.3.2 Occupational Exposure

Osterman-Golkar et al. (1996) monitored (using stationary and personal monitoring) 17 workers in the 1,3-butadiene production unit in a Swedish petrochemical plant to determine workplace exposure. Average exposure for workers handling 1,3-butadiene containers was 11.2 $\pm$ 18.6 mg/m^3 (5.06 $\pm$ 8.41 ppm). Maintenance and laboratory workers exposure was $\leq$1.2 mg/m^3 (0.54 ppm). These concentrations were determined by analyses of personal and area full shift air sample.

NIOSH conducted studies to determine 1,3-butadiene exposure in monomer, polymer, and end-user industries. Workers in 5 job areas were classified as having potentially higher exposure to 1,3-butadiene. These 5 areas included maintenance technician (0.026 - 94.38 mg/m^3; 0.012 - 42.7 ppm), loading (0.17 - 273 mg/m^3; 0.08 - 123 ppm), tank farm (0.02 - 52.8 mg/m^3; 0.009 - 24 ppm), process (i.e., purification, polymerization, and reaction) (< 0.011 - 76.78 mg/m^3; < 0.0050 - 34.7 ppm), and laboratory (< 0.0132 - 822.8 mg/m^3; < 0.006 - 372 ppm). Exposure concentrations were determined from personal or area full-shift air samples. Exposure of workers in the monomer industry, based on personal full-shift and short-term air samples (including subcategories of laboratory technician and process technician), ranged from < 0.02 to 374 ppm (< 0.04 - 827 mg/m^3). Personal exposure of workers in the polymer industry (including laboratory technician, tank farm operator, front end [reaction], maintenance technician, and back end [finishing]) ranged from < 0.005 to 42.9 ppm (< 0.01 - 94.9 mg/m^3) for full-shift samples and 0.087 to 280 ppm for short-term exposures. Full-shift (0.19 to 619 mg/m^3) area air samples in the polymer industry indicated 1,3-butadiene exposure ranging from less than 0.006 to 9.01 ppm (< 0.01 - 19.9 mg/m^3). For the monomer industry as a whole, 1,3-butadiene concentrations were > 10 ppm (22 mg/m^3) in 7.1% of the samples, 2-10 ppm (4 to 22 mg/m^3) in 12.8%, 1-2 ppm (2 - 4 mg/m^3) in 12.3% and < 1 ppm in 67.8% (the present OSHA permissible limit is 1 ppm). For

the polymer industry as a whole, the corresponding percentages for these 4 ranges were 3.3%, 7.7%, 3.3%, and 85.8%, respectively. The arithmetic mean exposure for personal full-shift exposures in the polymer plants was 1.14 ppm (2.57 mg/m^3) (Fajen et al., 1993).

In a study to test a new passive dosimeter for sampling workplace personal air, Yao et al. (1997) found that workers at the Shell Norco Refinery and Belpre Chemical Plant were exposed to 1,3-butadiene at concentrations of 0.0538 - 0.3720 ppm (0.1211 - 0.8370 mg/m^3). These results were similar to those found using the traditional NIOSH method sampling with SKC charcoal glass tubes (0.0746 - 0.3596 ppmv [0.168 - 0.7954 mg/m^3]).

The National Occupational Exposure Survey (NOES) conducted by the National Institute for Occupational Safety and Health (NIOSH) for 1981-1983 estimated that 51,971 total workers, including 1,411 women, at 2,201 facilities were potentially exposed to 1,3-butadiene (NIOSH, 1990) (**Table 2-1**).

Table 2-1. NIOSH National Occupational Exposure Survey (NOES, 1981-1983)[a]: By Industry

Industry	Number of Plants	Number of Employees	Number of Female Employees
Heavy Construction Contractors	19	3129	75
Special Trade Contractors	778	29860	244
Textile Mill Products	87	1619	806
Paper and Allied Products	7	21	7
Chemicals and Allied Products	208	3545	118
Petroleum and Coal Products	28	2070	161
Rubber and Misc. Plastics Products	123	926	
Machinery, Except Electrical	31	901	
Electric and Electronic Equipment	12	739	
Transportation Equipment	13	53	
Electric, Gas and Sanitary Services	166	4483	
Wholesale Trade - Durable Goods	174	3474	
Business Services	29	73	
Auto Repair, Services, and Garages	485	971	
Health Services	39	108	
Total	2201	51971	1411

[a] NIOSH (1990)

2.4 Regulations and Criteria

1,3-Butadiene is regulated by EPA under the Clean Air Act (CAA), Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Superfund Amendments and Reauthorization Act (SARA), and Toxic Substances Control Act (TSCA). Under CAA, it is designated as a hazardous air pollutant, and emission standards are given for the compound. Under CERCLA, a reportable quantity (RQ) of 1 lb has been established. FDA, under the Food, Drug, and Cosmetic Act (FD&CA), regulates 1,3-butadiene as an indirect food additive. NIOSH recommends that the exposure limit of the compound be the lowest feasible concentration. OSHA has lowered the permissible exposure limit (PEL) for 1,3-butadiene from 1000 ppm to 1 ppm as an 8-hour time-weight average (TWA), with a 15-minute short-term exposure limit (STEL) of 5 ppm. OSHA also regulates 1,3-butadiene under the Hazard Communication Standard and as a hazardous chemical in laboratories.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71.	The provisions of this part apply to the owner/operator of any stationary source which contains an affected facility (a stationary source with an apparatus to which a standard is applicable).
	40 CFR 61—PART 61—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS. Promulgated: 38 FR 8826, 04/06/73. U.S. Code: 7401, 7412, 7414, 7416, 7601.	This part lists substances that, pursuant to section 112 of the CAA, have been designated as hazardous air pollutants, and applies to the owner or operator of any stationary source for which a standard is prescribed under this part.
	40 CFR 61.01 ff.—Subpart A—Lists of pollutants and applicability of part 61. Promulgated: 59 FR 12429, 03/16/94. U.S. Code: 42 U.S.C. 7661.	Substances that, pursuant to section 112 of the CAA, have been designated as hazardous air pollutants. Substances for which a Federal Register notice has been published that included consideration of the serious health effects from ambient air exposure.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 et seq.; CAA.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
	40 CFR 63.70—Subpart D—Regulations Governing Compliance Extensions for Early Reductions of Hazardous Air Pollutants.	The provisions of this subpart apply to an owner/operator of an existing source wishing to obtain a compliance extension from a standard issued under section 112(d) of the CAA.
	40 CFR 63.74—Sec. 63.74 Demonstration of early reduction. Promulgated: 59 FR 53110, 10/21/94.	Procedures are given for demonstrating early reductions as required by 63.72(a)(1), including a description of the source and complete list of all emission points of HAPS in the source.
	40 CFR 63.100 ff.—Subpart F—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94. This subpart applies to chemical manufacturing process units that manufacture one or more of the chemicals listed in Table 1 and Table 2 of this subpart and are located at a plant site that is a major source as defined in section 112(a) of CAA.	Owners and operators of sources subject to this subpart shall comply with the requirements of subparts G and H of this part.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63.110 ff.—Subpart G—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry for Process Vents. Promulgated: 59 FR 04/22/94.	Specific process vent and methods and procedures provisions apply. The provisions of this subpart apply to all process vents, storage vessels, transfer racks, and wastewater streams within a source subject to subpart F of this part. Emission standard: Emissions of organic HAPs shall be controlled to the level represented by a given equation (see 40 CFR 63.112[a]).
	40 CFR 63.152—Sec. 63.152 General reporting and continuous records. Promulgated: 59 FR 19468, 04/22/94, as amended at 60 FR 63629, 12/12/95.	General reporting and continuous records requirements for the owner/operator of a source subject to this subpart.
	40 CFR 63.190—Subpart I—Nation Emission Standards for Organic Hazardous Air Pollutants for Certain Processes Subject to the Negotiated Regulation for Equipment leaks. Promulgated: 59 FR 19587, 04/22/94. Standards apply to owner/operators of pharmaceutical production processes and pertain to construction, maintenance, notification, and performance tests.	Specific compliance requirements are set forth in section 63.192 for owners/operators of a source subject to this subpart. The provisions of this subpart apply to emissions designated as organic HAPs from the processes specified in paragraphs (b)(1) through (b)(6) of section 63.190.
	40 CFR 63.680 ff.—Subpart DD—Applicability and designation of affected sources. Promulgated: 61 FR 34158, 07/01/96.	The provisions of this subpart apply to plant sites at which a major source of HAP emissions occurs as defined in 40 CFR 63.2, or at which is located one or more operations that receives offsite materials as specified in 40 CFR 63.680(b).

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63.780 ff—Subpart I—National Emission Standards for Organic Hazardous Air Pollutants for Certain Processes Subject to the Negotiated; Regulation for Equipment Leaks. Promulgated: 60 FR 64336, 12/15/95.	Application standards for all compounds listed in Table 2 of this subpart.
	40 CFR 63.800 ff.—Subpart JJ—National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95. Specific work and compliance requirements apply. The provisions of this subpart apply to each facility that is engaged in the manufacture of wood furniture or wood furniture components and that is a major source as defined in 40 CFR 63.2.	Emission limitations for existing sources presented in Table 3 of this subpart shall be met using any of the compliance methods in 40 CFR 63.804. Specific limitations apply to limiting VHAP emissions from contact adhesives.
	40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISIONS. Promulgated: 59 FR 4493, 01/31/94. U.S. Code: 42 U.S.C. 7412(r), 7601(a)(1), 7661-7661f.	This part lists regulated substances and thresholds, the petition process for adding or deleting substances, and specific requirements for preventing accidental releases.
	40 CFR 68.100 ff.—Subpart F—Regulated Substances for Accidental Release Prevention. Promulgated: 61 FR 31717, 06/20/96. U.S. Code: CAA, section 112(r)(3), (4), and (5).	This subpart designates substances to be listed, identifies their threshold quantity, and establishes the requirements for petitioning to add or delete substances from the list.
	40 CFR 68.130—Sec. 68.130 List of substances. Threshold quantities for listed toxic and flammable substances are specified in the tables. Substances listed as toxic and flammable are regulated under section 112(r) of the CAA (see tables 1, 2, 3, and 4).	This subpart designates substances to be listed, identifies their threshold quantity, and establishes the requirements for petitioning to add or delete substances from the list.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 79—PART 79—REGISTRATION OF FUELS AND FUEL ADDITIVES. Promulgated: 40 FR 52011, 11/07/75. U.S. Code: 42 U.S.C. 7414, 7524, 7545, and 7601.	The regulations of this part apply to the registration of fuel additives designated by the Administrator, pursuant to section 211 of the CAA.
	40 CFR 79.50 ff.—Subpart F—Testing Requirements for Registration. Promulgated: 59 FR 33093, 06/27/94. additives.	Specific testing requirements apply for the registration of fuels and fuel additives.
	40 CFR 79.52—Sec. 79.52 Tier 1. Manufacturers must characterize the emission products which are generated by evaporation and by combustion of the fuel or additive/base fuel mixture in a motor vehicle.	The regulations of this part apply to the registration of fuel additives designated by the Administrator, pursuant to section 211 of the CAA.
	40 CFR 80—PART 80—REGULATION OF FUELS AND FUEL ADDITIVES. Promulgated: 38 FR 1255, 01/10/73. U.S. Code: 42 U.S.C. 7414, 7545, and 7601(a).	This part prescribes regulations for the control and/or prohibition of fuels and additives for use in motor vehicles and motor vehicle engines.
	40 CFR 80.40—Subpart D—Reformulated Gasoline Promulgated: 59 FR 7813, 2/16/94.	Fuel certification procedures, and standards and requirements for compliance apply.
	40 CFR 80.42—Sec. 80.42 Simple emissions model. The model shall be used only in determining toxic emissions.	The equations that comprise the simple model for VOC emissions are found in this section.
	40 CFR 80.45—Sec. 80.45 Complex emissions model. Promulgated: 9 FR 7813, 02/16/94. Limits: See Tables 1, 2, and 3 of this section.	The weightings for normal and higher emitters (w1 and w2, respectively) given in Table 1 shall be used to calculate the exhaust emission performance of any fuel for the appropriate pollutant and phase.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 80.51—Sec. 80.51 Vehicle test procedures.	A specific test sequence is applicable when augmenting the emission models through vehicle testing.
	40 CFR 80.55—Sec. 80.55 Measurement methods for benzene and 1,3-butadiene. Expected values for benzene and 1,3-butadiene in bag samples for the baseline fuel are 4.0 ppm and 0.30 ppm, respectively. This procedure is detailed in 40 CFR 86.109.	Sampling for benzene and 1,3-butadiene must be accomplished by bag sampling as used for total hydrocarbons determination.
	40 CFR 80.90—Subpart E—Anti-Dumping. Promulgated: 50 FR 7860, 02/16/94.	Specific equations used to determine annual average conventional gasoline baseline emissions and individual baseline determination.
	40 CFR 132—PART 132—WATER QUALITY GUIDANCE FOR THE GREAT LAKES SYSTEM. Promulgated: 60 FR 15387, 03/23/95. U.S. Codes: 33 U.S.C. 1251 et seq.	RQs apply to discharges of substances listed, including isomers and hydrates and any solutions or mixtures containing the substances.
	40 CFR 173—PART 173—PROCEDURES GOVERNING THE RESCISSION OF STATE PRIMARY ENFORCEMENT RESPONSIBILITY FOR PESTICIDE USE VIOLATIONS. Promulgated: 46 FR 26059, 5/11/81. U.S. Codes: 7 U.S.C. 136w and 136w-2.	These procedures govern any proceeding to rescind a State's primary enforcement responsibility for pesticide use violations conducted under section 27(b) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (FIFRA).
	40 CFR 173.21 ff.—Subpart G—Gases; Preparation and Packaging.	Specific requirements for the transportation of butadiene.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 258—PART 258—CRITERIA FOR MUNICIPAL SOLID WASTE LANDFILLS. Promulgated: 56 FR 51016, 10/09/91. U.S. Code: 33 U.S.C. 1345(d) and (e); 42 U.S.C. 6907(a)(3), 6912(a), 6944(a) and 6949(a)(c).	The provisions of this part establish minimum national criteria under RCRA, as amended, for all MSWLF units and under the CWA, as amended, for MSWLF that are used to dispose of sewage sludge. The criteria ensure the protection of human health and the environment.
	40 CFR 266—PART 266—STANDARDS FOR THE MANAGEMENT OF SPECIFIC HAZARDOUS WASTES AND SPECIFIC TYPES OF HAZARDOUS WASTE MANAGEMENT FACILITIES. Promulgated: 50 FR 666, 01/04/85. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6924, and 6934.	Standards to control emissions are promulgated for generators, transporters, and users of materials used in a manner that constitutes disposal. Affected compounds are listed in 40 CFR 266.40.
	40 CFR 266—Subpart H—Hazardous Waste Burned in Boilers and Industrial Furnaces.	Appendix V to Part 266 lists a risk specific dose of 3.6×10^{-2} $\mu g/m^3$ for 1,3-butadiene: The sum for all compounds of the ratios of the actual ground level concentration to the level established in Appendix V cannot exceed 1.0.
	40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Superfund (CERCLA, SARA) reportable quantity (RQ) is 1 lb (0.45 kg).	EPA designated as hazardous those substances that when released into the environment may present substantial danger to the public health or welfare or the environment. Notification of EPA is required if the RQ is released to the environment.
	40 CFR 372—PART 372—TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013, 11028. This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986).	Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, to aid in the development of regulations, guidelines, and standards. See section 372.65 for chemicals and chemical categories to which this part applies.
	40 CFR 372—Subpart D—Specific Toxic Chemical Listings	
	40 CFR 372.65—Sec. 372.65 Chemicals and chemical categories to which this part applies.	
	40 CFR 414—PART 414—ORGANIC CHEMICALS, PLASTICS, AND SYNTHETIC FIBERS. Promulgated: 52 FR 42568, November 5, 1987. U.S. Code: 33 U.S.C. 1311, 1314, 1316, 1317, and 1361.	Limitations representing the degree of effluent reduction attainable by application of BAT. EPA gives pretreatment standards for existing sources (PSES) for metals and organics in effluents from several manufacturing categories.
	40 CFR 414.65—Subpart F—Commodity Organic Chemicals. Pretreatment Standards for Existing Chemicals (PSES).	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 716—PART 716—HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Code: 15 U.S.C. 2607(d).	The provisions of this part require the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of TSCA and on other chemicals for which EPA requires health and safety information in fulfilling the purposes of TSCA.
	40 CFR 716.105 ff.—Subpart B gives specific chemical listings, and gives substances and listed mixtures to which this subpart applies.	
	40 CFR 716.120—Substances and listed mixtures to which this subpart applies. Promulgated: 55 FR 39784, 09/28/90.	The list of substances identified in section 716.120 are subject to the reporting requirements of Subpart A for that substance.
	40 CFR 721—PART 721—SIGNIFICANT NEW USES OF CHEMICAL SUBSTANCES. U.S. Code: 15 U.S.C. 2604, 2607, and 2625(c).	Identifies uses of chemical substances which EPA has determined are significant new uses under the authority section 5(a)(2) of TSCA, and specifies procedures for manufacturers, importers, and processors to report on those significant new uses.
F D A	21 CFR 175—PART 175—INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS. Promulgated: 42 FR 14534, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.	The subparts A through C deal with components of adhesives and of coatings that may migrate into food from packaging.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
FDA	21 CFR 176—PART 176—INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS. Promulgated: 42 FR 14554, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 346, 348, and 379e.	1,3-Butadiene is regulated as an indirect food additive: adhesive coatings and components, paper and paperboard components, styrene block polymers and other polymers, adjuvants, production aids, and sanitizers.
	21 CFR 177—PART 177—INDIRECT FOOD ADDITIVES: POLYMERS. Promulgated: 42 FR 14572, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.	Subparts A through C govern polymers containing 1,3-butadiene used as components of single and repeated-use food-contact surfaces and components of articles for repeated use.
	21 CFR 178—PART 178—INDIRECT FOOD ADDITIVES: ADJUVANTS, PRODUCTION AIDS, AND SANITIZERS. Promulgated: 42 FR 14609, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.	Regulations in subparts A through C govern indirect food additives utilized to control the growth of microorganisms, antioxidants, stabilizers, adjuvants, and production aids.
NIOSH	2/9/84. Regards 1,3-butadiene as a potential carcinogen, teratogen, and possible reproductive hazard. Recommends that OSHA reexamine and lower the PEL.	Summary of NIOSH recommendation: exposure limit—Ca, lowest feasible concentration.
	2/9/84. Current Intelligence Bulletin #41, 1,3-Butadiene. Pub. No. 84-105, NTIS No. PB84-198 019.	
	1/86. NIOSH comments on OSHA Request for Comments: Occupational Exposure to 1,3-Butadiene. 29 CFR Part 1910, 50 FR 52952. NTIS No. PB91-152 280.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
N I O S H	12/86. Comments on OSH's advance notice of proposed rulemaking on occupational exposure to 1,3-butadiene. 1/90. Comments from the National Institute for Occupational Safety and Health on the Occupational Safety and Health Administration's proposed rule on occupational exposure to 1,3-butadiene. 11/9/90. NIOSH testimony on the OSHA Advance Notice of Proposed Rulemaking on Occupational Exposure to 1,3-Butadiene. 29 CFR Part 1910, Docket No. H-041. NTIS No. PB91-152 019. 1/17/91. NIOSH testimony on the OSHA Proposed Rule on Occupational Exposure to 1,3-Butadiene. 29 CFR 1910, Docket No. H-041. NTIS No. PB91-212 654. 9/27/91. NIOSH Risk Assessment: A Quantitative Assessment of the Risk of Cancer Associated with Exposure to 1,3-Butadiene. 29 CFR Part 1910, Docket No. H-041. 11/26/91. Additional Posthearing Comments from NIOSH on the OSHA Proposed Rule on Occupational Exposure to 1,3-Butadiene. 29 CFR Part 1910, Docket No. H-041. 2/10/92. Posthearing Brief from NIOSH on the OSHA Proposed Rule on Occupational Exposure to 1,3-Butadiene. 29 CFR 1910, Docket No. H-041.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
NIOSH	3/22/96. NIOSH testimony on the OSHA Proposed Rule on Occupational Exposure to 1,3-Butadiene. 29 CFR Parts 1910, 1915, and 1926, Docket No. H-041.	
OSHA	29 CFR 1910.1000—Sec. 1910.1000 Hazard Communication. Promulgated: 61 FR 9245, 03/07/96. OSH Act: Hazard Communication.	OSHA has lowered the PEL from 1000 ppm (2,200 mg/m^3) to 1 ppm (2.2 mg/m^3), 8-hr TWA, with a 15-min STEL of 5 ppm (11 mg/m^3).
	29 CFR 1910.1200, 1915, 1917, 1918, 1926, 1928. Promulgated 11/25/83. OSH Act: Hazard Communication.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. The Hazard Communication Program must include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450—Sec. 1910.1450 Occupational exposure to hazardous chemicals in laboratories. Promulgated: 61 FR 55508, 02/13/96. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2B), 1,3-butadiene is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan.

[a] The regulations in this table have been updated through January 17, 1997.

3.0 HUMAN STUDIES

IARC (1992, pp. 237-285; see Appendix A) evaluated pre-1992 published studies of workers occupationally exposed to 1,3-butadiene. Based on their review, IARC concluded that there was limited evidence for the carcinogenicity of 1,3-butadiene in humans. However, more recent epidemiological studies (summarized below and in **Table 3-1**) generally support the conclusion that 1,3-butadiene is a human carcinogen associated with an increased risk for lymphohematopoietic cancers. Excess mortality from leukemia has been found in styrene/butadiene-rubber workers (even after adjustment for styrene exposure) and excess mortality from lymphosarcoma or reticulosarcoma has been found in 1,3-butadiene production workers.

Two recent cohort studies (Ward et al., 1996; Divine and Hartman, 1996) indicate a significant increase in mortality associated with certain cancers among workers exposed to 1,3-butadiene. Ward et al. (1996) evaluated mortality among a cohort of 364 male 1,3-butadiene production workers employed between 1940 and 1979 in three 1,3-butadiene production facilities in West Virginia. Vital status of these workers was determined by (a) using the findings through December 31, 1978, of Rinsky et al. (1988, cited by Ward et al., 1996), and (b) an update using records through December 31, 1990, from the National Death Index. Workers not identified in these two sources were presumed alive as of December 31, 1990. The standardized mortality ratio (SMR) for all cancers was 1.05 [95% confidence interval (CI) = 0.78-1.40; 48 cancer deaths observed/45.5 expected]. The SMR for lymphosarcoma and reticulosarcoma among workers with at least 2 years of employment and ≥30 years since first employment was 19.8 (95% CI = 4.08-57.8; 3 observed/0.15 expected), while for the total cohort through January 1, 1990, it was 5.77 (95% CI = 1.57-14.8; 4 observed/0.69 expected). The study had a relatively small number of worker deaths, but the strong associations seen, especially with respect to exposure duration and intensity, were notable. The authors discussed several limitations of the study, most notably their emphasis on cancer mortality rather than cancer incidence. Also, because the production processes differed from facility to facility, there was significant potential for confounding by exposure of workers to other substances such as acetaldehyde. However, a mortality analysis of acetaldehyde-exposed workers did not yield any unique association with lymphosarcoma and reticulosarcoma.

Divine and Hartman (1996) conducted a follow-up study of a cohort of 2795 male former employees of a 1,3-butadiene production facility, who had been employed for ≥6 months between 1942 and 1994. The overall SMR for lymphohematopoietic cancers was significantly increased (SMR = 1.47; 95% CI = 1.06-1.98; 42 observed/28.6 expected), while the SMR for subsets of lymphohematopoietic cancers were increased but not significantly (lymphosarcoma: SMR = 1.91; 95% CI = 0.87-3.64; 9 observed/4.7 expected; Hodgkin's disease: SMR = 1.66; 95% CI = 0.45-4.24; 4 observed/2.4 expected; cancer of other lymphatic tissue: SMR = 1.52; 95% CI = 0.85-2.50; 15 observed/9.9 expected). There was only a weak increase in the risk for leukemia (SMR = 1.13; 95% CI = 0.60-1.93; 13 observed/11.5 expected). Workers were assigned to exposure groups (background, low varied) based on job title and work unit. Divine and Hartman (1996) also used the estimated cumulative exposure, based on calendar time and exposure classes defined by job and process, as a time-dependent explanatory variable for four cancer types. There was no significant association of exposure with any cancer, but cancer risk increased with age for most groups analyzed. Further analysis of the elevated SMRs for lymphohematopoietic cancers, particularly lymphosarcoma, showed that the association was concentrated among those workers employed fewer than 10 years, first hired during World War II, and with potential for varied exposure. An SMR of 2.49 (95%CI = 1.00-5.13; 7 observed/2.8 expected) for lymphosarcoma was found among varied exposure groups for those employed less than 10 years. Mortality from lymphosarcoma was also higher among those first employed prior to 1946 (SMR = 2.41; 95% CI = 0.97-4.97; 7 observed/2.9 expected). The study included a small number of lymphohematopoietic cancer deaths, had no industrial hygiene data, did not account for confounding exposures, and did not model other exposure measures such as peak

exposure. Nonetheless, the study suggested a moderate association between potential exposure to 1,3-butadiene and certain lymphohematopoietic cancers.

Two analyses of cancer mortality in a cohort of former employees of styrene-butadiene resin (SBR) manufacturing plants (Delzell et al., 1996; Macaluso et al., 1996) also indicate an increased risk of certain cancer types from exposure to 1,3-butadiene.

Delzell et al. (1996) studied the possible relationship between exposure to 1,3-butadiene and leukemia among 15,649 male workers who were employed for at least one year between 1943 and 1991 at one of eight North American SBR plants. These workers were potentially exposed to 1,3-butadiene and/or styrene. It was estimated that about 75% of the workers were exposed to 1,3-butadiene and 83% were exposed to styrene. The authors noted that many of the workers in their study had been included in previous studies conducted by Matanoski et al. (1990, cited by Matanoski et al., 1993, and Matanoski et al., 1993) and Meinhardt et al. (1982, cited by Matanoski et al. (1993)). However, the exact number of common subjects between this study and previous efforts was not provided. Detailed work histories were used to assign workers into 308 work area groups with similar jobs and processes. These were further combined into five process groups. Complete work histories were available for 97% of the workers, although subjects from two plants (n = 1354) were excluded due to a lack of data on specific work areas. As of January 1, 1992, the vital status of 95% of the workers was determined. The overall mortality due to cancer was slightly less than expected (SMR = 0.93; CI = 0.87-0.99; 950 observed/1024 expected). The overall SMR for lymphosarcoma was less than 1.00 (SMR = 0.80; 95% CI = 0.40-1.44; 11 observed/14 expected). Several process groups had elevated SMRs for lymphosarcoma although the estimates were rather imprecise. These process groups included field maintenance workers (SMR = 2.19; 95% CI = 0.88-4.51; 7 observed/3.2 expected), production laborers (SMR = 2.63; 95% CI = 0.32-9.51; 2 observed/0.8 expected), and maintenance laborers (SMR = 1.88; 95% CI = 0.39-5.48; 3 observed/1.6 expected).

The most notable result of the Delzell et al. (1996) study was a higher-than-expected incidence of deaths from leukemia (SMR = 1.31; 95% CI = 0.97-1.74; 48 observed/37 expected) in both white and black workers. Among ever-hourly employees, the SMR for leukemia was 1.43 (95% CI = 1.04-1.91; 45 observed/32 expected). The increase in mortality was mainly in workers with 10 or more years of employment and 20 to 29 years since hire, and was highest in workers in polymerization (SMR = 2.51, 95% CI = 1.40-4.14; 15 observed/6.0 expected), coagulation (SMR = 2.48, 95% CI =1.00-5.11; 7 observed/2.82 expected), maintenance labor (SMR = 2.65, 95% CI = 1.41-4.53; 13 observed/4.91 expected), and laboratories (SMR = 4.31, 95% CI = 2.07-7.93; 10 observed/2.32 expected). The authors acknowledged that work histories were incomplete for 12% of the workers, that workers who terminated their employment before 1950 were incompletely followed-up, and that there was a lack of medical records confirming death by leukemia and other lymphopoietic cancers. They concluded, however, that their study's large cohort size, long follow-up period, and objective study procedures for work history and cause of death determination support a positive association between leukemia and occupational exposure to 1,3-butadiene in SBR production. Workers in the SBR industry have potential exposure to a number of agents besides 1,3-butadiene. Based upon the presence of elevated SMRs for some but not all process groups, the authors suggest that the leukemia association is due to either 1,3-butadiene alone or 1,3-butadiene in combination with styrene.

Macaluso et al. (1996) analyzed quantitative exposure measures in relation to leukemia mortality for a cohort of 16,610 men employed for at least 1 year between 1943 and 1991 at one of six North American SBR manufacturing plants. The cohort overlaps with the cohort analyzed by Delzell et al. (1996). To estimate exposure, the authors identified work areas within each manufacturing process, historical changes in exposure potential, and specific tasks involving exposure. They used mathematical models to calculate job- and time-period specific average exposures and estimated cumulative exposure as parts per million x years exposed (ppm-yr). The Mantel-Haenszel rate ratios (RR), adjusted for race, age, and cumulative styrene exposure, increased with cumulative 1,3-butadiene exposure (RR for leukemia were 1, 2.0, 2.1, 2.4, and 4.5 for the 0, <1, 1-19, 20-79, and 80+ ppm-yr exposure groups, respectively; p for trend = 0.01). After exclusion of all subjects who had zero cumulative exposure to 1,3-butadiene and > 10 ppm-yr exposure to styrene, the respective RR were 1, 3.5, and 5.1 for the 0.1-19, 20-79, and 80+ ppm-yr exposure groups (p for trend = 0.03). This confirms the presence of a progression in risk with increasing 1,3-butadiene exposure level. There was some association between RR and styrene exposure, but the pattern was less clear than for exposure to 1,3-butadiene.

Matanoski et al. (1993) explored a previous nested case-control analysis and SMR analysis for a cohort of workers in eight SBR manufacturing plants (Matanoski et al., 1990; Santos-Burgoa et al., 1992; both cited by Matanoski et al., 1993). In the original case-control study, 26 workers who developed leukemia were compared with 84 workers in the same plants, individually matched by plant worked, age at hire, year of hire, and duration worked. Exposure experts evaluated job and work area for potential exposure to 1,3-butadiene and styrene. The calculated ORs from the earlier analysis was 7.6 (95% CI = 1.6-35.6) for 1,3-butadiene exposure, 2.9 (95% CI = 0.8-10.3) for styrene, and 7.4 (95% CI = 1.3-41.3) for potential exposure to both. [This study was reviewed in the NTP Bioassay for 1,3-butadiene (NTP, 1993)]. A new set of controls matched (3:1) for all factors except duration of employment resulted in a lower risk estimate for leukemia (OR = 6.0; 95% CI = 0.8-47.2). Analysis with a variable to account for duration of work resulted in an OR of 9.2 (95% CI = 1.04 - 81.1). The case-control data set matched for duration of employment was also analyzed using the same exposure cutpoint (OR = 8.5; 95% CI = 1.1-5.4). A hospital records review confirmed 25 of the 26 cases of leukemia.

The followup cohort analysis by Matanoski et al. (1993) involved the use of 1,3-butadiene and styrene exposure data for each of the eight plants. Three SBR manufacturing plants had geometric mean exposure levels higher than those in the other five plants; the data for 3429 former workers in these three plants hired before 1960 and with ≥10 years of employment were analyzed. The SMR by cause of death was 1.63 (95% CI = 1.13 - 2.27; 34 observed/20.9 expected) for all lymphohematopoietic cancers; 1.16 (95% CI = 0.37-2.70; 5 observed/4.31 expected) for lymphosarcoma and reticulosarcoma; 2.43 (95% CI = 0.78-5.68; 5 observed/2.06 expected) for Hodgkin's disease; 1.81 (95% CI = 1.01 - 2.99; 15 observed/8.29 expected) for leukemia and aleukemia; and 1.49 (95% CI = 0.68-2.82; 9 observed/6.04 expected) for tumors involving other lymphatic tissue.

In a study that combined retrospective mortality and prospective morbidity data with results from hematological evaluations, no excess cancer incidence was found among Shell Oil Company employees potentially exposed to 1,3-butadiene (Cowles et al., 1994). Workers with a minimum of five years of employment in jobs with potential 1,3-butadiene exposure, or those

who worked at least half of their total duration of employment in jobs with potential 1,3-butadiene exposure (three-month minimum in such jobs), were eligible for the study. The study included men who worked in production, maintenance, laboratories, and shipping at Shell Deer Park Manufacturing Complex from 1948 to 1989. 1,3-Butadiene was not produced at this facility between 1948 and 1970. Most 1,3-butadiene exposures between 1979 and 1992 did not exceed 10 ppm (2.2 mg/m^3) and most were below 1 ppm with an arithmetic mean of 3.5 ppm (7.7 mg/m^3).

SMRs were calculated based on the rates for the county in which the facility is located and adjusted for age, race, and calendar year. Analysis of mortality data from 614 employees yielded an all-cancer SMR of 34 (95% CI = 9-87), based on four observed deaths. The SMR for lung cancer was 42 (95% CI = 5-151). There were no deaths due to lymphohematopoietic cancer, whereas 1.2 were expected. The standardized morbidity rate (SMbR) of 438 members of the original cohort was 51 (95% CI = 22-100; 8 observed/15.7 expected) for all neoplasms. There was one observed illness absence, versus 0.8 expected, due to a lymphohematopoietic neoplasm (non-Hodgkin's lymphoma). No significant differences were seen between 429 members of a 1,3-butadiene morbidity study subcohort and a non-butadiene group in hematological test results. The authors acknowledge a lack of statistical stability, as illustrated by the wide range of 95% CIs, attributable to the small number of deaths from any specific cause in this study (Cowles et al., 1994). [Ratios and Cis should be divided by 100 to be comparable to data from other studies.]

Table 3-1. Recent Human Studies of Effects of Exposure to 1,3-Butadiene

Design	Population Groups	Exposure	Effects	Potential Confounders/Effects	References
Cohort	**Exposed:** 364 men who worked in three butadiene plant production units **Controls:** U.S. male general population and male general population in county where production plants were located	**Estimation:** used index that showed production units where 1,3-butadiene was the primary product and where neither benzene nor ethylene oxide was present **Duration:** 39 yr (workers from 1940-1979; from work records) **Categories:** employment duration (<2 yr; ≥2 yr) and latency (<30 yr; ≥30 yr). Latency = time since first employment in production process	**Evaluation:** compared mortality rates (U.S.: 1940-1979; county: 1960-1990) using a modified life-table analysis **SMR (95% CI; observed/expected cases):** 1.05 (0 .78-1.4; 48/45.5) for all cancers; 19.8 (4.08-57.8;3/0.15) for lymphosarcoma and reticulosarcoma in categories ≥2 yr employment and latency ≥30 yr; 5.77 (1.57-14.8; 4/0.69) for lymphosarcoma and reticulosarcoma of the total cohort through Jan. 1, 1990	The authors discussed several limitations of the study, most notably their emphasis on cancer mortality rather than cancer incidence. Also, because the production processes differed from facility to facility, there is significant potential for confounding by exposure of workers to other substances such as acetaldehyde, although a mortality analysis of acetaldehyde-exposed workers did not yield any unique association with lymphosarcoma and reticulosarcoma.	Ward et al. (1996)
Follow-up of cohort	**Exposed:** 2795 male former employees of a 1,3- butadiene facility **Controls:** 1) for SMR analysis, U.S. white male population 2) for modeling, ten controls from cohort matched with each case by date of birth	**Estimation:** 1) For SMR analysis, used qualitative job/unit exposure classification scheme **Duration:** ≥6 mo during 1942-1994 **Stratified by:** time period (<1946,1946-1949, 1950+) Duration (<5, 5-19, 20+ yr) Exposure (background, low, varied)	**Evaluation:** calculated SMR and fit regression models **1) SMR analysis (95% CI): observed/expected cases:** 1.47 (1.06-1.98; 42/28.6) for lymphohematopoietic cancers 1.66 (0.45-4.24; 4/2.4) for Hodgkin's disease 1.13 (0.60-1.93; 13/11.5) for leukemia 1.52 (0.85-2.50; 15/9.9) for cancer of other lymphatic tissue 1.91 (0.87-3.64; 9/4.7) for lymphosarcoma 2.49 (1.00-5.13; 7/2.8) for lymphosarcoma among varied exposure group (<10 yr) 2.41 (0.97-4.97; 7/2.9) for lymphosarcoma among first employed <1946 **2) Modeling**: used estimated cumulative exposure as a time-dependent explanatory variable for 4 cancer types; no significant association of exposure with any cancer, but cancer risk increased with age for most groups	The study included a small number of lymphohematopoietic cancer deaths, provided no industrial hygiene data, did not account for confounding exposures, and did not model other exposure measures such as peak exposure. Nonetheless, the study suggested a moderate association between potential exposure to 1,3 butadiene and certain lymphohematopoietic cancers.	Divine and Hartman (1996)

Table 3-1. Recent Human Studies of Effects of Exposure to 1,3-Butadiene (Continued)

Design	Population Groups	Exposure	Effects	Potential Confounders/Effects	References
Follow-up of cohort	**Exposed:** 15,649 men employed at any of eight North American styrene-butadiene rubber plants **Controls:** general U.S. or Ontario population	**Estimation:** identified work areas within each manufacturing process, noted historical changes in exposure potential and specific tasks involving exposure, used mathematical models to calculate job- and time-period specific average exposures **Duration:** ≥1 yr during 1943-1991	**Evaluation:** compared the overall and cause-specific mortality rates of the exposed group with control mortality rates using calculated standardized mortality ratios (SMRs), adjustments for cause-specific cancer deaths, age, race, calendar time-specific mortality rates of control **SMR (95% CI; observed/expected cases):** 0.93 (0.87-0.99; 950/1024) for overall mortality due to cancer; 0.80 (0.40-1.44; 11/14) for lymphosarcoma; 1.31 (0.97-1.74; 48/37) for leukemia in overall cohort; 1.43 (1.04-1.91; 45/32) for leukemia among ever-hourly subjects; 2.19 (0.88-4.51; 7/3.2) for field maintenance workers; 2.63 (0.32-9.51; 2/0.8) for production laborers; 1.88 (0.39-5.48; 3/1.6) for maintenance laborers. 2.51 (1.40-4.14; 15/6.0) for leukemia in polymerization group 2.48 (1.00-5.11; 7/2.8) for leukemia in coagulation group 2.65 (1.41-4.53; 13/4.9) for leukemia in maintenance labor group 4.31 (2.07-7.93; 10/2.3) for leukemia in laboratory group	Work histories were incomplete for 12% of the workers, workers who terminated their employment before 1950 were incompletely followed up, and there was a lack of medical records confirming death by leukemia and other lymphopoietic cancers. The authors concluded, however, that their study's large cohort size, long follow-up period, and objective study procedures for work history and cause of death determination support a positive association between leukemia and occupational exposure to SBR. Workers in the SBR industry have potential exposure to a number of agents besides 1,3-butadiene, including styrene and benzene. Based upon the presence of elevated SMRs for some but not all process groups, the authors suggests that the leukemia association is due to either 1,3-butadiene alone or 1,3-butadiene in combination with styrene.	Delzell et al. (1996)
Follow-up of cohort	**Exposed:** 16,610 men employed ≥ 1 yr during 1943-1991 at one of six North American SBR manufacturing plants **Controls:** U.S. male general population or Ontario male general population	**Estimation:** identified work areas in each process, noted historical changes in exposure potential and specific tasks involving exposure, used mathematical models to calculate job- and time-period specific average exposures **Duration:** ≥1 yr :1943-1991 **Categories:** estimated cumulative exposure as ppm-yr (0, <1, 1-19, 20-79, 80+)	**Evaluation:** analyzed leukemia mortality rates and exposure estimates using stratified and Poisson regression analyses **Evidence for Dose-Response:** Mantel-Haenszel rate ratios, adjusted for race, age, and cumulative styrene exposure, increased with cumulative 1,3-butadiene exposure. The rate ratios were 1, 2.0, 2.1, 2.4, and 4.5 for the 0, <1, 1-19, 20-79, and 80+ ppm-yr exposure groups, respectively (p for trend = 0.01). After exclusion of all subjects with zero cumulative exposure to butadiene and >10 ppm-yr styrene exposure, the respective rate ratios were 1, 3.5, and 5.1 for the 0.1-19, 20-79, and 80+ ppm-yr exposure groups (p for trend = 0.03).	There was some association with styrene exposure, but the pattern was less clear than for exposure to 1,3-butadiene.	Macaluso et al. (1996)

Table 3-1. Recent Human Studies of Effects of Exposure to 1,3-Butadiene (Continued)

Design	Population Groups	Exposure	Effects	Potential Confounders/Effects	References
Case-control; reanalysis of cohort	**Exposed:** A) 26 workers in SBR manufacturing plants who developed leukemia B) 3429 former workers in three SBR manufacturing plants hired before 1960 for ≥10 yr **Controls:** A) 84 workers in SBR manufacturing plants, individually matched to cases by plant worked, age at hire, year of hire, and duration worked B) general U.S. or Ontario population	**Estimation:** exposure score for each worker = assigned rank for each job x no. months in each job and summed for all jobs; validated by correlation between assigned ranks and exposure level derived from personal monitoring ($p < 0.001$) **Duration:** ≥10 yr	**Evaluation:** A) calculated OR for leukemia with logistic regression models using 1,3-butadiene exposure as a continuous variable. All ORs were significantly increased at $p < 0.05$. Model — Odds Ratio (95% CI) 1) mixed jobs and 1,3-butadiene — 5.6 (0.5 - 18.1) 2) controls matched for duration of work — 8.5 (1.1 - 5.4) 3) controls not matched for for duration of work — 6.0 (0.8 - 47.2) 4) controls not matched for duration of work, adjust for duration added — 9.2 (1.04 - 81.1) B) calculated **SMR** (95% CI; observed/expected cases) by cause of death 1.63 (1.13 - 2.27; 34/20.9) for all lymphohematopoietic cancers; 1.81 (1.01 - 2.99; 15/8.3) for leukemia and aleukemia 1.16 (0.37 - 2.70; 5/4.31) for lymphosarcoma and reticulosarcoma; 2.43 (0.78 - 5.68; 5/2.06) for Hodgkin's disease; 1.49 (0.68 - 2.82; 9/6.04) for other lymphatic tissue	Exposure to styrene.	Matanoski et al. (1993)
Cohort	**Exposed:** 614 male employees of a Shell Oil manufacturing facility **Controls:** male employees at the same facility not exposed to butadiene	**Estimation:** identified workers active for at least five years or half of their total employment duration (three month minimum) in areas with potential butadiene exposure **Duration:** 1948 to 1989	**Evaluation:** calculated SMRs (95% CI) for cancer based on rates for county where facility is located, as well as SMbRs and hematological tests **SMRs:** 34 (9-87) for all cancer 42 (5-151) for lung cancer **SMbR:** 51(22-100) for all neoplasms Data from the hematological tests showed no significant differences between workers potentially exposed to butadiene and those not exposed. **[Divide values by 100 to compare with other studies.]**	The authors acknowledge a lack of statistical stability, as illustrated by the wide range of 95% CIs, attributable to the small numbers of deaths from any specific cause in this study.	Cowles et al. (1994)

Abbreviations: SMR = standardized mortality ratio; SMbR = standardized morbidity rate; OR = odds ratio; CI = confidence interval

4.0 EXPERIMENTAL CARCINOGENICITY

Experimental carcinogenicity studies reported prior to 1992 were reviewed by IARC (1992, pp. 254-264; see Appendix A) and it was concluded that there was sufficient evidence for the carcinogenicity of 1,3-butadiene in experimental animals. This conclusion was based on four inhalation studies with mice and one with rats that resulted in a dose-related increase in the incidences of tumors at multiple sites (including tumors of the hematopoietic system and an uncommon neoplasm of the heart in male and female mice) in both sexes of both species. These studies indicated that mice were sensitive to lower levels than rats, so later studies further explored the carcinogenic effects of 1,3-butadiene in mice.

In an NTP-sponsored mouse inhalation study reviewed by IARC (1992), male and female mice were exposed by inhalation to 625 or 1250 ppm (1380 or 2765 mg/m^3) 1,3-butadiene, 6 hr/day, five days/wk (NTP, 1984). This study, originally scheduled for two years, was terminated at week 60 for males and week 61 for females due to reduced animal survival from malignant neoplasms in multiple organs. Malignant lymphomas were observed in males and females after 20 weeks of exposure. At study termination, a significant increase in the incidence of neoplasms was observed at several sites in males and females, including hemangiosarcoma of the heart, malignant lymphomas of the hematopoietic system, alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma (both separately and combined), papilloma of the forestomach, and squamous cell carcinoma of the forestomach. In female mice, mammary gland acinar cell carcinoma and ovarian granulosa cell tumors, and hepatocellular adenoma or carcinoma (combined) were also significantly increased. No neoplastic lesions of the nasal cavity were observed in males or females at any dose level.

Additional experimental inhalation carcinogenicity studies have been conducted by NTP to explore the relationship in mice between dose or exposure duration and 1,3-butadiene-induced tumor incidence (NTP, 1993, pp. 5-95; see Appendix B). In one study, B6C3F$_1$ mice were exposed by inhalation to 6.25, 20, 62.5, 200, or 625 ppm (13.8, 44, 138, 442, or 1380 mg/m^3) for 6 hr/day, 5 days/week for up to two years (NTP, 1993; see also Melnick and Huff, 1993). Interim sacrifices were conducted after 40 and 65 weeks of exposure. Survival was reduced among males and females exposed to 20 ppm (44 mg/m^3) or more, largely due to the development of fatal tumors. Multi-site neoplasms were observed in male and female mice. Malignant lymphomas of thymic origin occurred as early as week 23 in males exposed to 625 ppm (1380 mg/m^3) and in females exposed to 200 and 625 ppm (442 and 1380 mg/m^3). Hemangiosarcomas of the heart were increased in males exposed to 62.5, 200, or 625 ppm (138, 442, or 1380 mg/m^3), and in females exposed to 200 or 625 ppm (442 or 1380 mg/m^3). Lung neoplasms were increased in males exposed to 62.5 or 200 ppm (13.8 or 442 mg/m^3) and in females exposed to all doses. Other tumor sites included the forestomach, harderian gland, liver, preputial gland (males), mammary gland (females), and ovary (females).

This study also included stop-exposure studies to further examine the relationship between cancer development and exposure duration and level. In these studies, mice were exposed to 1,3-butadiene at 200 ppm (442 mg/m^3) for 40 weeks, 625 ppm (1380 mg/m^3) for 13 weeks, 312 ppm (690 mg/m^3) for 52 weeks, and 625 ppm (1380 mg/m^3) for 26 weeks. After each exposure was terminated, the mice were maintained in control chambers for the remainder of the 2-year study. Multi-site neoplasms developed in all groups. The incidence of lymphomas

was greater after exposure to a higher concentration for a short time than after exposure to a lower concentration for a longer period, for exposure groups with the same cumulative exposure. Survival was reduced in all exposed groups, but the incidence of other tumor types did not show the same relationship to exposure duration/level as the incidence of lymphomas.

To assess occupational situations of unavoidable but short-term exposure to high concentrations of 1,3-butadiene, $B6C3F_1$ mice were exposed by inhalation to 1000, 5000, or 10,000 ppm (2212, 11,060, 22,120 mg/m^3) 1,3-butadiene for two hours (Bucher et al., 1993). The exposed mice were then held for two years, and tumor incidence assessed (see **Table 4-1**). A statistically significant increase in the incidence of neoplastic lesions was not induced using this exposure protocol.

Table 4-1. Experimental Mammalian Carcinogenicity of 1,3-Butadiene

Species, Strain, and Age	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Exposure Duration	Results/Comments	Reference
Mice B6C3F$_1$ 8-10 wk old	60 M, 60 F	60 M, 60 F	Not Reported	1000, 5000 or 10,000 ppm (2212, 11,060, or 22,120 mg/m^3) in inhalation chamber	2 hr; then held for 2 yr	No statistically significant increased incidences of neoplastic lesions in males or females Lymphoma: M: 7/59, 8/58, 8/58, 10/58 F: 13/57, 19/56, 18/57, 13/58 Hemangiosarcoma: M: 1/59, 0/58, 0/58, 0/58 F: 0/56, 0/56, 0/57, 0/58 Alveolar-bronchiolar neoplasm: M: 8/59, 9/58, 12/57, 8/58 F: 3/56, 4/56, 0/57, 3/58 Squamous cell neoplasm: M: 0/59, 1/58, 1/58, 3/58 F: 0/57, 1/56, 0/57, 0/58 Acinar cell neoplasm: M: 0/59, 0/58, 1/58 F: 2/57, 1/56, 3/57, 4/58 Granulosa cell neoplasm: F: 0/53, 0/52, 1/53, 0/56 Hepatocellular neoplasm: M: 17/59, 21/58, 21/57, 18/58 F: 5/56, 6/55, 8/57, 3/58	Bucher et al. (1993)

5.0 GENOTOXICITY

Studies on the genotoxic effects of 1,3-butadiene published prior to 1992 have been reviewed by IARC (1992, pp. 264-272, see Appendix A) and prior to 1993 by NTP (1993, pp. 5-10, see Appendix B). More recent studies are summarized below and in **Table 5-1**.

5.1 Genotoxicity Studies Reviewed by IARC (1992)

In bacterial systems, 1,3-butadiene induced a positive increase in *his* gene mutations in *Salmonella typhimurium* strain TA1530 and a weak positive increase in strain TA1535, both only in the presence of S9 activation.

In lower eukaryotes, 1,3-butadiene did not induce wing spot mutations in *Drosophila melanogaster*.

Using cultured mammalian cells, 1,3-butadiene was weakly positive for the induction of sister chromatid exchanges (SCE) in Chinese hamster ovary cells in the presence but not the absence of rat liver S9. Conflicting results were obtained in mitogen-stimulated human lymphocyte cultures in which 1,3-butadiene with and without S9 activation was positive for SCE induction in one study but negative in another study.

In vivo, 1,3-butadiene induced both DNA-DNA and DNA-protein cross-links, as measured by alkaline elution, in the liver and lungs of $B6C3F_1$ mice but not Sprague-Dawley rats exposed via inhalation. Another study reported that it did not induce cross-links in the livers of either species. Following a 2-day inhalation exposure, 1,3-butadiene induced a positive increase in SCE in the bone marrow of male $B6C3F_1$ mice but not Sprague-Dawley rats. Unscheduled DNA synthesis was not induced in liver hepatocytes by 1,3-butadiene in either $B6C3F_1$ mice or Sprague-Dawley rats exposed via inhalation for 2 days. Chromosome aberrations were induced by 1,3-butadiene in the bone marrow of male $B6C3F_1$ and NIH Swiss mice exposed via inhalation for 6 hours. Likewise, 1,3-butadiene induced a positive increase in micronucleated polychromatic and normochromatic erythrocytes (PCE and NCE, respectively) of NMRI mice exposed via inhalation for 23 hours but not in the bone marrow of Sprague-Dawley rats exposed for 2 days. Lastly, 1,3-butadiene induced a positive increase in both dominant lethal mutations and sperm abnormalities in male Swiss CD-1 mice.

In humans, the frequency of SCE, chromosomal aberrations, or micronuclei was not induced in peripheral blood lymphocytes of workers in a Finnish 1,3-butadiene manufacturing facility.

5.2 Additional Genotoxicity Studies Reviewed by NTP (1993)

1,3-Butadiene was positive for the induction of *his* gene mutations in *S. typhimurium* strain TA1535 both with and without rat and hamster S9 activation when tested in a sealed desiccator for 48 hours. The authors speculated that the unexpected positive response without activation may have been induced by a volatile mutagenic intermediate since the plates with activation were tested in the same desiccator as those without S9. 1,3-Butadiene was negative in all other strains tested including TA97, TA98, and TA100 both with and without rat or hamster S9.

In lower eukaryotes, a negative response was observed for sex-linked recessive lethal mutations in 1,3-butadiene-treated *D. melanogaster*. Canton-S males were exposed to 1,3-butadiene vapors for 72 hours followed by mating to Basc females for three 2-3 day mating periods.

In vitro in mammalian cells, the frequency of *tk* gene mutations was not increased in 1,3-butadiene-exposed mouse lymphoma L5178Y cells in the presence or absence of rat liver S9 activation. The lack of mutagenic activity was attributed to the low solubility of gaseous 1,3-butadiene in the culture medium.

In vivo, 1,3-butadiene induced a positive increase in SCE and chromosome aberrations in the bone marrow of male $B6C3F_1$ mice exposed via inhalation for 10 days, at exposures as low as 6.25 ppm (Tice et al., 1987; cited by NTP, 1993, and by IARC, 1992). Likewise, 1,3-butadiene induced a positive increase in micronucleated PCE and NCE measured in peripheral blood of male $B6C3F_1$ mice exposed via inhalation for 10 days. A positive MN result was also obtained in the

peripheral blood (both PCE and NCE) of male and female B6C3F$_1$ mice exposed to 1,3-butadiene for either 13 weeks or 15 months.

5.3 Genotoxicity Studies Published Post IARC/NTP

Adler and Anderson (1994) reported a positive increase in dominant lethal mutations in male C3H mice exposed via inhalation (1300 ppm, 6 h/day for 5 days) for either one week or ten weeks prior to mating. Adler et al. (1995) later reported that 1,3-butadiene also induced heritable translocations in the late spermatids of C3H mice dosed via the same regimen.

Sorsa et al. (1996) found an increase in the level of hemoglobin adducts in the peripheral blood of 56 1,3-butadiene-exposed workers [generally <1 ppm (2.21 mg/m^3)] at three separate European manufacturing/processing plants. However, no significant increase in the levels of *ras* oncoproteins, as measured by an immunoconcentration technique, was found in the plasma of exposed workers. Anderson et al. (1996) observed a similar lack of increase in *ras* oncoproteins in the plasma of 10 exposed male workers [mean level of 1.8 ppm (4.0 mg/m^3)] at a Czech plant.

The frequency of *hprt* gene mutations in blood lymphocytes from 1,3-butadiene-exposed workers [median levels of 1.0-3.5 ppm (2.2-7.7 mg/m^3)] at a Chinese rubber plant was slightly but not significantly higher (statistically) than that of unexposed coworkers (Hayes et al., 1996). Ward et al. (1996), however, reported a significant increase in the frequency of *hprt* gene mutations in the lymphocytes of 1,3-butadiene-exposed workers [mean level of 3.5 ppm (7.7 mg/m^3)] compared to non-exposed coworkers at a recently modernized SBR plant.

No significant increase in SCE, chromosomal aberrations, or micronuclei was reported in mitogen-stimulated peripheral blood lymphocytes of workers in a Finnish 1,3-butadiene manufacturing facility (Sorsa et al., 1994). Ambient air concentrations were < 1 ppm (< 2.21 mg/m^3) and workers used protective clothing and respirators. In a similar study, forty 1,3-butadiene exposed workers at a U.S. production plant [< 2 ppm (< 4.4 mg/m^3)] did not show an increase in lymphocyte SCE frequency over controls (Kelsey et al., 1995). However, when lymphocytes were cultured from these workers and subsequently exposed *in vitro* to the diepoxide metabolite, six of the 40 workers (15%) exhibited a positive increase in SCE. In a later study, Sorsa et al. (1996) again found no increase in lymphocyte SCE in 56 1,3-butadiene-exposed workers [generally < 1 ppm (2.2 mg/m^3)] at three separate European manufacturing/processing plants.

The frequency of chromosome aberrations in blood lymphocytes from ten 1,3-butadiene-exposed workers [mean level of 3.5 ppm (7.7 mg/m^3)] was higher than ten matched nonexposed coworkers (Au et al., 1996). Blood samples were also cultured and challenged *in vitro* with 10 cGy gamma rays to assess DNA repair competency. Lymphocytes from exposed workers had a significantly higher percentage of radiation-induced aberrant cells and dicentrics, suggesting to the authors that the workers were deficient in strand break repair. Sorsa et al. (1996), however, found no increase in lymphocyte chromosome aberrations or micronuclei in 56 1,3-butadiene-exposed workers [generally < 1 ppm (2.2 mg/m^3)] at three separate European manufacturing/processing plants. In a similar study, 24 1,3-butadiene exposed workers (exposure level not given) did not show an increase in lymphocyte chromosome aberration frequency over controls (Hallberg et al., 1997). However, when lymphocytes were cultured from these workers and subsequently exposed *in vitro* to 1,3-butadiene, a consistent but nonsignificant increase in chromosome aberrations was observed. This increase suggested an abnormal DNA repair response to the authors.

Table 5-1. Summary of 1,3-Butadiene Genotoxicity Studies Published Post IARC/NTP

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response -/+ Activation	Comments	Reference
Mammalian Systems *In Vivo*							
C3H male mice	Dominant lethal mutations	NA	NG	1300 ppm (2876 mg/m^3) via inhalation - 6 hr/day for 5 days	Positive	Dominant lethal mutations were observed in mating weeks 2 and 3 after the end of exposure, corresponding to exposed spermatids.	Adler and Anderson (1994)
C3H male mice	Heritable translocations	NA	NG	1300 ppm (2876 mg/m^3) via inhalation – 6 hr/day for 5 days	Positive	1,3-Butadiene induced heritable translocations in late spermatids. The translocation frequency was 2.7%, 54 times higher than historical controls.	Adler et al. (1995)
Human Studies							
56 workers at 3 European manufacturing/ processing plants	Hemoglobin adducts	NA	NG	Generally <1 ppm (2.2 mg/m^3)	Positive	Hemoglobin adducts levels were increased among workers with higher potential exposure.	Sorsa et al. (1996)
56 workers at 3 European manufacturing/ processing plants	Increase in *ras* oncoproteins	NA	NG	Generally <1 ppm (2.2 mg/m^3)	Negative	No exposure related effect was seen in the *ras* oncoprotein levels of plasma samples from exposed workers.	Sorsa et al. (1996)
10 workers at a Czech manufacturing/ processing plant	Increase in *ras* oncoproteins	NA	NG	Mean level of 1.8 ± 2.8 ppm (4.0 ± 6.2 mg/m^3)	Negative	There was no statistically significant difference between the exposed and control groups.	Anderson et al. (1996)
7 workers at a recently modernized SBR plant	*hprt* gene mutations	NA	NG	Mean level of 3.5 ppm (7.7 mg/m^3)	Positive	The mean frequency of mutant lymphocytes in exposed workers was significantly higher when compared to nonexposed.	Ward et al. (1996)

Table 5-1. Summary of 1,3-Butadiene Genotoxicity Studies Published Post IARC/NTP (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response -/+ Activation	Comments	Reference
Human Studies (continued)							
Workers at a Chinese rubber production plant	*hprt* gene mutations	NA	NG	Median levels of 1.0-3.5 ppm (2.2-7.7 mg/m^3)	Negative	The frequency of mutations in blood lymphocytes of exposed workers was slightly but not significantly increased over controls.	Hayes et al. (1996)
Workers at a Finnish manufacturing plant	Sister chromatid exchanges (SCE)	NA	NG	<1 ppm (2.2 mg/m^3)	Negative	Workers used protective clothing and respirators.	Sorsa et al. (1994)
40 exposed workers at a U.S. production plant	SCE	NA	NG	<2 ppm (4.4 mg/m^3)	Negative	When lymphocytes were cultured from these workers and subsequently exposed *in vitro* to the diepoxide metabolite, 6 workers (15%) exhibited a positive increase.	Kelsey et al. (1995)
56 workers at 3 European manufacturing/ processing plants	SCE	NA	NG	Generally <1 ppm (2.2 mg/m^3)	Negative	17 exposed workers from a manufacturing plant in Portugal, 23 exposed worker from a polymerization plant in the Czech Republic, and 16 workers from a processing plant in the Czech Republic.	Sorsa et al. (1996)
Workers at a Finnish manufacturing plant	Chromosome aberrations	NA	NG	<1 ppm (2.2 mg/m^3)	negative	Workers used protective clothing and respirators	Sorsa et al. (1994)
56 workers at 3 European manufacturing/ processing plants	Chromosome aberrations	NA	NG	Generally <1 ppm (2.2 mg/m^3)	negative	17 exposed workers from a manufacturing plant in Portugal, 23 exposed worker from a polymerization plant in the Czech Republic, and 16 workers from a processing plant in the Czech Republic.	Sorsa et al. (1996)

Table 5-1. Summary of 1,3-Butadiene Genotoxicity Studies Published Post IARC/NTP (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response +/- Activation	Comments	Reference
10 exposed workers	Chromosome aberrations	NA	NG	3.5 ppm (7.7 mg/m^3)	Positive	When lymphocytes were cultured from these workers and subsequently exposed to 10 cGy gamma rays *in vitro*, a significant increase in aberrant cells was observed.	Au et al. (1996)
24 exposed workers at a manufacturing plant	Chromosome aberrations	NA	NG	NG in source used	Negative	When lymphocytes were cultured from these workers and subsequently exposed *in vitro*, a consistent but nonsignificant increase was observed.	Hallberg et al. (1997)
Workers at a Finnish manufacturing plant	Micronuclei induction	NA	NG	<1 ppm (2.2 mg/m^3)	Negative	Workers used protective clothing and respirators	Sorsa et al. (1994)
56 workers at 3 European manufacturing/ processing plants	Micronuclei induction	NA	NG	Generally <1 ppm (2.2 mg/m^3)	Negative	17 exposed workers from a manufacturing plant in Portugal, 23 exposed worker from a polymerization plant in the Czech Republic, and 16 workers from a processing plant in the Czech Republic.	Sorsa et al. (1996)

Abbreviations: NA = not applicable; NG = not given

6.0 OTHER RELEVANT DATA

6.1 Metabolism, Distribution, and Excretion

6.1.1 Metabolism

Cytochrome P450 oxidizes 1,3-butadiene to butadiene monoepoxide or monoxide (BMO), also known as 1,2-epoxy-3-butene. Human isoforms 2A6 and 2E1 exhibit the highest oxidation rates of all active cytochrome P450 isoforms (Elfarra et al., 1996). The metabolism of butadiene to BMO and the oxidation of BMO to diepoxybutane (butadiene diepoxide; BDE) has been demonstrated in human liver microsomes (Csanády et al., 1992; Seaton et al., 1995). The same conversion can be catalyzed by human or mouse myeloperoxidase, an enzyme which is plentiful in bone marrow (Maniglier-Poulet et al., 1995). Myeloperoxidase is also found in bronchioles isolated from $B6C3F_1$ mice and Sprague-Dawley rats, and exhibits about twice as much activity in mice as in rats (Seaton et al., 1996). A combination of human myeloperoxidase and 1,3-butadiene yields a mixture of BMO and crotonaldehyde (Duescher and Elfarra, 1992 abstr.). A highly sensitive gas chromatography (GC) assay with verification by GC/mass spectrometry revealed that liver microsomes from mice, rats, and humans exposed to 1,3-butadiene also produce BMO and crotonaldehyde, although the latter is present at only 2 to 5% of the level of BMO (Elfarra et al., 1996).

Oxidation of BMO to BDE, is catalyzed by cytochrome P450, specifically the 2E1 isoform. The 3A4 isoform, on the other hand, is active only at higher levels of 1,3-butadiene exposure (Seaton et al., 1995). BMO undergoes metabolic inactivation by epoxide hydrolase and GSH *S*-transferase (Csanády et al., 1992; Sabourin et al., 1992; Sharer and Elfarra, 1992; Bechtold et al., 1994; all cited by Osterman-Golkar and Bond, 1996). The same two enzymes catalyze the metabolic inactivation of BMO (Boogaard et al., 1996; Boogaard and Bond, 1995). GSH *S*-transferase converts BMO to 1-hydroxy-2-(*N*-acetylcysteinyl-*S*)-3-butene, a mercapurate called M-II (Sabourin et al., 1992), or *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-*L*-cysteine (Nauhaus et al., 1996). If epoxide hydrolase acts first, BMO is hydrolyzed to 3-butene-1,2-diol, which GSH *S*-transferase conjugates with GSH, forming 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*)butane, called M-I (Sabourin et al., 1992). Partial hydrolysis of BDE or oxidation of butenediol, formed from hydrolysis of BMO, can form a diol epoxide (3,4-epoxy-1,2-butanediol) (Henderson, 1996).

6.1.2. Distribution

The metabolites BMO and BDE were detected in various tissues in rats and mice following a 4-hour inhalation exposure to 62.5 ppm (138 mg/m^3) 1,3-butadiene (Thornton-Manning et al., 1996). Both epoxides were present at much higher concentrations in mice than in rats. Levels of BMO were highest in the fat, while BDE levels were highest in the blood. In mice, both BMO and BDE were detected in blood, heart, fat, liver, lung, spleen, thymus, and bone marrow. In rats, BMO was detected in the blood, heart, fat, spleen, thymus, and bone marrow, while BDE was detected in all tissues tested except for liver and bone marrow. However, the levels of BDE were very low and at least one rat did not have detectable levels of BDE in the lung, spleen, and thymus (Thornton-Manning et al., 1995). In two other studies, measurable levels of BDE were not detected in the blood of rats (Bechtold et al., 1995) or in the blood, liver, or lungs of rats following inhalation of 1,3-butadiene (Himmelstein et al., 1996).

6.1.3 Excretion

Following inhalation of 1,3-butadiene, mice and rats excreted about 50% of the dose in the urine, exhaled 15-20% as volatile metabolites or 1,3-butadiene, and retained 10-15% in the body. The remaining small fractions were excreted in the feces or exhaled as CO_2. In the monkey, however, twice as much of the original dose was exhaled as CO_2 as was excreted in the urine. The difference between the excretion pathways of the rodents and the primate possibly reflects the high epoxide hydrolase activity found in primate liver, which converts BDE or the diol epoxide to the tetrol, and then to CO_2 and water (Dahl et al., 1991).

The mercapturic acids M-I and M-II are the two major urinary metabolites found in mice, rats, and monkeys exposed to ^{14}C-labeled 1,3-butadiene. The ratio of M-I to the sum of M-I and M-II was proportional to the epoxide hydrolase activity in the liver of each species (Sabourin et al., 1992; Bechtold et al., 1994). The urine of humans exposed to 1,3-butadiene in the workplace was examined and found to contain M-I but not M-II (Bechtold et al., 1994). Urinary levels of M-II were higher in amount relative to the total urinary metabolites in mice as compared to rats and monkeys (Sabourin et al., 1992; Bechtold et al., 1994). This suggested to Henderson (1996) that conjugation of BMO with GSH is a major pathway for its removal in mice, while hydrolysis to the butenediol followed by GSH conjugation is a major mechanism for removal in primates.

Urinary metabolites were analyzed by nuclear magnetic resonance (NMR) in male Sprague-Dawley rats and male B6C3F$_1$ mice exposed to 800 ppm (1770 mg/m^3) [1,2,3,4-^{13}C] 1,3-butadiene by nose-only inhalation. Three metabolites accounted for 87% of the total metabolites in rat urine and 73% in mouse urine. These metabolites were *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-*L*-cysteine (44% in mouse, 8% in rat), M-I (7% in mouse, 26% in rat), and M-II (22% in mouse, 53% in rat). Three percent of the total metabolites in mouse and 5% in rat were 3-butene-1,2-diol, the butenediol created when BMO is hydrolyzed. A possible intermediate between BMO and M-II, *S*-(1-(hydroxymethyl)-2-propenyl)-*L*-cysteine, was detected as 5% of the metabolites excreted in mouse, but not rat, urine. Other metabolites found in mouse, but not rat, urine included; *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-*L*-cysteine (4%), *N*-acetyl-*S*-(3-hydroxypropyl)cysteine (5%), *N*-acetyl-*S*-(2-carboxyethyl)cysteine (5%), and *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-*L*-cysteine (5%). The first of these mouse urinary metabolites is the hemithioacetal of 3-butenal, an intermediate in the oxidation of butadiene to crotonaldehyde. *N*-Acetyl-*S*-(3-hydroxypropyl)cysteine and *N*-acetyl-*S*-(2-carboxyethyl)cysteine could be derived from conversion of 1,3-butadiene to acrolein followed by conjugation to *S*-(oxoethyl)glutathione and oxidation or reduction, respectively (Sanduja et al., 1989; Ramu et al., 1995; Patel et al., 1980; Kaye, 1973; Mitchell and Peterson, 1989; Draminski et al., 1983; all cited by Nauhaus et al., 1996) or by the intermediates in metabolism or further metabolism of M-I (Sabourin et al., 1992). *N*-Acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-*L*-cysteine is derived from the glutathione conjugate of BDE (Nauhaus et al., 1996). Rat urine, but not mouse urine, contained 1,3-dihydroxypropanone (5%) (Nauhaus et al., 1996).

6.2 Pharmacokinetics

Human and rat liver microsomes are half as active as those from mice at converting 1,3-butadiene to BMO (Schmidt and Loeser, 1985; cited by Henderson et al., 1996), while in lung microsomes, the difference is 10-fold (Csanády et al., 1992; cited by Henderson et al., 1996). When the removal of 1,3-butadiene from air was used as a measure of metabolism, Kreiling et al. (1986; cited by Henderson et al., 1996) concluded that the metabolism of 1,3-butadiene was twice as fast in mice as in rats.

Kohn and Melnick (1996) established a physiologically based pharmacokinetic (PBPK) model to study 1,3-butadiene metabolism. Compartments for blood, liver, lung, fat, GI tract, other rapidly perfused tissue, and slowly perfused tissue were included, with blood being distributed among compartments as arterial and venous blood and subcompartmentalized into vascular spaces for each tissue compartment. The viscera and GI tract were separated to more realistically represent perfusion of the liver, and gases were represented as being exchanged with ambient air in the alveolar space. Metabolic conversion of 1,3-butadiene to BMO was described as occurring in the liver and lung. Detoxification of BMO was catalyzed by epoxide hydrolase (Kohn and Melnick, 1996). The model was able to fit the uptake data for 1,3-butadiene and BMO gathered by Kreiling et al. (1986, 1987; both cited by Kohn and Melnick, 1996), but was more sensitive to the physiological values than to the biochemical parameter values.

Johanson and Filser (1996) constructed a model incorporating first-pass intrahepatic hydrolysis of BMO, reduced alveolar ventilation, and two-substrate Michaelis-Menten kinetics for the GSH conjugation of BMO. Relative internal doses of BMO (expressed as the relation between steady-state concentrations or AUCs in mixed venous blood) were reported to be 1.6, 1.0, and 0.3 under nonsaturating conditions in mouse, rat, and human, respectively. These doses are predicted

to result from inhalation exposure to 10 ppm butadiene for 12 hours in an open system. Neither of these studies took into account the conversion of BMO to crotonaldehyde, a carcinogen (Chung et al., 1986; cited by Elfarra et al., 1996), or the diepoxide BDE, a potent mutagen (Vgood et al., 1981; Cochrane and Skopek, 1994; cited by Thornton-Manning et al., 1995) because of the lack of biochemical data on the activities of these two substances in mouse, rat, and human tissue . While no models have yet been found incorporating crotonaldehyde, Csanády et al. (1996) designed a physiological toxicokinetic model taking into consideration formation of BDE in mice. Rate constants for BDE formation and elimination were those obtained in liver cell fractions (Csanády et al., 1992). Several other PBPK models for 1,3-butadiene have been published and are reviewed by Sweeney et al. (1996).

6.3 Structure-Activity Relationships

Isoprene is a 2-methyl analog of 1,3-butadiene (NTP, 1995 draft). Based on NTP rodent carcinogenicity data, IARC (1994) concluded that there was sufficient evidence for the carcinogenicity of isoprene in experimental animals and that isoprene was a possible carcinogen in humans. Human studies have not been conducted. Isoprene is a multiple-organ carcinogen in mice (NTP, 1995 draft; Melnick et al., 1994). Male and female mice exposed by inhalation to isoprene vapor for six months showed increases in the incidence of squamous cell papillomas and carcinomas of the forestomach, alveolar/bronchiolar adenomas and carcinomas, hepatocellular adenomas and carcinomas, and harderian gland adenomas and carcinomas (NTP, 1995 draft; Melnick et al., 1994).

Male rats exposed by inhalation to isoprene vapor for six months showed an increase in the incidence and severity of interstitial cell hyperplasia of the testes and a slight increase in the incidence of interstitial cell adenomas (NTP, 1995 draft; Melnick et al., 1994).

Only limited data on the genotoxicity of isoprene are available (IARC, 1994; NTP, 1995 draft). As reviewed by NTP (1995 draft), mutagenicity tests in *S. typhimurium* were negative, and no induction of SCE or chromosomal aberrations was observed in Chinese hamster ovary cells. In contrast to the negative *in vitro* results, isoprene induced significant increases in the frequency of SCE in bone marrow cells and of micronucleated PCE and NCE in peripheral blood of mice exposed by inhalation for 12 days over a 16-day period. Also, exposure of mice and rats by i.p. injection and mice by inhalation resulted in isoprene hemoglobin-protein adducts. The diepoxide intermediate of isoprene metabolism is mutagenic in *Salmonella.*

6.4 Metabolites

6.4.1 Butadiene Monoepoxide (BMO)

BMO is genotoxic in mammalian and bacterial assays (de Meester, 1988; cited by Thornton-Manning et al., 1995). *In vivo* experiments show it to be carcinogenic as well as genotoxic (van Duuren et al., 1963, 1966; Conner et al., 1983; Sharief et al., 1986; all cited by IARC, 1992).

6.4.2 Butadiene Diepoxide (BDE)

BDE is also genotoxic and carcinogenic in a variety of mammalian and bacterial assays (de Meester, 1988; cited by Thornton-Manning et al., 1995; IARC, 1992). Certain assays have shown BDE to be a more potent mutagen than BMO (Vgood et al., 1981; Cochrane and Skopek, 1994; both cited by Thornton-Manning et al., 1995). Studies done in human cells found that BDE was mutagenic *in vitro* at concentrations 10- to 100-fold lower than BMO (Cochrane and Skopek, 1994; cited by Bond et al., 1996).

7.0 MECHANISMS OF CARCINOGENESIS

The most likely mechanism for the carcinogenicity of 1,3-butadiene is the induction of mutagenic DNA damage by one or more reactive metabolites (Melnick and Kohn, 1995). As summarized in Section 5, exposure to 1,3-butadiene resulted in increased levels of DNA damage and mutations in experimentally exposed animals and occupationally exposed workers. In mice, exposure by inhalation to 1,3-butadiene resulted in increased levels of DNA-DNA and DNA-protein cross-links in liver and lung, N^7-alkylguanine adducts in liver DNA, SCE and chromosomal aberrations in bone marrow cells, micronucleated erythrocytes in peripheral blood, *hprt* mutations in lymphocytes, dominant lethal mutations, and sperm abnormalities. The mutational spectra of 1,3-butadiene at the *hprt* locus in mouse lymphocytes are similar to that for ethylene oxide, an alkylating agent classified by IARC (1994) as a human carcinogen (Melnick and Kohn, 1995). Furthermore, activated K-*ras* oncogenes (predominantly due to a specific codon 13 mutation and inactivated tumor suppressor genes were detected in 1,3-butadiene-induced tumors in mice (Goodrow et al., 1990; Wiseman et al., 1994; cited by Melnick and Kohn, 1995). These events are analogous to genetic alterations frequently observed in a wide variety of human cancers (Melnick and Kohn, 1995).

In workers, occupational exposure to 1,3-butadiene induced a significant increase in hemoglobin adducts, and in some but not all studies, *hprt* mutations and chromosomal aberrations in lymphocytes. In addition, the same N^7-alkylguanine adduct detected in liver DNA of mice exposed to 1,3-butadiene was identified in the urine of an occupationally exposed worker.

While *in vitro* studies show a lack of *in vitro* mutagenicity in *Salmonella* in the absence of metabolic activation, *in vivo* studies suggest that the genotoxicity of 1,3-butadiene depends on its metabolism to reactive species, presumably BMO and BDE. *In vivo* experiments show BMO and BDE to be carcinogenic as well as genotoxic (van Duuren et al., 1963; 1966; Conner et al., 1983; Sharief et al., 1986; all cited by IARC, 1992) and BDE is a more potent mutagen than BMO (Vgood et al., 1981; Cochrane and Skopek, 1994; both cited by Thornton-Manning et al., 1995). Both of these metabolites are produced by rat, mouse, and human tissue (Melnick and Kohn, 1995; Bond et al., 1995; Seaton et al., 1995). Species differences in tumor response are probably a result of differences in the rate of metabolism and/or detoxification of 1,3-butadiene and its genotoxic intermediates and subsequent levels of genotoxic damage induced *in vivo*.

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APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 54 (Occupational Exposure to Mists and Vapors from Strong Inorganic Acids; and Other Industrial Chemicals) 1,3-Butadiene pp. 237-285, 1992

1,3-BUTADIENE

This substance was considered by previous working groups in June 1985 (IARC, 1986a; see also correction, IARC, 1987a) and March 1987 (IARC, 1987b). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 106-99-0
Chem. Abstr. Name: 1,3-Butadiene
IUPAC Systematic Name: 1,3-Butadiene
Synonyms: Biethylene; bivinyl; butadiene; buta-1,3-diene; α,γ-butadiene; *trans*-butadiene; divinyl; erythrene; pyrrolylene; vinylethylene

$$CH_2=CH-CH=CH_2$$

C_4H_6 Mol. wt: 54.09

1.1.2 *Chemical and physical properties*

(*a*) *Description*: Colourless gas with mildly aromatic odour; easily liquefied (Sax & Lewis, 1987)
(*b*) *Boiling-point*: –4.4 °C (Weast, 1989)
(*c*) *Melting-point*: –108.9 °C (Weast, 1989)
(*d*) *Density*: 0.6211 g/ml at 20 °C/liquefied (Kirshenbaum, 1978; Verschueren, 1983)
(*e*) *Spectroscopy data*: Ultraviolet (Grasselli & Ritchey, 1975), infrared (Sadtler Research Laboratories, 1980; prism [893[a]], grating [36758]), nuclear magnetic resonance and mass spectral data (US National Institutes of Health/Environmental Protection Agency Chemical Information System, 1983) have been reported.
(*f*) *Solubility*: Very slightly soluble in water (735 mg/l at 20 °C); soluble in ethanol, diethyl ether and organic solvents (Verschueren, 1983; Sax & Lewis, 1987; Budavari, 1989)
(*g*) *Volatility*: Vapour pressure, 1790 mm Hg (239 kPa) at 20 °C (Santodonato, 1985); relative vapour density (air = 1), 1.87 (Verschueren, 1983)

[a]Spectrum number in Sadtler compilation

(*h*) *Stability*: Flash-point, –76 °C (Sax & Lewis, 1987); slowly dimerizes to 4-vinyl-1-cyclohexene (US Occupational Safety and Health Administration, 1990); may form peroxides upon exposure to air (Kirshenbaum, 1978)
(*i*) *Reactivity*: Polymerizes readily, particularly if oxygen is present (Sax & Lewis, 1987)
(*j*) *Conversion factor*[b]: $mg/m^3 = 2.21 \times ppm$

1.1.3 *Technical products and impurities*

1,3-Butadiene is available commercially as a liquefied gas under pressure in several grades of purity, including a special purity or instrument grade of 99.4–99.5 mol% purity, a research grade of 99.86 mol% purity, a technical–commercial grade of 98 mol% purity and a rubber grade (Santodonato, 1985). Analytical, polymer, rubber and liquid grades (Aldrich Chemical Co., 1990; Kuney, 1990) range in minimal purity from 99.0 to 99.5%, with the following typical impurities: 1,2-butadiene, acetaldehyde (see IARC, 1987b), acetylenes (alpha, vinyl), propadiene, butadiene dimer (4-vinylcyclohexene, see IARC, 1986b), peroxides, sulfur and C_5 hydrocarbons. Oxidation/polymerization of 1,3-butadiene is inhibited by addition of hydroquinone, di-*n*-butylamine, *tert*-butylcatechol, aliphatic mercaptans or *ortho*-dihydroxybenzene (Exxon Chemical Co., 1973; Kirshenbaum, 1978; Lyondell Petrochemical Co., 1988; Budavari, 1989).

Crude 1,3-butadiene is also available from many producers for use as a feedstock. Such grades contain a minimum of 36–65% 1,3-butadiene, with specifications typically given for acetylenes, C_3 compounds and lighter hydrocarbons, C_5 compounds and heavier, peroxides, carbonyl compounds, sulfur and organic chlorides. Inhibitors (e.g., *tert*-butylcatechol, 50–200 ppm) are also added (Vista Chemical Co., 1985; Union Carbide Corp., 1987).

1.1.4 *Analysis*

Selected methods for the analysis of 1,3-butadiene in various matrices are listed in Table 1 (methods used previously are given in section 1.3.2).

The specificity and the detection limit of methods for determining simple, small molecules present in packaging materials which migrate into packaged goods have been discussed (Vogt, 1988). 1,3-Butadiene can be determined in plastic polymers, foods and food simulants by chromatographic methods.

Several gas detector tubes are used in conjunction with common colorimetric reactions to detect 1,3-butadiene. The reactions include the reduction of chromate or dichromate to chromous ion and the reduction of ammonium molybdate and palladium sulfate to molybdenum blue (Saltzman & Harman, 1989).

1.2 Production and use

1.2.1 *Production*

1,3-Butadiene was first produced in 1886 by the pyrolysis of petroleum hydrocarbons (Kirshenbaum, 1978). Commercial production started in the 1930s (Kosaric *et al.*, 1987).

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[b]Calculated from: mg/m^3 = (molecular weight/24.45) × ppm, assuming normal temperature (25°C) and pressure (760 mm Hg [101.3 kPa])

Table 1. Methods for the analysis of 1,3-butadiene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Collect on solid sorbent tube; desorb with dichloromethane; chill in ice	GC/FID	0.044 mg/m^3	Eller (1987)
	Collect on solid sorbent tube of charcoal coated with *tert*-butylcatechol; desorb with carbon disulfide	GC/FID	0.35 mg/m^3	Hendricks & Schultz (1986)
	Inject sample into GC using a temperature-programmed, fused-silica, porous layer, open tubular Al_2O_3/KCl column	GC/FID	0.01 ppm by volume (0.01 µl/l)	Locke *et al.* (1987)
	Assay directly	FT-IR	5 ppm (10 mg/m^3)	Harman (1987)
Plastics, liquid foods	Dissolve in *ortho*-dichlorobenzene; inject headspace sample	GC/FID	2–20 µg/kg	US Food and Drug Administration (1987)
Solid foods	Cut or mash sample; inject headspace sample	GC/FID	2–20 µg/kg	US Food and Drug Administration (1987)

Abbreviations: FT-IR, Fourier transform–infrared absorption spectroscopy; GC, gas chromatograph; GC/FID, gas chromatography/flame ionization detection

1,3-Butadiene has been produced commercially by three processes: catalytic dehydrogenation of *n*-butane and *n*-butene (the Houdry process), oxidative dehydrogenation of *n*-butene (the Oxo-D or O-X-D process) and recovery from the C_4 co-product (by-product) stream from the steam cracking process used to manufacture ethylene (the ethylene co-product process). All three processes involve the production of 1,3-butadiene from a C_4 hydrocarbon stream, and solvent extraction and extractive distillation are used in all three to further concentrate the 1,3-butadiene. There has recently been a shift to the use of cheaper, heavier feedstocks for ethylene production, with a concomitant increase in the volume of co-product containing 1,3-butadiene (Krishnan & Corwin, 1987). The ethylene co-product process accounts for approximately 95% of US and 85% of worldwide production (Morrow, 1990).

The production of 1,3-butadiene is thus a two-stage process: (i) production of a C_4 co-product during ethylene manufacture and (ii) recovery of 1,3-butadiene from the co-product. The first stage consists of cracking a hydrocarbon such as naphtha to produce ethylene as the primary product and a co-product stream composed of C_4 hydrocarbons. The amount of 1,3-butadiene in the co-product depends on the feedstock used and the severity of the cracking process: the heavier the feedstock and the more severe the cracking, the more 1,3-butadiene is produced. The 1,3-butadiene content of the co-product C_4 stream is 20–70%; the C_4 feed streams are usually blended with a feed stream containing 40–50% 1,3-butadiene for processing. In the extraction plants, solvents such as dimethylformamide, acetonitrile, furfural, dimethylacetamide and methylpyrrolidone are used (US Occupational Safety and Health Administration, 1990) to alter the volatility of components in a fractional distillation

selectively and to produce a high purity (> 99.0%) 1,3-butadiene monomer (Krishnan & Corwin, 1987).

In 1987, worldwide production of 1,3-butadiene was approximately 5.5 million tonnes (Morrow, 1990). A more detailed accounting of the production of 1,3-butadiene in several countries in 1980–90 is presented in Table 2. Global 1,3-butadiene consumption in 1987 was estimated at 5.5 million tonnes, 1.5 million tonnes of which were used in the USA. As in most years, the US demand exceeded its supply, so approximately 227 thousand tonnes of 1,3-butadiene monomer were imported in 1987 (Morrow, 1990).

Table 2. Trends in production of 1,3-butadiene in several countries (thousand tonnes)

Country	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
Canada	NA	126	118	133	127	132	146	167	182	175	192
France	259	266	258	281	303	288	291	307	335	329	281
Germany[a]	NA	NA	579	717	754	840	683	701	761	717	771
Italy	183	166	159	195	181	NA	NA	NA	NA	NA	NA
Japan	574	518	522	556	627	639	656	707	780	827	827
Mexico	17	12	15	19	20	18	18	21	12	NA	NA
United Kingdom	192	207	228	237	259	297	192	231	239	226	195
USA[b]	1270	1356	869	1068	1113	1062	1156	1329	1437	1417	1435

From Anon. (1984, 1986, 1988, 1991b); NA, not available
[a]Figures prior to 1990 are for western Germany only
[b]Rubber grade

Information available in 1988 indicated that 1,3-butadiene was produced by nine companies in Germany, eight in Japan, four in the United Kingdom and in Brazil, three in France, two in Australia, Belgium, Canada, the Netherlands and Spain, and one each in Argentina, Austria, Bulgaria, China, Czechoslovakia, Finland, India, Italy, Mexico, Poland, Saudi Arabia, Singapore, Taiwan and Yugoslavia (Chemical Information Services, 1988). It was produced by eight companies in the USA in 1991 (Anon., 1991a).

1.2.2 *Use*

1,3-Butadiene is used principally as a monomer in the manufacture of a wide range of polymers and copolymers. Polymerization of styrene and 1,3-butadiene yields styrene–butadiene rubber, the largest single use of butadiene; almost 80% of the styrene–butadiene rubber produced is used in tyres and tyre products. Polymerization of 1,3-butadiene produces polybutadiene, almost all of which used for car and bus tyres. Nitrile rubber is produced by copolymerizing 1,3-butadiene and acrylonitrile; it is used in hoses, gaskets, seals, latexes, adhesives and footwear. Acrylonitrile–butadiene–styrene resins are graft terpolymers of polybutadiene on a styrene-acrylonitrile copolymer; they are used in automotive parts, pipes, appliances, business machines and telephones. Styrene–butadiene latexes are suspensions of particles or globules of the elastomer in water and are used in paper coatings and paints and as carpet backing (Santodonato, 1985; US Occupational Safety and Health Administration, 1990).

1,3-Butadiene is used as a chemical intermediate in the production of a number of important chemicals. Neoprene is made by chlorinating 1,3-butadiene and treating the resultant chloroprene with sodium hydroxide; two-thirds of the neoprene produced is used for industrial and automotive rubber goods. Adiponitrile is produced by chlorinating 1,3-butadiene and cyanating the product to 1,4-dicyanobutene, which is then reduced to adiponitrile; this is converted to hexamethylenediamine for the production of Nylon 66. 1,4-Hexadiene, made by reacting 1,3-butadiene with ethylene, is used as a monomer for ethylene–propylene terpolymer. Sulfolane, produced by reacting sulfur dioxide and 1,3-butadiene and dehydrogenating the product, is a valuable solvent for extraction. 1,5,9-Cyclodecatriene is produced by trimerizing 1,3-butadiene and is used for the production of various nylon fibres and resins. Some other nonpolymer applications include manufacture of agricultural fungicides (captan and captafol) and anthraquinone dyes (Santodonato, 1985; US Occupational Safety and Health Administration, 1990).

In 1990, 1,3-butadiene was used in the USA 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., 1991a).

(For more detailed discussions of the production and use of 1,3-butadiene, see Miller, 1978; Leviton, 1983; Greek, 1984.)

1.3 Occurrence

1.3.1 *Natural occurrence*

1,3-Butadiene is not known to occur as a natural product (Santodonato, 1985).

1.3.2 *Occupational exposure*

On the basis of a National Occupational Exposure Survey, the US National Institute for Occupational Safety and Health (1990) estimated that 52 000 workers were potentially exposed to 1,3-butadiene in the USA in 1981–83. Potential exposure to 1,3-butadiene can occur in the following industrial activities: petroleum refining and related operations (production of C_4 fractions containing 1,3-butadiene, production and distribution of gasoline), production of purified 1,3-butadiene monomer, production of various 1,3-butadiene-based rubber and plastics polymers and other derivatives, and the rubber and plastics products manufacturing industry (production of tyres, hoses and a variety of moulded objects).

In the descriptions below, the accuracy of the levels of exposure to 1,3-butadiene may have been affected by inability to distinguish between 1,3-butadiene and other C_4 compounds, low desorption efficiency at low concentrations, possible sample breakthrough in charcoal tubes and possible loss during storage, in methods used until the mid-1980s (Lunsford *et al.*, 1990). No data are available on levels of exposure to 1,3-butadiene before the 1970s, when different processes and working conditions (e.g., during the Second World War) would have resulted in exposure conditions different from those now prevalent in developed countries.

(a) Petroleum refining and production of crude 1,3-butadiene

Gasoline contains a small percentage of 1,3-butadiene, and exposures of workers in various job groups in the production and distribution of gasoline are shown in Table 3. Table 4 shows the exposures since 1984 of workers in different areas of petroleum refineries and petrochemical facilities where crude 1,3-butadiene is produced (usually a C_4 stream obtained as a by-product of ethylene production).

Table 3. Personal exposures (mg/m^3) to 1,3-butadiene associated with gasoline during 1984–85 in 13 European countries

Activity	Mean	Range	Exposure duration (TWA)
Production on-site (refining)	0.3	ND–11.4	8 h
Production off-site (refining)	0.1	ND–1.6	8 h
Loading ships (closed system)	6.4	ND–21.0	8 h
Loading ships (open system)	1.1	ND–4.2	8 h
Loading barges	2.6	ND–15.2	8 h
Jettyman	2.6	ND–15.9	8 h
Bulk loading road tankers			
Top loading < 1 h	1.4	ND–32.3	< 1 h
Top loading > 1 h	0.4	ND–4.7	8 h
Bottom loading < 1 h	0.2	ND–3.0	< 1 h
Bottom loading > 1 h	0.4	ND–14.1	8 h
Road tanker delivery (bulk plant to service station)	ND		
Railcar top loading	0.6	ND–6.2	8 h
Drumming	ND		
Service station attendant (dispensing fuel)	0.3	ND–1.1	8 h
Self-service station (filling tank)	1.6	ND–10.6	2 min

From CONCAWE (1987); ND, not detected; TWA, time-weighted average

Table 4. Mean 8-h time-weighted average concentrations of 1,3-butadiene to which workers in different jobs in petroleum refineries and petrochemical facilities have been exposed since 1984

Job area	No. of facilities	Mean[a]		Range	
		ppm	mg/m^3	ppm	mg/m^3
Production	7	0.24	0.53	0.008–2.0	0.02–4.4
Maintenance	6	0.11	0.24	0.02–0.37	0.04–0.82
Distribution	1	2.9	64.1		
Laboratory	4	0.18	0.40	0.07–0.4	0.16–0.88

From Heiden Associates (1987)
[a]Weighted by number of exposed workers

(*b*) *Monomer production*

Detailed industrial hygiene surveys were conducted by the US National Institute for Occupational Safety and Health in 1985 in four of 10 US facilities where 1,3-butadiene was produced by solvent extraction of C_4 fractions originating as ethylene co-product streams (Krishnan *et al.*, 1987). Levels of 1,3-butadiene to which workers in various job categories were exposed are summarized in Table 5. Jobs that require workers to handle or transport containers, such as voiding sample cylinders or loading and unloading tank trucks or rail cars, present the greatest potential exposure. Geometric means of full-shift exposure levels for other job categories were below 1 ppm [2.2 mg/m^3]. Short-term samples showed that such activities as open-loop sampling and cylinder voiding were associated with peak exposures of 100 ppm [220 mg/m^3]. Full-shift area samples indicated that ambient concentrations of 1,3-butadiene were greatest in the railcar terminals (geometric mean, 1.77 [3.4 mg/m^3]) and in the tank storage farm (2.12 ppm [3.4 and 4.7 mg/m^3]).

Table 5. Full-shift, time-weighted average exposure levels in personal breathing-zone samples at four US 1,3-butadiene monomer production facilities, 1985

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician Control room	10	0.45 [1.0]	0.09 [0.20]	< 0.02–1.87 [< 0.04–4.1]
Process technician Process area Loading area	28	2.23 [4.9]	0.64 [1.4]	< 0.08–34.9 [< 0.18–77.1]
Railcar	9	14.64 [32.4]	1.00 [2.2]	0.12–123.57 [0.27–273.1]
Tank truck	3	2.65 [5.9]	1.02 [2.3]	0.08–5.46 [0.18–12.1]
Tank farm	5	0.44 [0.97]	0.20 [0.44]	< 0.04–1.53 [< 0.09–3.4]
Laboratory technician	29	1.06 [2.3]	0.40 [0.88]	0.03–6.31 [0.07–14.0]
Cylinder voiding	3	125.52 [277.4]	7.46 [16.5]	0.42–373.54 [0.93–825.5]

From Krishnan *et al.* (1987)

In 1984, the US Chemical Manufacturers' Association obtained data on personal exposure to 1,3-butadiene before 1984 from 13 monomer-producing companies, categorized broadly by job type (Table 6). These data were collected by an older method and provide a historical perspective on the data reported in Table 5. The highest exposures were in the maintenance and distribution jobs. Out of a total of 1287 samples, 91% were less than or equal to 10 ppm [22.1 mg/m^3] and 68% were less than or equal to 5 ppm [11.1 mg/m^3]. Factors that limit generalization of these data are unspecified sampling and analytical techniques, lack of detailed job descriptions and different or unspecified average times of sampling (JACA Corp., 1987).

Monitoring in a Finnish plant generally indicated ambient air levels of less than 10 ppm [22.1 mg/m^3] at different sites (33 samples; mean sampling time, 5.3 h). In personal samples for 16 process workers, the concentration ranged from < 0.1 to 477 ppm [< 0.22–1054.2

Table 6. Time-weighted average exposure to 1,3-butadiene in 13 US monomer production plants before 1984

Job area	No. of samples	Exposure (ppm [mg/m^3])											
		0.00–5.00 [0–11.05]		5.01–10.00 [11.07–22.12]		10.01–25.00 [22.12–55.25]		25.01–50.00 [55.27–110.50]		50.01–100.00 [110.52–221.23]		> 100.00 [> 221.23]	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Production	562	446	79.4	111	19.7	5	0.9						
Maintenance	329	247	75.1			47	14.3	35	10.6				
Supervisory	64	60	93.8	4	6.2								
Distribution	206	60	29.1	121	58.7	16	7.8	5	2.4	2	1.0	2	1.0
Laboratory	126	58	46.0	68	54.0								
Total	1287	871	67.8	304	23.6	68	5.3	40	3.1	2	0.1	2	0.1

From JACA Corp. (1987)

mg/m^3] (mean, 11.5 ppm [25.4 mg/m^3]; median, < 0.1 ppm [< 0.22 mg/m^3]; 46 samples; mean sampling time, 2.5 h). The highest concentrations were measured during sample collection. Protective clothing and respirators were used during this operation (Arbetsmiljöfonden, 1991).

Potential exposure in the monomer industry other than to 1,3-butadiene includes extraction solvents and components of the C_4 feedstock. Extraction solvents differ among facilities; some common ones are dimethylformamide, dimethylacetamide, acetonitrile, β-methoxypropylnitrile (Fajen, 1985a), furfural and cuprous ammonium acetate (US Occupational Safety and Health Administration, 1990). Stabilizers are commonly used to prevent formation of peroxides in air and polymerization (see p. 238). No information was available on these other exposures, or on exposures to chemicals other than 1,3-butadiene that are produced in some facilities, such as butylenes, ethylene, propylene, polyethylene and polypropylene resins, methyl-*tert*-butyl ether and aromatic hydrocarbons (Fajen, 1985b,c).

(c) *Production of polymers and derivatives*

Detailed industrial hygiene surveys were conducted in 1986 in five of 17 US facilities where 1,3-butadiene was used to produce styrene–butadiene rubber, nitrile–butadiene rubber, polybutadiene rubber, neoprene and adiponitrile (Fajen, 1988). Levels of 1,3-butadiene to which workers in various job categories were exposed are summarized in Table 7. Process technicians in unloading, the tank farm, purification, polymerization and reaction, laboratory technicians and maintenance technicians were exposed to the highest levels. Short-term sampling showed that activities such as sampling a barge and laboratory work were associated with peak exposures to more than 100 ppm [221 mg/m^3]. Full-shift area sampling indicated that geometric mean ambient concentrations of 1,3-butadiene were less than 0.5 ppm [1.1 mg/m^3] and usually less than 0.1 ppm [0.22 mg/m^3] in all locations at the five plants.

Eight-hour time-weighted average (TWA) exposures to 1,3-butadiene in the polymer industry were obtained by personal sampling in 11 North American synthetic rubber plants in 1978–84 and reported by the International Institute of Synthetic Rubber Producers in 1984 (JACA Corp., 1987) (Table 8). The highest exposures were found for tank car loaders (15% of exposures, > 10 ppm [> 22.1 mg/m^3]), reactor operators (18% of exposures, > 10 ppm) and laboratory technicians (6% of exposures, > 10 ppm). Sampling and analytical techniques and job descriptions were not available.

Other data on levels of exposure to 1,3-butadiene have been collected during health surveys and epidemiological studies (Table 9). In a US styrene–butadiene rubber manufacturing plant in 1979, the only two departments in which levels were greater than 10 ppm [22.1 mg/m^3] were tank farm (53.4 ppm [118 mg/m^3]) and maintenance (20.7 ppm [45.8 mg/m^3]) (Checkoway & Williams, 1982). In samples taken at one of two US styrene–butadiene rubber plants in 1976, levels above 100 ppm [221 mg/m^3] were encountered by technical services personnel (114.6 ppm [253.3 mg/m^3]) and an instrument man (174.1 ppm [384.78 mg/m^3]) (Meinhardt *et al.*, 1978). Overall mean 8-h TWA exposure levels differed considerably between the two plants, however: 1.24 ppm [2.74 mg/m^3] in one plant and 13.5 ppm [29.84 mg/m^3] in the other (Meinhardt *et al.*, 1982).

Table 7. Full-shift time-weighted average exposure levels in personal breathing-zone samples at five US plants producing 1,3-butadiene-based polymers and derivatives, 1986

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician				
Unloading area	2	14.6 [32.27]	4.69 [10.37]	0.770–28.5 [1.7–63.0]
Tank farm	31	2.08 [4.60]	0.270 [0.60]	< 0.006–23.7 [< 0.01–52.4]
Purification	18	7.80 [17.24]	6.10 [13.48]	1.33–24.1 [3.0–53.3]
Polymerization or reaction	81	0.414 [0.92]	0.062 [0.14]	< 0.006–11.3 [< 0.01–25.0]
Solutions and coagulation	33	0.048 [0.11]	0.029 [0.06]	< 0.005–0.169 [< 0.01–0.4]
Crumbing and drying	35	0.033 [0.07]	0.023 [0.05]	< 0.005–0.116 [< 0.01–0.26]
Packaging	79	0.036 [0.08]	0.022 [0.05]	< 0.005–0.154 [< 0.01–0.34]
Warehouse	20	0.020 [0.04]	0.010 [0.02]	< 0.005–0.068 [< 0.01–0.15]
Control room	6	0.030 [0.07]	0.019 [0.04]	< 0.012–0.070 [< 0.03–0.16]
Laboratory technician	54	2.27 [5.02]	0.213 [0.47]	< 0.006–37.4 [< 0.01–82.65]
Maintenance technician	72	1.37 [3.02]	0.122 [0.27]	< 0.006–43.2 [< 0.01–95.47]
Utilities operator	6	0.118 [0.26]	0.054 [0.12]	< 0.006–0.304 [< 0.01–0.67]

From Fajen (1988)

The manufacture of butadiene-based polymers and butadiene derivatives implies potential occupational exposure to a number of other chemical agents, which varies according to product and process. These include other monomers (styrene (see IARC, 1987b), acrylonitrile (see IARC, 1987b), chloroprene (see IARC, 1979)), solvents, additives (e.g., activators, antioxidants, modifiers), catalysts, mineral oils (see IARC, 1987b), carbon black (see IARC, 1987b), chlorine, inorganic acids and caustic solution (Fajen, 1986a,b; Roberts, 1986). Styrene, benzene (see IARC, 1987b) and toluene (see IARC, 1989) were measured in various departments of a US styrene–butadiene rubber manufacturing plant in 1979: mean 8-h TWA levels of styrene were below 2 ppm [8.4 mg/m^3], except for tank farm workers (13.7 ppm [57.5 mg/m^3], 8 samples); mean benzene levels did not exceed 0.1 ppm [0.3 mg/m^3], and those of toluene did not exceed 0.9 ppm [3.4 mg/m^3] (Checkoway & Williams, 1982). Meinhardt *et al.* (1982) reported that the mean 8-h TWA levels of styrene were 0.94 ppm [3.9 mg/m^3] (55 samples) and 1.99 ppm [8.4 mg/m^3] (35 samples) in two styrene–butadiene rubber manufacturing plants in 1977; the average benzene level measured in one of the plants was 0.1 ppm [0.3 mg/m^3] (3 samples). Average levels of styrene, toluene, benzene, vinyl cyclohexene and cyclooctadiene were reported to be lower than 1 ppm in another styrene–butadiene rubber plant in 1977 (Burroughs, 1977).

(*d*) *Rubber and plastics products manufacturing industries*

Unreacted 1,3-butadiene was detected as only a trace (0.04–0.2 ng/mg) in 15 of 37 bulk samples of polymers and other chemicals synthesized from 1,3-butadiene and analysed in 1985–86. Only two samples contained measurable amounts of 1,3-butadiene: tetrahydrophthalic anhydride (53 ng/mg) and vinyl pyridine latex (16.5 ng/mg) (JACA Corp., 1987).

Table 8. Time-weighted average exposures to 1,3-butadiene in 11 North American plants producing synthetic rubber, 1978–84

Occupational group	No. of samples	Exposure (ppm [mg/m^3])															
		0.00–5.00 [0–11.05]		5.01–10.00 [11.07–22.12]		10.01–25.00 [22.12–55.25]		25.01–50.00 [55.27–110.50]		50.01–100.00 [110.52–221.23]		100.01–200.00 [221.23–420.00]		200.01–500.00 [442.02–1105.00]		500.01–1000.00 [1105.02–2210.00]	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tank car loader	102	78	76.5	9	8.8	9	8.8	4	3.9	2	2.0						
Vessel cleaner	214	199	93	9	4.2	4	1.9	2	0.9								
Charge solution make-up	89	83	93.2	3	3.4			2	2.3	1	1.1						
Reactor operator	190	133	70	22	11.6	14	7.4	7	3.7	7	3.7	5	2.6	1	0.5	1 1	0.5
Recovery operator	108	100	92.6	5	4.6	2	1.9	1	0.9								
Coagulation operator	185	173	93.5	9	4.9	2	1.1	1	0.5								
Dryer operator	85	84	98.8	1	1.2												
Baler and packager	167	164	98.2	2	1.2	1	0.6										
Warehouseman	22	22	100														
Laboratory technician	116	103	88.8	6	5.2	6	5.2	1	0.9								
Maintenance technician	262	241	92.0	12	4.6	4	1.5	2	0.8	3	1.1						
Supervisor	123	111	90.2	6	4.9	6	4.9										
Waste treatment operator	9	9	100														
Total	1672	1500	89.7	84	5.0	48	2.9	20	1.2	13	0.78	5	0.30	1	0.06	1	0.06

From JACA Corp. (1987)

Table 9. Mean 8-h time-weighted average concentrations of 1,3-butadiene measured in two US styrene–butadiene rubber manufacturing plants

Job classification or department	No. of samples	Concentration		Year of sampling	Reference
		ppm	mg/m^3		
Instrument man	3	58.62	129.55	1976	Meinhardt *et al.* (1978)
Technical services personnel	12	19.85	43.87		
Head production operator	5	15.50	34.26		
Carpenter	4	7.80	17.24		
Production operator	24	3.30	7.29		
Maintenance mechanic	17	3.15	6.96		
Common labourer	17	1.52	3.36		
Production foreman	1	1.16	2.56		
Operator helper	3	0.79	1.75		
Pipefitter	8	0.74	1.64		
Electrician	5	0.22	0.49		
Tank farm	8	20.03	44.3	1979	Checkoway & Williams (1982)
Maintenance	52	0.97	2.14		
Reactor and recovery	28	0.77	1.7		
Solution	12	0.59	1.3		
Factory service	56	0.37	0.82		
Shipping and receiving	2	0.08	0.18		
Storeroom	1	0.08	0.18		

Detailed industrial hygiene surveys were conducted in 1984–87 in a US rubber tyre plant and a US industrial hose plant where styrene–butadiene rubber, polybutadiene and acrylonitrile–butadiene rubber were processed. No 1,3-butadiene was detected in any of a total of 124 personal full-shift samples from workers in the following job categories, which were identified as involving potential exposure to 1,3-butadiene: Banbury operators, mill operators, extruder operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al.*, 1990).

Measurements taken in 1978 and 1979 in personal 8-h samples in companies where acrylonitrile–butadiene–styrene moulding operations were conducted showed levels of < 0.05–1.9 mg/m^3 (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980). In a polybutadiene rubber warehouse, levels of 0.003 ppm [0.007 mg/m^3] were found in area samples; area and personal samples taken in tyre plants contained 0.007–0.05 ppm [0.016–0.11 mg/m^3] (Rubber Manufacturers' Association, 1984). In a US tyre and tube manufacturing plant in 1975, a cutter man/Banbury operator was reported to have been exposed to 1,3-butadiene at 2.1 ppm [4.6 mg/m^3] (personal 6-h sample) (Ropert, 1976).

Occupational exposures to many other agents in the rubber goods manufacturing industry were reviewed in a previous monograph (IARC, 1982).

1.3.3 *Air*

In 1989, total emissions of 1,3-butadiene to the air in the USA were estimated at approximately 2512 tonnes from 158 locations; total land releases were estimated at 6.7 tonnes (US National Library of Medicine, 1991).

Data on annual emissions of 1,3-butadiene from US facilities producing 1,3-butadiene, polybutadiene, neoprene/chloroprene and styrene–butadiene rubber and from miscellaneous facilities where 1,3-butadiene was used were collected in 1984 by the US Environmental Protection Agency. Data on episodic emissions were collected from most of the same facilities in 1985–86 (US Environmental Protection Agency, 1987; Mullins, 1990). Average annual emissions, the average rates and durations of episodic emissions and the highest rates for specific types of emissions are presented in Table 10.

Table 10. 1,3-Butadiene emissions from US manufacturing facilities in 1984–86

Activity of facility	No. of facilities	Total emissions (tonnes/year)		Episodic emissions (1986)		
		Average	Range	Average rate (kg/min)	Highest average rate (kg/min)	Average duration (min)
1,3-Butadiene production	10[a]	135.9	6.8–752	355	1600[b] 1100[c]	2170
Polybutadiene production	7	57.4	22.1–176	24	81.4[c] 24.0[d]	7.5
Chloroprene/-neoprene production	2	10, 32.2		2.9	181[b]	38.8
Styrene–butadiene rubber production	17	49.3	0.9–145	3.9	9.9[e] 9.2[c]	49.6
Using 1,3-butadiene	11[f]	63.5	2.2–350	NR	NR	NR

From US Environmental Protection Agency (1987); NR, not reported
[a]Episodic emissions reported for eight facilities
[b]Pressure relief discharges
[c]Accidental liquid releases
[d]Equipment openings
[e]Accidental gas releases
[f]Episodic emissions reported for five facilities

Few data are available on levels of 1,3-butadiene in ambient air; reported concentrations in urban air generally range from less than 1 to 10 ppb [2–22 μg/m^3] (Neligan, 1962; Cote & Bayard, 1990). In the USA, combined levels of 1,3-butadiene and 2-butene were 5.9–24.4 ppb (0.01–0.05 mg/m^3) in 1978 in Tulsa, OK (Arnts & Meeks, 1981), and 0–0.019 ppm (0–0.042 mg/m^3) in 1973–74 in Houston, TX (Siddiqi & Worley, 1977). Levels of 1,3-butadiene were 0.004 mg/m^3 in Denver, CO, and < 0.001–0.028 mg/m^3 in various cities in Texas (Hunt *et al.*, 1984); urban air in Los Angeles and Riverside, CA, contained levels as high as 9 ppb [0.02 mg/m^3] (Parsons & Wilkins, 1976).

1,3-Butadiene was found in 32% of 24-h ambient air samples taken in 19 US cities in 1987–88, at a mean concentration of 1.39 μg/m^3 (range, 0.11–6.94) (US Environmental Protection Agency, 1989).

1.3.4 *Water*

1,3-Butadiene has been detected in drinking-water in the USA (US Environmental Protection Agency, 1978; Kraybill, 1980). Total releases to ambient water in 1989 were estimated to be 65 tonnes (US National Library of Medicine, 1991).

1.3.5 *Food*

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 & Gilbert, 1984).

1.3.6 *Miscellaneous*

The US Environmental Protection Agency (1990) estimated that 1,3-butadiene is emitted in automobile exhaust at 8.9–9.8 mg/mile [5.6–6.1 mg/km] and comprises about 0.35% of total hydrocarbon in exhaust emissions. It has been detected in smoke generated during house fires at up to 15 ppm [33 mg/m^3] (Berg *et al.*, 1978).

Sidestream cigarette smoke contains 1,3-butadiene at approximately 0.4 mg/cigarette, and levels of 1,3-butadiene in smoky indoor environments are typically 10–20 μg/m^3 (Löfroth *et al.*, 1989).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for 1,3-butadiene in some countries and regions are presented in Table 11. Exposure limits were lowered in many countries in the late 1980s.

1,3-Butadiene is regulated by the US Food and Drug Administration (1989) for use in resinous and polymeric coatings in can-end cements; for use only as a coating or coating component and limited to a level not to exceed 1% by weight of paper or paperboard in contact with foods; for use in semi-rigid and rigid acrylic and modified acrylic plastics in repeat-use articles; for use in acrylonitrile–butadiene–styrene copolymers used in closures with sealing gaskets for food containers; and for use in textiles and textile fibres that come in contact with food.

2. Studies of Cancer in Humans

2.1 Cohort studies

The rubber industry, i.e., the manufacture of finished rubber goods, in which there is potential exposure to 1,3-butadiene, among other chemicals, has been evaluated previously; it was concluded that exposure in the rubber industry is carcinogenic to humans (IARC, 1982, 1987b). The epidemiological studies that were evaluated did not, however, include specific information on styrene–butadiene rubber manufacture, and it is these that are summarized below. In these descriptions, the histological descriptions of observed tumours given by the authors are used, with ICD codes when available.

Table 11. Occupational exposure limits and guidelines for 1,3–butadiene

Country or region	Year	Concentration (mg/m^3)	Interpretation[a]
Australia	1990	22 (carcinogen)	
Austria	1982	2200	TWA
Belgium	1990	22 (carcinogen)	TWA
Brazil	1978	1720	TWA
Bulgaria	1984	100	TWA
Czechoslovakia	1990	20	TWA
		40	STEL
Denmark	1990	22 (carcinogen)	TWA
Finland	1987	73 (carcinogen)	TWA
Germany	1989	0 (carcinogen in animals; III A2)	
Hungary	1990	10 (carcinogen)	STEL
Indonesia	1978	2200	TWA
Italy	1978	1000	TWA
Mexico	1983	2200	TWA
Netherlands	1989	110	TWA
Norway	1990	2.2 (carcinogen)	TWA
Poland	1984	100	TWA
Romania	1975	1500[b]	TWA
		2000[b]	Ceiling
Sweden	1990	20 (carcinogen)	TWA
		40	STEL (15-min)
Switzerland	1990	11 (carcinogen)	TWA
Taiwan	1981	2200	TWA
United Kingdom	1991	22	TWA
USA			
ACGIH	1991	22 (suspected human carcinogen; A2)	TWA
OSHA	1989	2200[c]	TWA
USSR	1984	100	MAC
Venezuela	1978	2200	TWA
		2750	Ceiling
Yugoslavia	1971	500	TWA

From Cook (1987); US Occupational Safety and Health Administration (OSHA) (1989); Direktoratet for Arbeidstilsynet (1990); Dutch Expert Committee for Occupational Standards (1990); American Conference of Governmental Industrial Hygienists (ACGIH) (1991); Health and Safety Executive (1991); International Labour Office (1991)

[a]TWA, 8-h time-weighted average; STEL, short-term exposure limit; MAC, maximum allowable concentration

[b]Skin notation

[c]The US OSHA has proposed to reduce the permissible exposure limits to 4.4 mg/m^3 for an 8-h TWA, 22 mg/m^3 for a 15-min STEL and 2.2 mg/m^3 for an 8-h TWA 'action level'; for a detailed discussion of this proposal, see US Occupational Safety and Health Administration (1990).

Follow-up of mortality in a cohort of workers who manufactured 1,3-butadiene monomer in Texas (USA) (Downs *et al.*, 1987) was extended through 1985 (Divine, 1990). The cohort comprised men who had been employed for six months or more between the opening of the plant in 1943 and 31 December 1979. Vital status was ascertained through the Social Security Administration or from state health departments. Of 2582 male employees, 1.9% were lost to follow-up and 32.0% were dead, 6% of these with no death certificate. Using US white men as the comparison population, the standardized mortality ratio (SMR) for mortality from all causes was 0.84 (826 deaths; 95% confidence interval [CI], 0.79–0.90) and that for all cancers was 0.80 (163 deaths; 95% CI, 0.69–0.94). The only significantly elevated SMR was for lymphosarcoma and reticulosarcoma (ICD8, 200) (2.29; 9 deaths; 95% CI, 1.04–4.35), thus confirming the earlier report (Downs *et al.*, 1987). Seven of the nine subjects had first been employed before 1946. When analysis was carried out by years of employment, there was no trend in SMR with increasing length of employment for lymphosarcoma or reticulosarcoma, and the only excess was seen for men with fewer than 10 years of employment. On the basis of the department listed on workers' personnel records, exposure to 1,3-butadiene was classified as low (not normally exposed to 1,3-butadiene), routine (exposed to 1,3-butadiene on a daily basis), non-routine (exposed intermittently to 1,3-butadiene, with possible exposure to peak concentrations higher than those with routine exposure) or unknown. Workers ever employed with routine exposure had a significant excess of lympho- and reticulosarcoma (5 deaths; SMR, 5.61; 95% CI, 1.81–13.10); all five deaths were seen in workers who had been employed fewer than 10 years. The rates for cancers of the kidney and large intestine were nonsignificantly increased among men who had worked for more than 10 years. Men who had had non-routine exposure had non-significantly increased risks for leukaemia (ICD8, 204–207) (SMR, 1.85; 6 deaths; 95% CI, 0.68–4.03) and lymphosarcoma and reticulosarcoma (SMR, 1.26; 2 deaths; 95% CI, 0.14–4.54).

Results were available from a study on the mortality of white male workers who had been employed for at least six months in two US styrene–butadiene rubber plants (Meinhardt *et al.*, 1982). A total of 1662 workers employed in plant A between 1943 and 1976 and 1094 workers employed in plant B between 1950 and 1976 were followed-up through 31 March 1976. Nine deaths from cancer of the lymphatic and haematopoietic tissues (ICD7, 200–205) were seen in workers in plant A (SMR, 1.55 [95% CI, 0.71–2.95]); all these deaths occurred among men who had first been employed between January 1943 and December 1945 (SMR, 2.12; [95% CI, 0.97–4.02]), after which the process changed from batch to continuous feed operation. No information was available, however, on the work histories of the subjects. The SMR for leukaemia (ICD7, 204) in plant A among workers employed between 1943 and 1945 was 2.78 (5 deaths [95% CI, 0.65–4.72]); two of the deaths had occurred within three years of first employment. In plant B, the numbers were very small: one death from leukaemia was observed (0.99 expected), which occurred within four years of first employment; the SMR for lymphatic and haematopoietic neoplasms was 0.78 (2 deaths [95% CI, 0.10–2.83]). Time-weighted average exposure to 1,3-butadiene was estimated after 1976 to be about 10 times higher in plant B (mean, 13.5 ppm; SD, 29.9; range, 0.34–174) than in plant A (mean, 1.24 ppm; SD, 1.20; range, 0.11–4.17). Concomitant exposure to styrene had occurred in plants A and B, and to traces of benzene at least in plant A.

Matanoski *et al.* (1990a) investigated mortality patterns from 1943 (synthetic rubber production began in 1942) through 1982 of employees from eight styrene–butadiene rubber plants in Canada and the USA, previously followed up through 1979 by Matanoski and Schwartz (1987). The study included all men who had been employed for at least one year between 1943—or when their plant records were complete—and 1976. Canadian workers were included in the more recent study only if they had worked 10 or more years or had reached age 45 while still employed, since this enabled more complete ascertainment of their vital status through the company's insurance records. Of 12 113 employees, 2441 (20.2%) were deceased, 416 (3.4%) had unknown vital status and 9256 (76.4%) were still living at the end of follow-up. Death certificates were obtained for 97.2% of deceased individuals. On the basis of US death rates for black and white men (since Ontario rates were similar to US rates), the SMRs for the entire cohort were as follows: 0.81 for all causes (2441 deaths; 95% CI, 0.78–0.85); 0.85 for all cancers (518 deaths; 95% CI, 0.78–0.93), 0.61 for lymphosarcoma (ICD8, 200) (seven deaths; 95% CI, 0.25–1.26), 1.20 for Hodgkin's disease (ICD8, 201) (eight deaths; 95% CI, 0.52–2.37), 0.96 for leukaemia (ICD8, 204–207) (22 deaths; 95% CI, 0.60–1.46) and 1.11 for 'other lymphatic' system cancers (ICD8, 202, 203, 208) (17 deaths; 95% CI, 0.64–1.77). The SMR for lymphatic or haematopoietic cancers showed no clear trend of increasing with increasing number of years worked or years since first exposure. When employees were classified according to the job held longest, production workers (presumed by the authors to be those with highest exposures to 1,3-butadiene) had an SMR for deaths from all causes of 0.88 (594 deaths; 95% CI, 0.81–0.95) and a significant excess of other lymphatic cancer (SMR, 2.60; nine deaths; 95% CI, 1.19–4.94). When mortality among production workers was examined by race, the only significant excess was seen for leukaemia in blacks (three deaths; SMR, 6.56; 95% CI, 1.35–19.06). Of 92 deaths among black production workers, six were due to all lymphopoietic cancers (5.07; 1.87–11.07), and three of these were leukaemias (6.56; 1.35–19.06). The rates for haematopoietic cancers among maintenance workers were lower than those of the production workers. Maintenance workers showed increased risk for some digestive cancers, which were not evident in production workers. Workers in the two other job classification categories ('utility' and 'other') showed no significant increase in SMR for any type of cancer. A limitation of this study, pointed out by the authors, was that missing information on 2391 employees meant that they were excluded from the analysis of job department. Since many of these men were active in 1976 and are thus more likely to be alive than dead, the analysis by job is biased toward including more dead workers. The SMRs in this analysis may therefore be higher than those in the total cohort and are thus not directly comparable.

2.2 Case–control studies

In a case–control study nested within a cohort of 6678 US male rubber workers, deaths from cancers at the following sites were compared to those in a sample of the whole cohort: stomach (ICD8, 151) (41 deaths), colorectal (ICD8, 153–154) (63), respiratory tract (ICD8, 160–163) (119), prostate (ICD8, 185) (52), urinary bladder (ICD8, 188) (13), lymphatic and haematopoietic (ICD8, 200–209) (51) and lymphatic leukaemia (ICD8, 204) (14) (McMichael *et al.*, 1976). A 6.2-fold increase in risk for lymphatic and haematopoietic cancers (99.9% CI, 4.1–12.5) and a 3.9-fold increase for lymphatic leukaemia (99.9% CI,

2.6–8.0) were found in association with more than five years' work in manufacturing units producing mainly styrene–butadiene rubber during 1940–60. Of the five other cancer sites investigated, only cancer of the stomach was associated with a significant (two-fold) increase in risk. [The Working Group noted that, although the confidence limits were calculated by a method not used commonly, the results are significant at the 5% level.]

A case–control study nested within the US and Canadian cohort study described above (Matanoski *et al.*, 1990a) involved 59 workers with lymphopoietic cancers, identified using both underlying and contributing causes listed on death certificates. Controls were 193 workers without cancer, matched to the cases for plant, age, sex, date of hire, duration of work and survival up to date of death of the case (Santos-Burgoa, 1988; Matanoski *et al.*, 1990b). Since the exposures to 1,3-butadiene and to styrene were highly correlated, an attempt was made to discern to what extent each exposure contributed to the risk for leukaemia. Four industrial engineers who had no knowledge of the case or control status of the subjects estimated the intensity of exposure in each job, and duration of work was determined from job histories. The sum of the product of intensity and duration for each job resulted in a cumulative ranked exposure index for 1,3-butadiene and styrene separately. When the log of the ranked exposure indexes was dichotomized above and below the mean score for each exposure, 1,3-butadiene alone was associated with a risk for leukaemia (26 deaths) of 7.61 (95% CI, 1.62–35.64), and styrene alone gave a risk of 2.92 (95% CI, 0.83–10.27), each without adjustment for the other chemical. The relative risk for exposure to styrene, adjusted for 1,3-butadiene, was 1.06 (95% CI, 0.23–4.96), while the risk for 1,3-butadiene, adjusted for styrene, was 7.39 (95% CI, 1.32–41.33). The same type of analysis for other lymphatic cancers (18 deaths), including non-Hodgkin's lymphoma (ICD8, 202) and multiple myeloma (ICD8, 203), gave a risk of 0.81 (95% CI, 0.28–2.38) for styrene adjusted for 1,3-butadiene and a risk of 1.68 (95% CI, 0.55–5.15) for 1,3-butadiene adjusted for styrene.

In the population-based case–control study of cancers at multiple sites (excluding leukaemia) carried out in Montréal, Canada (Siemiatycki, 1991), described in detail on p. 95, 4% of the entire study population had been exposed at some time to styrene–butadiene rubber. Elevated odds ratios were seen for cancer of the kidney: 2.0 (90% CI, 1.2–3.4) for 12 cases with 'any' exposure and 2.9 (1.0–8.3) for three cases with 'substantial' exposure. For non-Hodgkin's lymphoma, the odds ratios were 0.9 (0.5–1.7) for seven cases with 'any' exposure and 1.5 (0.4–5.1) for two cases with 'substantial' exposure.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, eight to nine weeks of age, were exposed to 625 or 1250 ppm (1380 or 2760 mg/m^3) 1,3-butadiene (minimum purity, > 98.9%) for 6 h per day on five days per week for 60 weeks (males) or 61 weeks (females). An equal number of animals sham-exposed in chambers served as controls. The study was terminated after 61 weeks because of a high incidence of lethal neoplasms in the exposed animals. The numbers

of survivors were: males—49/50 controls, 11/50 low-dose and 7/50 high-dose; females—46/50 controls, 14/50 low-dose and 30/50 high-dose. Haemangiosarcomas originating in the heart with metastases to various organs were found in: males—0/50 controls, 16/49 ($p < 0.001$) low-dose and 7/49 ($p = 0.006$) high-dose—and females—0/50 controls, 11/48 ($p < 0.001$) low-dose and 18/49 ($p < 0.001$) high-dose (Fisher exact test). [The Working Group noted that the incidence of haemangiosarcomas of the heart in historical controls was 1/2372 in males and 1/2443 in females.] Other types of neoplasm for which the incidences were increased (Fisher exact test) in animals of each sex were malignant lymphomas: males—0/50 controls, 23/50 ($p < 0.001$) low-dose and 29/50 ($p < 0.001$) high-dose; females—1/50 controls, 10/49 ($p = 0.003$) low-dose and 10/49 ($p = 0.003$) high-dose; alveolar bronchiolar adenomas or carcinomas of the lung: males—2/50 controls, 14/49 ($p < 0.001$) low-dose and 15/49 ($p < 0.001$) high-dose; females—3/49 controls, 12/48 ($p = 0.01$) low-dose and 23/49 ($p < 0.001$) high-dose; papillomas or carcinomas of the forestomach: males—0/49 controls, 7/40 ($p = 0.003$) low-dose and 1/44 ($p = 0.473$) high-dose; females—0/49 controls, 5/42 ($p = 0.018$) low-dose and 10/49 ($p < 0.001$) high-dose. Tumours that occurred with statistically significantly increased incidence in females only included hepatocellular adenoma or carcinoma of the liver: 0/50 controls, 2/47 ($p = 0.232$) low-dose and 5/49 ($p = 0.027$) high-dose; acinar-cell carcinoma of the mammary gland: 0/50 controls, 2/49 low-dose and 6/49 ($p = 0.012$) high-dose; and granulosa-cell tumours of the ovary: 0/49 controls, 6/45 ($p = 0.01$) low-dose and 12/48 ($p < 0.001$) high-dose (US National Toxicology Program, 1984; Huff *et al.*, 1985).

Groups of 60 male B6C3F$_1$ and 60 male NIH Swiss mice, four to six weeks of age, were exposed to 0 or 1250 ppm (2760 mg/m^3) 1,3-butadiene (> 99.5% pure) for 6 h per day on five days per week for 52 weeks. A group of 50 male B6C3F$_1$ mice was exposed similarly to 1,3-butadiene for 12 weeks and held until termination of the experiment at 52 weeks. The incidence of thymic lymphomas was 1/60 control B6C3F$_1$ mice, 10/48 B6C3F$_1$ mice exposed for 12 weeks, 34/60 B6C3F$_1$ mice exposed for 52 weeks and 8/57 NIH Swiss mice exposed for 52 weeks. Haemangiosarcomas of the heart were observed in 5/60 B6C3F$_1$ mice and 1/57 NIH Swiss mice (Irons *et al.*, 1989). [The Working Group noted the absence of reporting on NIH Swiss control mice.]

In studies designed to characterize exposure–response relationships further, groups of 70–90 male and 70–90 female B6C3F$_1$ mice, 6.5 weeks of age, were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm (0.14, 44, 138, 440 or 1380 mg/m^3) 1,3-butadiene (purity, > 99%) for 6 h per day on five days per week for up to two years. Ten animals per group were killed and evaluated after 40 and 65 weeks of exposure. Survival was significantly reduced ($p < 0.05$) in all groups of mice exposed to 1,3-butadiene at 20 ppm or higher; terminal survivors were: males, 35/70 controls, 39/70 at 6.25 ppm, 24/70 at 20 ppm, 22/70 at 62.5 ppm, 3/70 at 200 ppm and 0/90 at 625 ppm; females, 37/70 controls, 33/70 at 6.25 ppm, 24/70 at 20 ppm; 11/70 at 62.5 ppm; 0/70 at 200 ppm and 0/90 at 625 ppm. Tumours for which the rates were significantly increased by exposure to 1,3-butadiene are shown in Table 12 (Melnick *et al.*, 1990).

Groups of 50 male B6C3F$_1$ mice, 6.5 weeks of age, were exposed to 1,3-butadiene (purity, > 99%) for 6 h per day on five days per week at 200 ppm (442 mg/m^3) for 40 weeks, 625 ppm (1380 mg/m^3) for 13 weeks, 312 ppm (690 mg/m^3) for 52 weeks or 625 ppm

Table 12. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in mice exposed to 1,3-butadiene for up to two years

Tumour	Sex	Exposure concentration (ppm)											
		0		6.25		20		62.5		200		625	
		I	R	I	R	I	R	I	R	I	R	I	R
Lymphoma	M	4/70	8	3/70	6	8/70	19	11/70	25[a]	9/70	27[a]	69/90	97[a]
	F	10/70	20	14/70	30	18/70	41[a]	10/70	26	19/70	58[a]	43/90	89[a]
Haemangiosarcoma of the heart	M	0/70	0	0/70	0	1/70	2	5/70	13[a]	20/70	57[a]	6/90	53[a]
	F	0/70	0	0/70	0	0/70	0	1/70	3	20/70	64[a]	26/90	84[a]
Alveolar-bronchiolar adenoma and carcinoma[b]	M	22/70	46	23/70	48	20/70	45	33/70	72[a]	42/70	87[a]	12/90	73[a]
	F	4/70	8	15/70	32[a]	19/70	44[a]	27/70	61[a]	32/70	81[a]	25/90	83[a]
Forestomach papilloma and carcinoma	M	1/70	2	0/70	0	1/70	2	5/70	13	12/70	36[a]	13/90	75[a]
	F	2/70	4	2/70	4	3/70	8	4/70	12	7/70	31[a]	28/90	85[a]
Harderian gland adenoma and adenocarcinoma	M	6/70	13	7/70	15	11/70	25	24/70	53[a]	33/70	77[a]	7/90	58[a]
	F	9/70	18	10/70	21	7/70	17	16/70	40[a]	22/70	67[a]	7/90	48
Preputial gland adenoma and carcinoma	M	0/70	0	0/70	0	0/70	0	0/70	0	5/70	17[a]	0/90	0
Hepatocellular adenoma and carcinoma	M	31/70	55	27/70	54	35/70	68	32/70	69	40/70	87[a]	12/90	75
	F	17/70	35	20/70	41	23/70	52[a]	24/70	60[a]	20/70	68[a]	3/90	28
Adenocarcinoma of the mammary gland	F	0/70	0	2/70	4	2/70	5	6/70	16[a]	13/70	47[a]	13/90	66[a]
Benign and malignant granulosa-cell tumour of the ovary	F	1/70	2	0/70	0	0/70	0	9/70	24[a]	11/70	44[a]	6/90	44

From Melnick *et al.* (1990)

[a]Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

[b]The Working Group noted that the incidence in control males and females was in the range of that in historical controls (Haseman *et al.*, 1985).

(1380 mg/m^3) for 26 weeks. After the exposures were terminated, the animals were placed in control chambers for up to 104 weeks. A group of 70 males served as chamber controls (0 ppm). Survival was reduced in all treated groups; the numbers of survivors at the end of the study were 35 controls, nine exposed to 200 ppm, five exposed to 625 ppm for 13 weeks, one exposed to 312 ppm and none exposed to 625 ppm for 26 weeks. Tumours for which the rates were significantly increased by exposure to 1,3-butadiene are shown in Table 13 (Melnick *et al.*, 1990).

3.1.2 *Rat*

Groups of 100 male and 100 female Sprague–Dawley rats, five weeks of age, were exposed to 0, 1000 or 8000 ppm (2200 or 17 600 mg/m^3) 1,3-butadiene (minimal purity, 99.2%) for 6 h per day on five days per week for 111 weeks (males) or 105 weeks (females). Survival was reduced in low- and high-dose females and in high-dose males; the numbers of survivors were: males—45 control, 50 low-dose and 32 high-dose; females—46 control, 32 low-dose and 24 high-dose. Tumours that occurred at significantly increased incidence in males were exocrine adenomas and carcinomas of the pancreas (3 control, 1 low-dose, 11 ($p < 0.05$) high-dose) and Leydig-cell tumours of the testis (0 control, 3 low-dose, 8 ($p < 0.01$) high-dose). Those that occurred at significantly increased incidence (Fisher exact test) in females were follicular-cell adenomas and carcinomas of the thyroid gland (0 control, 4 low-dose, 11 ($p < 0.001$) high-dose) and benign and malignant mammary gland tumours (50 control, 79 low-dose and 81 high-dose, with a significant, dose-related trend ($p < 0.001$); most of the latter were fibroadenomas: 40 control, 75 ($p < 0.001$) low-dose, 67 ($p < 0.01$) high-dose. Tumours that occurred only with positive trends (Cochran–Armitage trend test) in females were sarcomas of the uterus ($p < 0.05$; 1 control, 4 low-dose, 5 high-dose) and carcinomas of the Zymbal gland ($p < 0.01$; 0 control, 0 low-dose, 4 high-dose) (Owen *et al.*, 1987; US Occupational Safety and Health Administration, 1990). [The Working Group noted that differences in tumour incidence between groups were not analysed using statistical methods that took into account differences in mortality between control and treated groups.]

3.2 Carcinogenicity of metabolites

Mouse: D,L-1,2:3,4-Diepoxybutane (IARC, 1976), an intermediate of 1,3-butadiene metabolism, induced 10/30 papillomas and 6/30 squamous-cell carcinomas of the skin when applied at 3 mg three times per week for life to the skin of female Swiss mice (Van Duuren *et al.*, 1965). 1,2-Epoxy-3-butene (vinyloxirane), another intermediate in 1,3-butadiene metabolism, induced 4/30 skin tumours when applied at 100 mg three times per week to the skin of male Swiss mice (Van Duuren *et al.*, 1963). Subcutaneous injection of D,L-1,2:3,4-diepoxybutane at 0.1 and 1.1 mg/animal in tricaprylin once per week for one year induced local fibrosarcomas in 5/50 and 5/30 female Swiss mice; no tumour was observed in three solvent-treated control groups. Administration of D,L-1,2:3,4-diepoxybutane at 1 mg/animal in tricaprylin once per week for one year induced local fibrosarcomas in 9/50 Sprague–Dawley rats, compared with none in controls (Van Duuren *et al.*, 1966).

Table 13. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in male mice exposed to 1,3-butadiene in stop-exposure studies (After exposures were terminated, animals were placed in control chambers until the end of the study at 104 weeks.)

Tumour	Exposure									
	0		200 ppm, 40 wk		625 ppm, 13 wk		312 ppm, 52 wk		625 ppm, 26 wk	
	I	R	I	R	I	R	I	R	I	R
Lymphoma	4/70	8	12/50	35[a]	24/50	61[a]	15/50	55[a]	37/50	90[a]
Haemangiosarcoma of the heart	0/70	0	15/50	47[a]	7/50	31[a]	33/50	87[a]	13/50	76[a]
Alveolar–bronchiolar adenoma and carcinoma	22/70	46	35/50	88[a]	27/50	87[a]	32/50	88[a]	18/50	89[a]
Forestomach squamous-cell papilloma and carcinoma	1/70	2	6/50	20[a]	8/50	33[a]	13/50	52[a]	11/50	63[a]
Harderian gland adenoma and adenocarcinoma	6/70	13	27/50	72[a]	23/50	82[a]	28/50	86[a]	11/50	70[a]
Preputial gland carcinoma	0/70	0	1/50	3	5/50	21[a]	4/50	21[a]	3/50	31[a]
Renal tubular adenoma	0/70	0	5/50	16[a]	1/50	5	3/50	15[a]	1/50	11

From Melnick *et al.* (1990)

[a]Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

3.3 Activated oncogenes

Tumours from the study of Melnick *et al.* (1990) were evaluated in independent studies for the presence of oncogenes. Activated K-*ras* oncogenes were detected in 6/9 lung adenocarcinomas, 3/12 hepatocellular carcinomas and 2/11 lymphomas obtained from B6C3F$_1$ mice exposed to 1,3-butadiene. A specific codon 13 mutation (guanine to cytosine transversion) was found in most of the activated K-*ras* genes (Goodrow *et al.*, 1990). Activated K-*ras* genes have not been found in spontaneously occurring liver tumours or lymphomas from B6C3F$_1$ mice (Reynolds *et al.*, 1987; Goodrow *et al.*, 1990) and were observed in only 1/10 spontaneous lung tumours in this strain of mice (Goodrow *et al.*, 1990).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

1,3-Butadiene was reported to be metabolized to 1,2-epoxy-3-butene by a single human postmitochondrial liver preparation; no metabolism was observed in a single lung sample (Schmidt & Loeser, 1985). [The Working Group was unable to determine whether the lung and the liver samples were from the same individual.] Incubations of 1,3-butadiene with human liver microsomes from four subjects produced the chiral antipodes 1,2-epoxy-3-butene at ratios of 52–56% *R*- to 44–48% *S*-epoxybutene (Wistuba *et al.*, 1989).

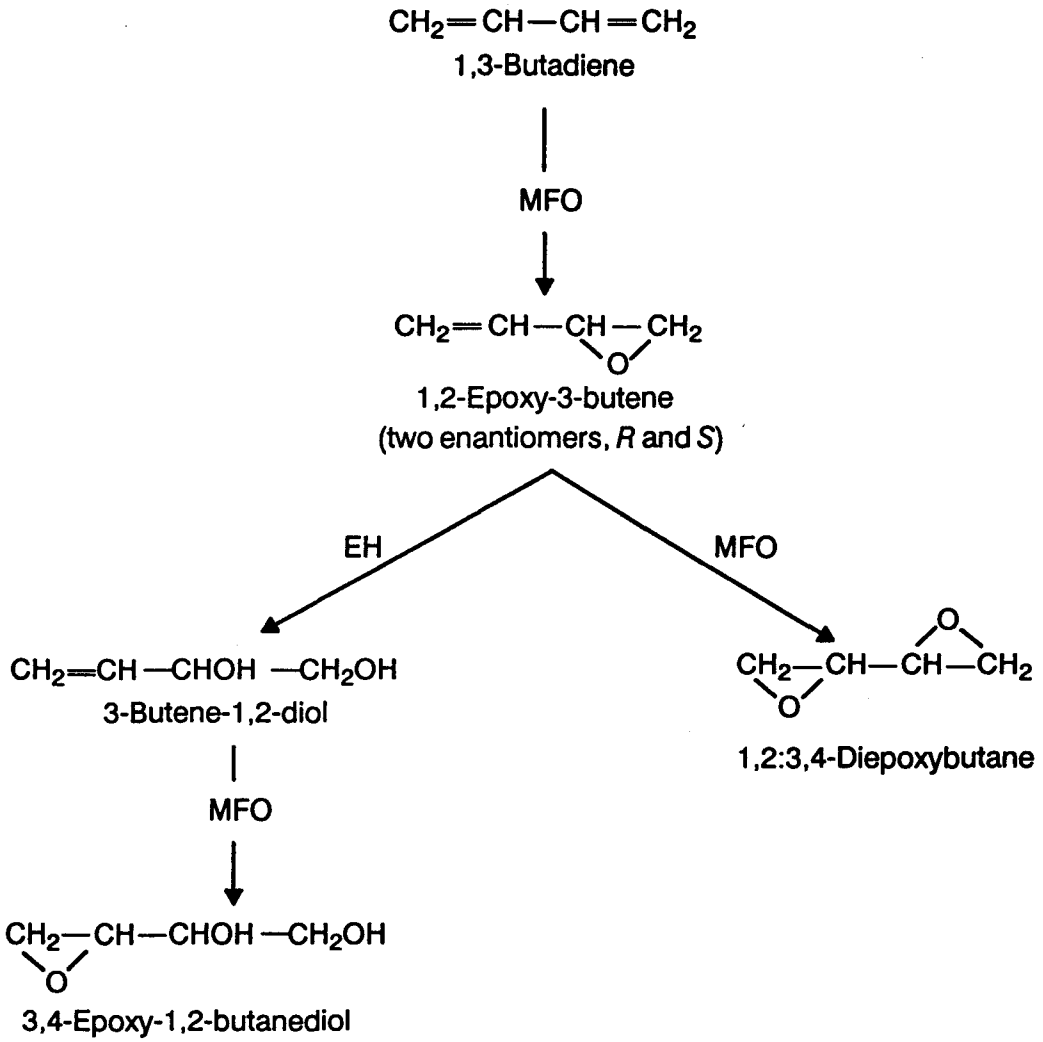
1,2-Epoxy-3-butene is further transformed by epoxide hydrolase and glutathione *S*-transferase, as measured by disappearance of the epoxide by human liver microsomes and cytosol (Kreuzer *et al.*, 1991).

4.1.2 *Experimental systems*

Male Sprague-Dawley rats were exposed in closed inhalation chambers to various initial concentrations of 1,3-butadiene to study the pharmacokinetic behaviour of the compound. Analysis of the resulting concentration decline curves of 1,3-butadiene in the gas phase revealed that its metabolism was saturable. At less than 800–1000 ppm [1800–2200 mg/m^3], 1,3-butadiene was metabolized according to first-order kinetics; at higher exposure concentrations (> 1500 ppm [> 3300 mg/m^3], saturation range), a maximal metabolic rate of 220 μmol/h per kg bw was observed; this was enhanced by pretreatment with Aroclor 1254 (Bolt *et al.*, 1984). In similar experiments in male B6C3F$_1$ mice, saturation of 1,3-butadiene metabolism was observed at higher exposure concentrations (> 2000 ppm [> 4400 mg/m^3]) at a maximal metabolic rate of 400 μmol/h per kg bw. Pharmacokinetic analysis of the data suggested that the species-related difference in the effect of 1,3-butadiene was due to more rapid uptake of the compound from the gas phase by mice (Kreiling *et al.*, 1986a).

1,3-Butadiene is converted to 1,2-epoxy-3-butene by mixed-function oxidases in rat liver microsomes *in vitro*. Pretreatment of rats with phenobarbital increases enzyme activity (Malvoisin *et al.*, 1979; Bolt *et al.*, 1983). 1,2-Epoxy-3-butene is further metabolized to 1,2:3,4-diepoxybutane and 3-butene-1,2-diol; the latter product is metabolized by mixed-function oxidases to 3,4-epoxy-1,2-butanediol (Malvoisin & Roberfroid, 1982) (Fig. 1).

Fig. 1. Possible pathways for metabolism of 1,3-butadiene by rat liver microsomes



From Malvoisin and Roberfroid (1982); MFO, mixed-function oxidases; EH, epoxide hydrolase

Cytochrome P-450-mediated formation of 1,2-epoxy-3-butene from 1,3-butadiene also occurs in the presence of mouse liver microsomes, and crotonaldehyde has been shown to be a further metabolite (Elfarra *et al.*, 1991).

1,2-Epoxy-3-butene is present in the expired air of rats and mice exposed to 1,3-butadiene (Bolt *et al.*, 1983; Kreiling *et al.*, 1987). When male Sprague-Dawley rats were exposed in closed exposure chambers to concentrations of 1,3-butadiene higher than 2000 ppm [4400 mg/m^3], which result in the maximum possible metabolic rate, about 4 ppm [8.8 mg/m^3] 1,2-epoxy-3-butene were measured in the gas phase under steady-state conditions. Kinetic analysis revealed that only 29% of the predicted value of 1,3-butadiene metabolite under these conditions was available systemically as 1,2-epoxy-3-butene, which was considered to be related to a first-pass metabolism of the 1,2-epoxy-3-butene originating in the

liver (Filser & Bolt, 1984). The exhalation of 1,2-epoxy-3-butene by two male Sprague–Dawley rats and six male B6C3F$_1$ mice exposed in a closed system to 2000–4000 ppm [4400–8800 mg/m^3] 1,3-butadiene for 15 h was compared (Kreiling *et al.*, 1987). After about 2 h, rats had built up a constant concentration of 1,2-epoxy-3-butene at about 4 ppm [8 mg/m^3], with no sign of toxicity. 1,2-Epoxy-3-butene concentrations in the experiment with mice increased to about 10 ppm [22 mg/m^3] after 10 h; and after 12 h, animals showed signs of acute toxicity.

Studies on the disposition of inhaled (nose only) ^{14}C-labelled 1,3-butadiene in Sprague–Dawley rats and B6C3F$_1$ mice confirmed that mice metabolize 1,3-butadiene to a greater extent than rats. Radiolabelled metabolites present in blood were separated according to their volatility by vacuum line–cryogenic distillation (Dahl *et al.*, 1984). Blood samples taken from mice during exposure to 13 000 mg/m^3 (7100 ppm) (*sic*) for 6 h contained two to five times more radiolabelled 1,2-epoxy-3-butene than did the blood of rats (Bond *et al.*, 1987). Three male cynomolgus monkeys (*Macaca fascicularis*) were exposed by nose only to 10, 310 or 7760 ppm [22, 680 or 17 150 mg/m^3] ^{14}C-butadiene for 2 h. For exposures equivalent to those in mice and rats, the concentrations of total 1,3-butadiene metabolites in blood were 5–50 times lower in monkeys than in mice. The ranking of species was thus mice > rats > monkeys (Dahl *et al.*, 1991).

Metabolic species differences were also investigated *in vitro* using liver preparations from rats (Sprague–Dawley, Wistar), mice (NMRI and B6C3F$_1$), rhesus monkeys and humans (Schmidt & Loeser, 1985). The ranking of species for 1,2-epoxy-3-butene formation was: female mice > male mice > rats (humans) > monkeys. [The Working Group noted that the quantitative data on the human rate were derived from a single sample of liver.]

Repeated pretreatment of male Sprague–Dawley rats and male B6C3F$_1$ mice (inhalation by nose only) with 1,3-butadiene at 13 600 mg/m^3 (7600 ppm) for 6 h per day for five days had no effect on the ability of liver microsomes isolated from these animals to metabolize 1,3-butadiene. The metabolism of 1,3-butadiene *in vitro* was depressed significantly, however, in microsomes from lungs of pre-exposed rats and mice compared to unexposed controls (Bond *et al.*, 1988). Formation of 1,2-epoxy-3-butene was also observed after incubation of 1,3-butadiene with mouse and rat lung tissue but not after incubation with lung tissue from monkeys or humans (Schmidt & Loeser, 1985). [The Working Group noted that the quantitative data on the human rate were derived from a single sample of lung.]

The inhalation pharmacokinetics of the metabolite 1,2-epoxy-3-butene were studied in male Sprague–Dawley rats and male B6C3F$_1$ mice in closed chambers. Whereas in rats no indication of saturation kinetics could be obtained up to exposure concentrations of 5000 ppm [11 000 mg/m^3], saturation occurred in mice exposed to 500 ppm [1100 mg/m^3] or more (Kreiling *et al.*, 1987; Laib *et al.*, 1990).

4.2 Toxic effects

4.2.1 *Humans*

The toxic effects of combined exposures to 1,3-butadiene and other agents (e.g., styrene, chloroprene, hydrogen sulfide, acrylonitrile) have been reviewed (Parsons & Wilkins, 1976). Concentrations of several thousand parts per million of 1,3-butadiene irritate the skin, eyes, nose and throat (Carpenter *et al.*, 1944; Wilson *et al.*, 1948; Parsons & Wilkins, 1976).

Several studies have been reported on the effects of occupational exposure to 1,3-butadiene, mainly from the ex-USSR and Bulgaria. Few are substantiated by details on the atmospheric concentration or duration of exposure, and control data are generally not provided. The effects reported include haematological disorders (Batkina, 1966; Volkova & Bagdinov, 1969), kidney malfunction, laryngotracheitis, irritation of the upper respiratory tract, conjunctivitis, gastritis, various skin disorders, a variety of neuraesthenic symptoms (Parsons & Wilkins, 1976) and hypertension and neurological disorders (Spasovski *et al.*, 1986).

Checkoway and Williams (1982) reported minimal changes in haematological indices among eight workers exposed to about 20 ppm (44.2 mg/m^3) 1,3-butadiene, 14 ppm (59.5 mg/m^3) styrene and 0.03 ppm (0.1 mg/m^3) benzene, as compared to those among 145 workers exposed to less than 2 ppm (4.4 mg/m^3) 1,3-butadiene, 2 ppm (8.5 mg/m^3) styrene and 0.1 ppm (0.3 mg/m^3) benzene. Changes included a slight decrease in haemoglobin level and a slight increase in red-cell mean corpuscular volume. [The Working Group considered that these changes cannot be interpreted as an effect of 1,3-butadiene on the bone marrow, particularly as alcohol intake was not evaluated.]

4.2.2 *Experimental systems*

LC_{50} values for 1,3-butadiene were reported to be 270 000 mg/m^3 [122 170 ppm] in mice exposed for 2 h and 285 000 mg/m^3 [129 000 ppm] in rats exposed for 4 h; after 1 h of exposure, rats were in a state of deep narcosis (Shugaev, 1969). Oral LD_{50} values of 5.5 g/kg bw for rats and 3.2 g/kg bw for mice have been reported (US National Toxicology Program, 1984).

In female rats exposed to 1–30 mg/m^3 (0.45-14 ppm) 1,3-butadiene for 81 days, morphological changes were observed in liver, kidney, spleen, nasopharynx and heart (G.K. Ripp reported in Crouch *et al.*, 1979). In groups of 24 rats exposed to 600–6700 ppm [1300–14 800 mg/m^3] 1,3-butadiene for 7.5 h per day on six days per week for eight months, no adverse effect was noted, except for a slight retardation in growth with the highest concentration (Carpenter *et al.*, 1944). Rats exposed to 2200–17 600 mg/m^3 (1000–8000 ppm) 1,3-butadiene for 6 h per day on five days per week for three months showed no treatment-related effect other than increased salivation in females (Crouch *et al.*, 1979).

Groups of 110 male and 110 female CD Sprague–Dawley rats were exposed to atmospheres containing 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] 1,3-butadiene for 6 h per day on five days per week. The study was terminated when it was predicted that survival would drop to 20–25% (105 weeks for females, 111 weeks for males). Ten animals of each sex from each group were killed at 52 weeks. Treatment was associated with changes in clinical condition and lowering of body weight gain during the first 12 weeks, then nonsignificant changes, reduced survival and increases in certain organ weights and in the incidences of uncommon tumour types (for details, see p. 257). Increased mortality in high-dose males was accompanied by an increase in the severity of nephropathy (Owen *et al.*, 1987; Owen & Glaister, 1990).

B6C3F$_1$ mice exposed to 0, 625 or 1250 ppm [1380 or 2760 mg/m^3] 1,3-butadiene for 6 h per day on five days per week for 60–61 weeks had increased prevalences of atrophy of the ovary and testis, atrophy and metaplasia of the nasal epithelium, hyperplasia of the

respiratory and forestomach epithelium and liver necrosis (see also pp. 254–255) (US National Toxicology Program, 1984).

Haematological changes in male B6C3F$_1$ mice exposed to 62.5, 200 or 625 ppm [138, 440 or 1375 mg/m^3] 1,3-butadiene for 6 h per day on five days per week for 40 weeks included decreased red blood cell count, haemoglobin concentration and packed red cell volume and increased mean corpuscular volume. Similar changes occurred in female mice exposed to 625 ppm [1375 mg/m^3] 1,3-butadiene (for details, see pp. 255–257) (Melnick *et al.*, 1990).

The role of murine retroviruses on the induction of leukaemias and lymphomas following inhalation of 1,3-butadiene was evaluated in a series of studies reviewed by Irons (1990). Exposure of groups of male B6C3F$_1$ mice, which have the intact ecotropic murine leukaemia virus, to 1250 ppm [2750 mg/m^3] 1,3-butadiene for 6 h per day on 6 days per week for 6–24 weeks resulted in a decrease in the number of circulating erythrocytes, in total haemoglobin and in haematocrit and an increase in mean corpuscular volume. Leukopenia, due primarily to a decrease in the number of segmented neutrophils, and an increase in the number of circulating micronuclei were observed (Irons *et al.*, 1986a). Persistent immunological defects were not detectable after this treatment (Thurmond *et al.*, 1986). Exposure of male NIH Swiss mice, which do not possess intact endogenous ecotropic murine leukaemia virus, produced similar results (Irons *et al.*, 1986b).

A further study was conducted to examine the expression and behaviour of endogenous retroviruses in these strains during the preleukaemic phase of 1,3-butadiene exposure. Chronic exposure of B6C3F$_1$ mice to 1,3-butadiene (1250 ppm [2740 mg/m^3]) for 6 h per day on five days per week for 3–21 weeks increased markedly the quantity of ecotropic retrovirus recoverable from the bone marrow, thymus and spleen. Expression of other endogenous retroviruses (xenotropic, MCF-ERV) was not enhanced. No virus of any type was found in similarly treated NIH Swiss mice (Irons *et al.*, 1987a).

Enhanced susceptibility to 1,3-butadiene-induced leukaemogenesis as a result of the ability to express the retrovirus was suggested by the finding that exposure to 1250 ppm 1,3-butadiene for one year resulted in a 57% incidence of thymic lymphoma in B6C3F$_1$ mice (with expression of the virus) and a 14% incidence in NIH Swiss (without viral expression) (Irons *et al.*, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Fertility was reported to be unimpaired in mating studies in rats, guinea-pigs and rabbits exposed to 600, 2300 or 6700 ppm [1300, 5000 or 14 800 mg/m^3] 1,3-butadiene by inhalation for 7.5 h per day on six days per week for eight months (Carpenter *et al.*, 1944). [The Working Group noted the incomplete reporting of this study].

Pregnant Sprague–Dawley rats (24–28 per group) and Swiss (CD-1) mice (18–22 per group) were exposed to atmospheric concentrations of 0, 40, 200 or 1000 ppm [0, 88, 440 or 2200 mg/m^3] 1,3-butadiene for 6 h per day on days 6–15 of gestation and killed on gestation day 18 (mice) or 20 (rats). Subsequently, the uterine contents were evaluated; individual fetal

body weights were recorded; and external, visceral and skeletal examinations were performed. In rats, maternal toxicity was observed in the 1000-ppm group in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain. Under these conditions, there was no evidence of developmental toxicity. Maternal toxicity was observed in mice given 200 and 1000 ppm 1,3-butadiene; 40 ppm and higher concentrations of 1,3-butadiene caused significant exposure-related reductions in the mean body weights of male fetuses. Mean body weights of female fetuses were reduced at the 200 and 1000 ppm exposure levels. No increased incidence of malformations was observed in either species. The frequency of fetal variations (supernumerary ribs, reduced sternebral ossification) was significantly increased in mice exposed to 200 and 1000 ppm. In a study of sperm-head morphology, groups of 20 male B6C3F$_1$ mice were exposed to atmospheric concentrations of 0, 200, 1000 or 5000 ppm [0, 440, 2200 or 11 000 mg/m^3] 1,3-butadiene for 6 h per day for five consecutive days. Small, concentration-related increases in the frequency of abnormal sperm morphology were seen five weeks after exposure (the only time of examination) (Hackett *et al.*, 1987; Morrissey *et al.*, 1990). [The Working Group noted that sequential examinations were not conducted after exposure to determine the effect of 1,3-butadiene on all stages of gamete development.]

4.4 Genetic and related effects

4.4.1 *Humans*

In an abstract of a study of workers engaged in the manufacture of 1,3-butadiene in Finland, cytogenetic analysis revealed no increase in the frequency of sister chromatid exchange, chromosomal aberrations or micronucleus formation in peripheral blood. The ambient air concentrations of 1,3-butadiene were generally < 1 ppm [< 2.2 mg/m^3], and the workers used protective clothing and respirators (Sorsa *et al.*, 1991).

4.4.2 *Experimental systems* (see also Tables 14–16 and Appendices 1 and 2)

The genetic toxicology of 1,3-butadiene has been reviewed (Rosenthal, 1985; de Meester, 1988; Brown, 1990). Additional information on 1,3-butadiene is included in a review by the Dutch Expert Committee for Occupational Standards (1990). The genetic and related effects of two main metabolites of 1,3-butadiene (1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane) were reviewed by Ehrenberg and Hussain (1981) and de Meester (1988).

(a) 1,3-Butadiene

1,3-Butadiene was mutagenic to *Salmonella typhimurium* TA1530 in the presence of liver S9 from phenobarbital- or Aroclor 1254-pretreated rats but was not mutagenic in the presence of uninduced rat liver S9 (de Meester *et al.*, 1980). It was also mutagenic to TA1535 in the presence of Arcolor 1254-induced rat S9, uninduced rat S9 and uninduced mouse S9 but was not mutagenic in the presence of uninduced human S9 (Arce *et al.*, 1990).

1,3-Butadiene gave negative results in tests for somatic mutation and recombination in *Drosophila melanogaster*.

1,3-Butadiene was not active in the L5178Y mouse lymphoma forward mutation assay. A weak positive response was reported for sister chromatid exchange induction in Chinese hamster ovary (CHO) cells.

In one study, sister chromatid exchange was induced weakly in human whole blood lymphocyte cultures after treatment with 1,3-butadiene in the presence and absence of Aroclor-1254-induced rat liver S9. No sister chromatid exchange was induced in another study in which S9 from a variety of sources was used, including mouse and human.

When $B6C3F_1$ mice and Wistar rats were exposed to ^{14}C-1,3-butadiene in a closed exposure system, radiolabel was associated with hepatic nucleoproteins and DNA from both species. The association of radiolabel with nucleoproteins was about two times stronger in mice than in rats, but the association with DNA was similar in the two species (Kreiling *et al.*, 1986b). Acid hydrolysis of DNA isolated from the livers of mice exposed to ^{14}C-1,3-butadiene revealed the presence of two identifiable alkylation products: 7-*N*-(1-hydroxy-3-buten-2-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine. These were not found in similarly exposed rats (Jelitto *et al.*, 1989).

After a 7-h exposure of mice and rats to 1,3-butadiene at 250, 500 or 1000 ppm (550, 1100 or 2200 mg/mg^3), alkaline elution profiles from the livers and lungs showed the occurrence of protein–DNA and DNA–DNA cross-links with all doses of 1,3-butadiene in mice but not in rats. This finding was interpreted as a biological effect in mice of the bifunctional alkylating metabolite, 1,2:3,4-diepoxybutane (Jelitto *et al.*, 1989). In another study, there was no evidence of the formation of cross-links in DNA isolated from the livers of 1,3-butadiene-treated mice or rats (Ristau *et al.*, 1990).

No unscheduled DNA synthesis was evident in the livers of either Sprague-Dawley rats or $B6C3F_1$ mice after exposure to 10 000 ppm [22 000 mg/m^3] 1,3-butadiene.

1,3-Butadiene increased the frequency of sister chromatid exchange in bone-marrow cells of mice, but not of rats, exposed *in vivo*. Chromosomal aberrations and micronuclei, but not aneuploidy, were induced in mice by 1,3-butadiene, but, in a single study, micronuclei were not induced in rats.

In a study of dominant lethal mutations, male Swiss CD-1 mice were exposed to 0, 70, 200, 1000 or 5000 ppm [155, 440, 2200 or 11 050 mg/m^3] 1,3-butadiene for 6 h per day for five days and then mated weekly for eight weeks. After one week, a significant increase was observed in the number of dead implants in females mated with males exposed to 1000 ppm (smaller increases were seen at 200 and 5000 ppm). Two weeks after exposure, the proportion of dead implants was increased in the 200- and 1000-ppm groups [details not given]. Sperm-head abnormalities were induced in exposed males (Morrissey *et al.*, 1990).

(*b*) *1,2-Epoxy-3-butene*

1,2-Epoxy-3-butene reacts with DNA to give two main alkylated products, 7-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine (Citti *et al.*, 1984).

1,2-Epoxy-3-butene was mutagenic to bacteria in the absence of an exogenous metabolic system. It did not induce unscheduled DNA synthesis in rat or mouse hepatocytes but induced sister chromatid exchange in CHO cells and in cultured human lymphocytes. In a single study, it induced sister chromatid exchange and chromosomal aberrations in mouse bone marrow *in vivo*.

(*c*) *1,2:3,4-Diepoxybutane*

1,2:3,4-Diepoxybutane induced interstrand cross-links in DNA by reaction at the *N*7 position of guanine (Lawley & Brookes, 1967).

Table 14. Genetic and related effects of 1,3-butadiene

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1300.0000	Arce *et al.* (1990)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	+	86.0000	de Meester *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	650.0000	Arce *et al.* (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1300.0000	Arce *et al.* (1990)
SA8, *Salmonella typhimurium* TA97, reverse mutation	–	–	1300.0000	Arce *et al.* (1990)
DMM, *Drosophila melanogaster*, wing spot mutation	–	0	10000.0000	Victorin *et al.* (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	–	–	650.0000	McGregor *et al.* (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	(+)	1.3500	Sasiadek *et al.* (1991a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	2160.0000	Arce *et al.* (1990)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	108.0000	Sasiadek *et al.* (1991b)
DVA, DNA-DNA cross-links, Sprague-Dawley rats *in vivo*	–	0	310.0000 inhal. 8 h/d, 7 d	Ristau *et al.* (1990)
DVA, DNA-DNA cross-links, B6C3F1 mice *in vivo*	–	0	3100.0000 inhal. 8 h/d, 7 d	Ristau *et al.* (1990)
BVD, DNA alkylation, male Wistar rat liver cells *in vivo*	–	0	550.0000	Jelitto *et al.* (1989)
BVD, DNA alkylation, male B6C3F1 mouse liver cells *in vivo*	+	0	680.0000	Jelitto *et al.* (1989)
DVA, DNA-DNA cross-links, Sprague-Dawley rat liver/lung *in vivo*	–	0	550.0000	Jelitto *et al.* (1989)
DVA, DNA-DNA cross-links, B6C3F1 mouse liver/lung *in vivo*	+	0	680.0000	Jelitto *et al.* (1989)
UPR, Unscheduled DNA synthesis, Sprague-Dawley rats *in vivo*	–	0	4000.0000 inhal.[c]	Arce *et al.* (1990)
UPR, Unscheduled DNA synthesis, Sprague-Dawley rats *in vivo*	–	0	4000.0000 inhal.[d]	Arce *et al.* (1990)
UVM, Unscheduled DNA synthesis, B6C3F1 mice *in vivo*	–	0	11600.0000 inhal.[c]	Arce *et al.* (1990)
UVM, Unscheduled DNA synthesis, B6C3F1 mice *in vivo*	–	0	11600.0000 inhal.[d]	Arce *et al.* (1990)
SVA, Sister chromatid exchange, male B6C3F1 mouse bone marrow *in vivo*	+	0	116.0000 inhal. 6 h/d[e]	Cunningham *et al.* (1986)
SVA, Sister chromatid exchange, male Sprague–Dawley rat bone marrow *in vivo*	–	0	4000.0000 inhal. 6 h/d[e]	Cunningham *et al.* (1986)
SVA, Sister chromatid exchange, male B6C3F1 mouse bone marrow *in vivo*	+	0	7.0000 inhal. 6 h/d, 10 d	Tice *et al.* (1987)
MVM, Micronucleus test, male B6C3F1 mouse bone marrow *in vivo*	+	0	116.0000 6 h/d[e]	Cunningham *et al.* (1986)
MVM, Micronucleus test, male B6C3F1 mouse peripheral blood *in vivo*	+	0	70.0000 inhal. 6 h/d, 10 d	Tice *et al.* (1987)
MVM, Micronucleus test, male B6C3F1 mouse peripheral blood *in vivo*	+	0	7.0000[f]	Jauhar *et al.* (1988)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	+	0	35.0000 inhal. 23 h	Victorin *et al.* (1990)

Table 14 (contd)

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVR, Micronucleus test, male Sprague–Dawley rat bone marrow *in vivo*	–	0	4000.0000[e]	Cunningham *et al.* (1986)
CBA, Chromosomal aberrations, male B6C3F1 mouse bone marrow *in vivo*	+	0	1500.0000[g] inhal. 6 h[g]	Irons *et al.* (1987b)
CBA, Chromosomal aberrations, male NIH Swiss mouse bone marrow *in vivo*	+	0	1500.0000[g] inhal. 6 h[g]	Irons *et al.* (1987b)
CBA, Chromosomal aberrations, male B6C3F1 mouse bone-marrow *in vivo*	+	0	700.0000	Tice *et al.* (1987)
*Aneuploidy, male NIH Swiss mouse bone marrow *in vivo*	–	0	1500.0000 inhal. 6 h[g]	Irons *et al.* (1987b)
*Aneuploidy, male B6C3F1 mouse bone marrow *in vivo*	–	0	1500.0000 inhal. 6 h[g]	Irons *et al.* (1987b)
DLM, Dominant lethal test, Swiss CD–1 mouse	+	0	233.0000	Morrissey *et al.* (1990)
SPM, Sperm abnormality test, mouse	+	0	1165.0000	Morrissey *et al.* (1990)

[a]+, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)
[b]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[c]6 h treatment on day 1, 3 h on day 2, liver sampled 2 h later
[d]6 h treatment on days 1 and 2, liver sampled 18 h later
[e]For two days, killed 24 h after the second exposure
[f]Five days/week for 13 weeks
[g]Killed at 24, 48, 72 and 96 h after cessation of exposure
*Data not displayed on profiles

Table 15. Genetic and related effects of 1,2-epoxy-3-butene

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	350.0000	de Meester *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	26.0000	Gervasi *et al.* (1985)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	0	175.0000	de Meester *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	1750.0000	de Meester *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	0	8750.0000	de Meester *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	0	8750.0000	de Meester *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	8750.0000	de Meester *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	105.0000	Gervasi *et al.* (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	0	0.0000	Hemminki *et al.* (1980)
KPF, *Klebsiella pneumoniae*, fluctuation test	+	0	70.0000	Voogd *et al.* (1981)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	-	0	1000.0000	Arce *et al.* (1990)
UIA, Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	-	0	1000.0000	Arce *et al.* (1990)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	0.0700	Sasiadek *et al.* (1991a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	1.7500	Sasiadek *et al.* (1991b)
SVA, Sister chromatid exchange, male C57Bl/6 mouse bone marrow *in vivo*	+	0	25.0000	Sharief *et al.* (1986)
CBA, Chromosomal aberrations, male C57Bl/6 mouse bone marrow *in vivo*	+	0	25.0000	Sharief *et al.* (1986)

[a] +, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)
[b] In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

Table 16. Genetic and related effects of 1,2:3,4-diepoxybutane

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, *Bacillus megaterium*	+	0	0.0000	Lwoff (1953)
PRB, Prophage induction, *Pseudomonas pyocyanea*	+	0	0.0000	Lwoff (1953)
PRB, Prophage induction, *Escherichia coli* K-12	+	0	7.5000	Heinemann & Howard (1964)
ECB, *Escherichia coli* H540, DNA repair induction	+	0	2500.0000	Thielmann & Gersbach (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	50.0000	Dunkel *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	20.0000	Gervasi *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	25.0000	McCann *et al.* (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5.0000	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5.0000	Dunkel *et al.* (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	167.0000	Dunkel *et al.* (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	50.0000	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	167.0000	Dunkel *et al.* (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	167.0000	Dunkel *et al.* (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	60.0000	Gervasi *et al.* (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	(+)	(+)	167.0000	Dunkel *et al.* (1984)
ECR, *Escherichia coli* B, reverse mutation	+	0	1720.0000	Glover (1956)
ECR, *Escherichia coli* B/r, reverse mutation	+	0	860.0000	Glover (1956)
KPF, *Klebsiella pneumoniae*, fluctuation test	+	0	4.0000	Voogd *et al.* (1981)
**Saccharomyces cerevisiae* D7, gene conversion	+	+	130.0000	Sandhu *et al.* (1984)
SCH, *Saccharomyces cerevisiae* D4, mitotic gene conversion	+	0	430.0000	Zimmermann (1971)
SCH, *Saccharomyces cerevisiae* D81, mitotic crossing-over	+	0	2000.0000	Zimmermann & Vig (1975)
SCH, *Saccharomyces cerevisiae* D3, mitotic recombination	+	+	400.0000	Simmon (1979)
**Saccharomyces cerevisiae* D7, mitotic crossing-over	+	+	130.0000	Sandhu *et al.* (1984)
**Saccharomyces cerevisiae*, reverse mutation	+	0	4000.0000	Polakowska & Putrament (1979)

Table 16 (contd)

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCF, *Saccharomyces cerevisiae*, cytoplasmic petite mutation	-	0	4000.0000	Polakowska & Putrament (1979)
**Saccharomyces cerevisiae*, mitochondrial mutation	+	0	4000.0000	Polakowska & Putrament (1979)
**Saccharomyces cerevisiae* D7, reverse mutation	+	+	130.0000	Sandhu *et al.* (1984)
NCR, *Neurospora crassa*, reverse mutation	+	0	4300.0000	Kölmark & Westergaard (1953)
NCR, *Neurospora crassa*, reverse mutation	+	0	1720.0000	Pope *et al.* (1984)
DMM, *Drosophila melanogaster*, recombination and mutation, spot test	+	0	1000.0000	Graf *et al.* (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	100.0000	Bird & Fahmy (1953)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	175.0000	Sankaranarayanan *et al.* (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	1000.0000	Fahmy & Fahmy (1970)
DMC, *Drosophila melanogaster*, chromosomal deletion	+	0	1000.0000	Fahmy & Fahmy (1970)
DIA, DNA–DNA cross-links, B6C3F1 mouse liver DNA *in vitro*	+	0	4.0000	Ristau *et al.* (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	0	0.3000	McGregor *et al.* (1988)
SIC, Sister chromatid exchange, Chinese hamster CHO cells *in vitro*	+	0	0.0250	Perry & Evans (1975)
SIC, Sister chromatid exchange, Chinese hamster CHO cells *in vitro*	+	+	0.0100	Sasiadek *et al.* (1991a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.1250	Wiencke *et al.* (1982)
SHL, Sister chromatid exchange, human lymphocytes[c] *in vitro*	-	0	0.0100	Porfirio *et al.* (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.0100	Porfirio *et al.* (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	0.0400	Sasiadek *et al.* (1991b)
CHF, Chromosomal aberrations, human skin fibroblasts[d] *in vitro*	+	0	0.0100	Auerbach & Wolman (1978)
CHF, Chromosomal aberrations, human skin fibroblasts *in vitro*	-	0	0.0100	Auerbach & Wolman (1978)
CHL, Chromosomal aberrations, human lymphoblastoid cell lines[e]	+	0	0.0100	Cohen *et al.* (1982)
CHL, Chromosomal aberrations, human lymphocytes[a] *in vitro*	+	0	0.1000	Marx *et al.* (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	(+)	0	0.1000	Marx *et al.* (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	-	0	0.0100	Porfirio *et al.* (1983)
CHL, Chromosomal aberrations, human lymphocytes[c] *in vitro*	+	0	0.0100	Porfirio *et al.* (1983)

Table 16 (contd)

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIH, Chromosomal aberrations, human bone-marrow cells[c] *in vitro*	(+)	0	0.1000	Marx *et al.* (1983)
CIH, Chromosomal aberrations, normal human bone-marrow cells *in vitro*	(+)	0	0.1000	Marx *et al.* (1983)
HMM, Host-mediated assay, mutation, *S. typhimurium* TA1530 in mice	+	0	444.0000	Simmon (1979)?
HMM, Host-mediated assay, mitotic recombination, *S. cerevisiae* D3 in mice	-	0	56.0000	Simmon *et al.* (1979)
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+	0	1.0000	Conner *et al.* (1983)
SVA, Sister chromatid exchange, mouse alveolar macrophages *in vivo*	+	0	1.0000	Conner *et al.* (1983)
SVA, Sister chromatid exchange, mouse regenerating liver cells *in vivo*	+	0	1.0000	Conner *et al.* (1983)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+	0	22.0000[f]	Walk *et al.* (1987)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+	0	29.0000	Walk *et al.* (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+	0	34.0000[g]	Walk *et al.* (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+	0	32.0000	Walk *et al.* (1987)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+	0	22.0000[f]	Walk *et al.* (1987)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+	0	29.0000	Walk *et al.* (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+	0	34.0000[g]	Walk *et al.* (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+	0	32.0000	Walk *et al.* (1987)

[a] +, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)
[b] In-vitro tests, mg/ml; in-vivo tests, mg/kg bw
[c] Fanconi's anaemia (homozygotes and heterozygotes)
[d] Fanconi's anaemia (heterozygotes)
[e] Fanconi's anaemia (homozygotes and heterozygotes), ataxia telangiectasia, xeroderma pigmentosum, normal
[f] Calculated to give 22 (F) and 23 (M) mg/kg
[g] Calculated to give 34 (F) and 42 (M) mg/kg
* Not displayed on profile

Addition of an exogenous metabolic system was not required for genotoxic activity of this compound *in vitro*. In bacteria, it induced prophage, DNA repair and mutation. It induced mutation, gene conversion and mitotic recombination in yeast and mutation in fungi. In *Drosophila melanogaster*, it induced mutation and small chromosomal deletions.

1,2:3,4-Diepoxybutane induced DNA cross-links in mouse hepatocytes, dose-related increases in the frequency of sister chromatid exchange in cultured CHO cells and, in a single study, mutations in cultured mouse lymphoma L5178Y cells at the *tk* locus. It induced a dose-related increase in the frequency of sister chromatid exchange in cultured human lymphocytes from normal donors and from patients with a variety of solid tumours, but not from Fanconi's anaemia homozygotes or heterozygotes. It induced chromosomal aberrations in early-passage skin fibroblasts from Fanconi's anaemia heterozygotes, in primary lymphocytes from Fanconi's anaemia homozygotes and heterozygotes and in long-established lymphoblastoid cell lines from normal donors, Fanconi's anaemia homozygotes and heterozygotes and patients with xeroderma pigmentosum and ataxia telangiectasia. Bone-marrow cultures from Fanconi's anaemia patients and control individuals also showed increased frequencies of chromosomal aberrations after exposure to 1,2:3,4-diepoxybutane. Chromosomal aberrations were not induced in normal lymphocytes in two studies, but small increases were observed in another one.

1,2:3,4-Diepoxybutane induced mutations in *S. typhimurium* TA1530 in the mouse host-mediated assay, but it did not induce mitotic recombination in *Saccharomyces cerevisiae* D3.

Significant, dose-related increases in the frequency of sister chromatid exchange were observed in bone marrow and in alveolar macrophages from both intact and partially hepatectomized mice and in the regenerating liver of hepatectomized mice. 1,2:3,4-Diepoxybutane induced chromosomal aberrations and sister chromatid exchange in bone-marrow cells of male and female NMRI mice and Chinese hamsters exposed by inhalation or intraperitoneal injection.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,3-Butadiene has been produced on a large scale since the 1930s. It is used to manufacture a wide range of polymers and copolymers, including styrene–butadiene rubber, polybutadiene, nitrile rubber, acrylonitrile–butadiene–styrene resins and styrene–butadiene latexes. It is also an intermediate in the production of various other chemicals.

Occupational exposure to 1,3-butadiene occurs in the production of monomeric 1,3-butadiene, of butadiene-based polymers and butadiene-derived products. The mean concentrations reported have usually been < 10 ppm (< 22 mg/m^3), although that level may be exceeded during some short-term activities. 1,3-Butadiene is not usually found at detectable levels in the manufacture of finished rubber and plastic products. Because gasoline contains 1,3-butadiene, loading of gasoline and other gasoline-related operations entail exposure to 1,3-butadiene.

1,3-Butadiene has also been detected in automobile exhaust and, at levels of < 0.02 ppm (< 0.04 mg/m^3), in urban air.

5.2 Human carcinogenicity data

One US cohort study of workers who manufactured 1,3-butadiene monomer showed a significant excess risk for lymphosarcoma and reticulosarcoma. Although there was no overall excess risk for leukaemia, there was a suggested increase in risk in a subgroup of workers with 'non-routine' exposure to 1,3-butadiene.

In a US study of workers employed in two styrene–butadiene rubber plants, there was a suggested increase of risk for leukaemia with exposure to 1,3-butadiene in one of the plants. No increase in risk was seen for cancers of the lymphatic and haematopoietic system other than leukaemia.

In a study of styrene–butadiene rubber workers in eight plants in the USA and Canada, there was no overall increased risk for leukaemia; however, a subgroup of production workers had a significantly increased risk. There was no apparent increased risk for 'other lymphatic system' cancers, although a significant risk was seen for production workers.

In a case–control study nested within this cohort of styrene–butadiene rubber workers, a large excess of leukaemia was found which was associated with exposure to 1,3-butadiene and not to styrene.

In a case–control study in the rubber industry, a large excess of lymphatic and haematopoietic cancers, including lymphatic leukaemia, was seen among workers employed in styrene-butadiene rubber production.

One study, therefore, specifically related increased risks for leukaemia to exposure to 1,3-butadiene and not to styrene. In other studies, the increased risks for leukaemia and other lymphatic cancers occurred among workers whose exposure had been in the manufacture of 1,3-butadiene or styrene–butadiene rubber.

5.3 Animal carcinogenicity data

1,3-Butadiene was tested for carcinogenicity by inhalation exposure in four experiments in mice and one in rats. Tumours were induced at all exposure concentrations studied, ranging from 6.25 to 8000 ppm (13.8–17 600 mg/m^3). 1,3-Butadiene produced tumours at multiple organ sites in animals of each sex of both species, including tumours of the haematopoietic system and an uncommon neoplasm of the heart in male and female mice. Neoplasms at multiple organ sites were induced in mice after only 13 weeks of exposure. 1,3-Butadiene induced dose-related increases in the incidence of tumours at many sites.

Two metabolites, 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane, were carcinogenic to mice and rats when administered by skin application or subcutaneous injection.

Activated K-*ras* oncogenes have been detected in lymphomas and in liver and lung tumours induced in mice by 1,3-butadiene.

5.4 Other relevant data

In rats, mice and monkeys, 1,3-butadiene is metabolized to an epoxide, 1,2-epoxy-3-butene, for which quantitative differences in metabolic rates (mice > rat > monkey) have been observed. Because 1,2-epoxy-3-butene is exhaled by rats and mice exposed to 1,3-butadiene, the epoxide must undergo systemic circulation. Two experiments with human liver tissue demonstrated conversion of 1,3-butadiene to 1,2-epoxy-3-butene, suggesting that humans are not qualitatively different from animals in terms of epoxide formation.

Developmental toxicity, in the form of reduced fetal weight and skeletal variations, has been observed in mice, but not rats, exposed by inhalation to 1,3-butadiene.

Genotoxic effects were generally observed in mice but not in rats *in vivo*. This apparent species difference was highlighted in a comparison of liver DNA adducts from the two species. 1,3-Butadiene induced dominant lethal effects, sperm-head abnormalities, chromosomal aberrations, micronucleus formation and sister chromatid exchange *in vivo* in mice; it did not induce micronuclei or sister chromatid exchange in rats. Unscheduled DNA synthesis was not induced in either rats or mice after exposure of 1,3-butadiene. The compound did not induce mutation in the mouse lymphoma forward mutation assay and was not genotoxic to *Drosophila melanogaster*. It induced mutation in bacteria in the presence of an exogenous metabolic system.

1,2-Epoxy-3-butene, one of the main metabolites of 1,3-butadiene, induced sister chromatid exchange and chromosomal aberrations in mice *in vivo* and sister chromatid exchange in cultured human lymphocytes and rodent cells. It did not induce unscheduled DNA synthesis in isolated rat or mouse hepatocytes. 1,2-Epoxy-3-butene induced point mutation in bacteria in the absence of exogenous metabolic systems. It also reacted with purified DNA.

1,2:3,4-Diepoxybutane, another metabolite of 1,3-butadiene, induced chromosomal aberrations and sister chromatid exchange in mice and Chinese hamsters exposed *in vivo*. It induced chromosomal aberrations and sister chromatid exchange in cultured human cells and both sister chromatid exchange and mutation in cultured mammalian cells. 1,2:3,4-Diepoxybutane induced chromosomal deletions and gene mutation in *Drosophila*. It was mutagenic to bacteria in a mouse host-mediated assay as well as *in vitro*. It induced bacterial prophage and DNA repair. In one study, it induced DNA–DNA cross-links in mouse liver DNA *in vitro*; it induced DNA interstrand cross-links *in vitro*.

5.5 Evaluation[1]

There is *limited evidence* for the carcinogenicity in humans of 1,3-butadiene.

There is *sufficient evidence* for the carcinogenicity in experimental animals of 1,3-butadiene.

Studies *in vitro* suggest that the metabolism of 1,3-butadiene is qualitatively similar in humans and experimental animals. 1,3-Butadiene is metabolized in mammals to epoxy metabolites which interact with DNA. Base-substitution mutations are induced in bacteria. Similar mutations in the K-*ras* oncogene have been reported in tumours induced in mice by 1,3-butadiene.

Overall evaluation

1,3-Butadiene is *probably carcinogenic to humans (Group 2A)*.

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[1]For definition of the italicized terms, see Preamble, pp. 26–29.

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APPENDIX B

Excerpts from the NTP Technical Report Toxicology and Carcinogenesis Studies of 1,3-Butadiene in B6C3F$_1$ Mice (Inhalation Studies) pp. 5-95, 1993

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 1,3-BUTADIENE

(CAS NO. 106-99-0)

IN B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

May 1993

NTP TR 434

NIH Publication No. 93-3165

ABSTRACT

$$CH_2=CH-CH=CH_2$$

1,3-BUTADIENE

CAS No. 106-99-0

Chemical Formula: C_4H_6 Molecular Weight: 54.09

Synonyms: α,γ-Butadiene; bivinyl; divinyl; erythrene; vinylethylene; biethylene; pyrrolylene

1,3-Butadiene is produced in large volumes for use in the manufacture of synthetic rubber and of thermoplastic resins. In previous inhalation studies conducted by the NTP (NTP, 1984) there was clear evidence of multiple organ carcinogenicity in male and female mice exposed to 625 or 1,250 ppm 1,3-butadiene for 60 or 61 weeks. To better characterize exposure-response relationships for neoplasms and nonneoplastic lesions, toxicology and carcinogenesis studies were conducted by exposing groups of male and female $B6C3F_1$ mice to air containing 1,3-butadiene (greater than 99% pure) for up to 2 years. An additional study in male $B6C3F_1$ mice, in which exposure to 1,3-butadiene was stopped after limited exposure periods (13, 26, 40, or 52 weeks), was performed to assess the effects of varying concentration and duration of exposure on the incidences of 1,3-butadiene-induced neoplasms. *In vitro* genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse lymphoma cells. *In vivo* genetic effects were assayed in germ cells of male *Drosophila melanogaster* and in bone marrow and peripheral blood cells of $B6C3F_1$ mice.

2-Year Studies: Groups of 70 male and 70 female mice were exposed to air containing 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene for 6 hours per day, 5 days per week for up to 2 years; groups of 90 male and 90 female mice were exposed to 625 ppm 1,3-butadiene on the same schedule. Up to 10 animals from each group were examined after 9 and 15 months of exposure.

Survival and Body Weight in the 2-Year Studies: Two-year survival was decreased for males and females exposed to concentrations of 20 ppm or above, primarily due to the development of chemical-related malignant neoplasms. No female mice exposed to 200 or 625 ppm or males exposed to 625 ppm survived to the end of the studies (males: 35/50, 39/50, 24/50, 22/50, 4/50, 0/70; females: 37/50, 33/50, 24/50, 11/50, 0/50, 0/70). Mean body weights of exposed male and female mice were similar to those of the controls.

Hematologic Effects in the 2-Year Studies: Hematologic parameters were evaluated after 9 and 15 months of exposure. At 9 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume were observed in male mice exposed to 62.5 ppm or above and in female mice exposed to 200 or 625 ppm. Mean erythrocyte volume was increased in male mice exposed to 625 ppm and in females exposed to 200 or 625 ppm. At 15 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume and increases in mean erythrocyte volume were observed in male and female mice exposed to 625 ppm.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Exposure of mice to 1,3-butadiene induced benign and malignant neoplasms at multiple sites. Statistically significant increases in the incidences of neoplasms at one or more sites were seen at concentrations of 20 ppm and higher in males and 6.25 ppm and higher in females. There was no exposure level in this study at which a significant carcinogenic response was not observed. Statistically significant increases occurred in the incidences of malignant lymphoma; histiocytic sarcoma; cardiac hemangiosarcoma; harderian gland adenoma; hepatocellular adenoma and carcinoma; alveolar/bronchiolar adenoma and carcinoma; mammary gland carcinoma, adenoacanthoma, and malignant mixed tumor (females only); benign and malignant ovarian granulosa cell tumor; and forestomach squamous cell papilloma and carcinoma.

Low incidences of uncommon neoplasms also occurred in exposed male and female mice, including intestinal carcinomas in males, renal tubule adenomas in males and females, skin sarcomas (all types combined) in females, and Zymbal's gland adenomas and carcinomas in females.

Lymphocytic lymphomas appeared as early as week 23 and were the principal cause of death of male and female mice exposed to 625 ppm 1,3-butadiene. The early and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm resulted in a reduced number of mice at risk for neoplasms developing later at other sites. Exposure-response relationships for 1,3-butadiene-induced neoplasms were more clearly characterized at concentrations below 625 ppm and after adjustment for intercurrent mortality.

Increased incidences of nonneoplastic lesions in exposed mice included bone marrow atrophy; testicular atrophy; ovarian atrophy, angiectasis, germinal epithelial hyperplasia, and granulosa cell hyperplasia; uterine atrophy; cardiac endothelial hyperplasia and mineralization; alveolar epithelial hyperplasia; forestomach epithelial hyperplasia; and harderian gland hyperplasia.

Stop-Exposure Study: The stop-exposure study consisted of groups of 50 male mice exposed to 1,3-butadiene at concentrations of 200 ppm for 40 weeks, 625 ppm for 13 weeks, 312 ppm for 52 weeks, or 625 ppm for 26 weeks. After the exposures were completed, these groups were placed in control chambers for the remainder of the 2-year study. The total exposure of 1,3-butadiene (concentration times duration of exposure) of the 13- and 40-week stop-exposure groups was approximately 8,000 ppm · weeks, while that of the 26- and 52-week stop-exposure groups was approximately 16,000 ppm · weeks.

The survival of all stop-exposure groups was markedly lower than that of the controls. The incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma and carcinoma, forestomach squamous cell papilloma and carcinoma, hepatocellular adenoma, harderian gland adenoma and adenocarcinoma, and preputial gland carcinoma were significantly increased. Neoplasms were induced at most of these sites after only 13 weeks of exposure to 1,3-butadiene. Additionally, low numbers of malignant gliomas and neuroblastomas of the brain and Zymbal's gland carcinomas occurred in one or more stop-exposure groups.

At similar total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for an extended period (34% at 625 ppm for 13 weeks versus 12% at 200 ppm for 40 weeks; 60% at 625 ppm for 26 weeks versus 8% at 312 ppm for 52 weeks).

Genetic Toxicology: 1,3-Butadiene has been tested both *in vitro* and *in vivo* for mutagenic activity. *In vitro*, positive results were obtained in the *Salmonella typhimurium* gene mutation assay with strain TA1535; mutagenic activity was not observed in other *S. typhimurium* strains (TA100, TA97, and TA98). 1,3-Butadiene was negative in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells with and without S9.

In vivo, 1,3-butadiene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*; however, it did induce significant increases in chromosomal aberrations and sister chromatid exchanges in bone marrow cells of mice exposed for 2 weeks by inhalation. In addition, significant increases in micronucleated erythrocytes were observed in peripheral blood samples obtained

from male and female mice exposed to 1,3-butadiene for 2 or 13 weeks or 15 months by inhalation.

Conclusions: The previous inhalation studies of 1,3-butadiene in male and female $B6C3F_1$ mice provided *clear evidence of carcinogenicity** at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies — 2-year exposures of 6.25, 20, 62.5, 200, or 625 ppm or shorter duration exposures of 200, 312, or 625 ppm — provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male $B6C3F_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, preputial gland, brain, and kidney. There was *clear evidence of carcinogenicity* of 1,3-butadiene in female $B6C3F_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, ovary, and mammary gland.

Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also have been related to administration of 1,3-butadiene.

* Explanation of Level of Evidence of Carcinogenic Activity is on page 11. A summary of peer review comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene

	Male B6C3F$_1$ Mice (2-Year Study)	Male B6C3F$_1$ Mice (Stop-Exposure Study)	Female B6C3F$_1$ Mice
Doses	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks	200 ppm for 40 weeks, 312 ppm for 52 weeks, 625 ppm for 13 weeks, or 625 ppm for 26 weeks by inhalation for 6 hours daily, 5 days per week	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks
Body weights	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	35/50, 39/50, 24/50, 22/50, 4/50, 0/70	9/50, 1/50, 5/50, 0/50	37/50, 33/50, 24/50, 11/50, 0/50, 0/70
Nonneoplastic effects	Bone marrow: atrophy (0/50, 0/50, 0/50, 0/48, 0/49, 23/73) Heart: endothelial hyperplasia (0/50, 1/49, 0/50, 2/48, 4/48, 5/73); mineralization (0/50, 0/49, 0/50, 1/48, 3/48, 20/73) Alveolar epithelium: hyperplasia (2/50, 9/50, 6/50, 13/49, 17/50, 12/73) Forestomach epithelium: hyperplasia (4/50, 3/50, 3/50, 6/48, 4/48, 40/72) Harderian gland: hyperplasia (1/50, 3/49, 4/50, 6/47, 8/47, 5/40) Testicle: atrophy (1/50, 3/50, 4/50, 2/48, 6/49, 53/72)	Heart: endothelial hyperplasia (6/50, 3/50, 7/50, 7/50); mineralization (0/50, 6/50, 9/50, 14/50) Alveolar epithelium: hyperplasia (18/50, 14/50, 10/50, 11/50) Forestomach epithelium: hyperplasia (10/48, 20/48, 8/50, 15/50) Harderian gland: hyperplasia (4/48, 6/48, 3/42, 7/36) Testicle: atrophy (5/50, 3/50, 3/50, 5/50)	Bone marrow: atrophy (0/50, 0/49, 0/48, 0/49, 0/50, 11/79) Heart: endothelial hyperplasia (0/50, 2/50, 1/50, 4/49, 5/50, 8/80); mineralization (0/50, 2/50, 0/50, 2/49, 2/50, 11/80) Alveolar epithelium: hyperplasia (5/50, 5/50, 3/50, 9/50, 11/50, 11/78) Forestomach epithelium: hyperplasia (4/50, 5/49, 4/47, 7/48, 14/50, 47/79) Liver: hepatocellular foci (8/49, 14/49, 19/50, 12/50, 5/50, 4/80) Harderian gland: hyperplasia (1/50, 5/49, 9/48, 4/49, 4/49, 7/66) Ovary: angiectasis (4/49, 6/49, 3/48, 13/50, 14/50, 17/79); granulosa cell hyperplasia (1/49, 0/49, 2/48, 3/50, 4/50, 2/79); germinal epithelial hyperplasia (2/49, 3/49, 8/48, 15/50, 14/50, 18/79); atrophy (4/49, 19/49, 32/48, 42/50, 43/50, 69/79) Uterus: atrophy (1/50, 0/49, 1/50, 1/49, 8/50, 41/78)

(continued)

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)

	Male $B6C3F_1$ Mice (2-Year Study)	Male $B6C3F_1$ Mice (Stop-Exposure Study)	Female $B6C3F_1$ Mice
Neoplastic effects	Lymphoma (all lymphomas) (4/50, 2/50, 4/50, 6/50, 2/50, 51/73) Lymphocytic lymphoma (2/50, 0/50, 2/50, 4/50, 2/50, 49/73) Histiocytic sarcoma (0/50, 0/50, 4/50, 5/50, 7/50, 4/73) Heart: hemangiosarcoma (0/50, 0/49, 1/50, 5/48, 20/48, 4/73) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (21/50, 23/50, 19/50, 31/49, 35/50, 3/73) Forestomach: squamous cell papilloma or squamous cell carcinoma (1/50, 0/50, 0/50, 1/50, 8/50, 4/73) Liver: hepatocellular adenoma or carcinoma (21/50, 23/50, 30/50 25/48, 33/48, 5/72) Harderian gland: adenoma or carcinoma (6/50, 7/50, 9/50, 20/50, 31/50, 6/73) Preputial gland: carcinoma (0/50, 0/50, 0/50, 0/50, 5/50, 0/73) Kidney: renal tubule adenoma (0/50, 1/50, 0/50, 3/48, 1/49, 0/73)	Lymphoma (all lymphomas) (8/50, 8/50, 22/50, 33/50) Lymphocytic lymphoma (6/50, 4/50, 17/50, 30/50) Histiocytic sarcoma (5/50, 7/50, 2/50, 2/50) Heart: hemangiosarcoma (15/50, 33/50, 7/50, 13/50) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (36/50, 32/50, 28/50, 17/50) Forestomach: squamous cell papilloma or squamous cell carcinoma (3/50, 9/50, 7/50, 10/50) Liver: hepatocellular adenoma (27/49, 19/50, 19/49, 11/50) Harderian gland: adenoma or carcinoma (27/50, 30/50, 23/50, 13/50) Preputial gland: carcinoma (1/50, 4/50, 4/50, 3/50) Kidney: renal tubule adenoma (4/48, 3/49, 1/50, 1/50) Brain: malignant glioma (0/50, 0/50, 2/50, 1/50); neuroblastoma (0/50, 0/50, 2/50, 0/50)	Lymphoma (all lymphomas) (6/50, 12/50, 11/50, 7/50, 9/50, 32/80) Lymphocytic lymphoma (1/50, 3/50, 6/50, 3/50, 8/50, 31/80) Histiocytic sarcoma (3/50, 2/50, 7/50, 4/50, 7/50, 4/80) Heart: hemangiosarcoma (0/50, 0/50, 0/50, 1/49, 21/50, 23/80) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (4/50, 15/50, 19/50, 24/50, 25/50, 22/78) Forestomach: squamous cell papilloma or squamous cell carcinoma (0/50, 0/50, 3/50, 2/50, 4/50, 22/80) Liver: hepatocellular adenoma or carcinoma (15/49, 14/49, 15/50, 19/50, 16/50, 2/80) Harderian gland: adenoma or carcinoma (8/50, 10/50, 7/50, 15/50, 20/50, 9/80) Ovary: benign or malignant granulosa cell tumor (1/49, 0/49, 1/48, 9/50, 8/50, 6/79); adenoma or benign mixed tumor (2/49, 4/49, 1/48, 4/50, 6/50, 2/79) Mammary gland: adeno-acanthoma, carcinoma, or malignant mixed tumor (0/50, 2/50, 4/50, 12/50, 15/50, 16/80)

(continued)

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)

	Male $B6C3F_1$ Mice		Female $B6C3F_1$ Mice
	(2-Year Study)	(Stop-Exposure Study)	
Uncertain findings	Small intestine: carcinoma (0/50, 1/50, 1/50, 1/50, 2/50, 0/73)	Zymbal's gland: carcinoma (1/50, 0/50, 2/50, 2/50)	Kidney: renal tubule adenoma (0/49, 0/49, 0/48, 0/50, 2/50, 0/80) Skin, subcutaneous tissue: neurofibrosarcoma or sarcoma (1/50, 2/50, 3/50, 5/50, 3/50, 3/80) Zymbal's gland: adenoma or carcinoma (0/50, 0/50, 0/50, 0/50, 0/50, 2/80)
Level of evidence of carcinogenic activity	Clear evidence		Clear evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Positive in strain TA1535 Negative in strains TA100, TA97, and TA98		
Mouse lymphoma gene mutation:	Negative with and without S9		
Sex-linked recessive lethal mutations			
Drosophila melanogaster:	Negative by inhalation		
Chromosomal aberrations			
Mouse bone marrow *in vivo*:	Positive		
Sister chromatid exchanges			
Mouse bone marrow *in vivo*:	Positive		
Micronuclei			
Mouse peripheral blood erythrocytes *in vivo*:	Positive		

INTRODUCTION

$$CH_2=CH-CH=CH_2$$

1,3-BUTADIENE

CAS No. 106-99-0

Chemical Formula: C_4H_6 Molecular Weight: 54.09

Synonyms: α,γ-Butadiene; bivinyl; divinyl; erythrene; vinylethylene; biethylene; pyrrolylene

PHYSICAL AND CHEMICAL PROPERTIES, PRODUCTION, USE, AND EXPOSURE

1,3-Butadiene is a colorless, noncorrosive gas with a boiling point of -4.4° C and a vapor pressure of 1,900 mm Hg at 20° C (Kirshenbaum, 1978). The conversion factor for 1,3-butadiene at 25° C and 760 mm Hg is 1 ppm = 2.21 mg/m^3. 1,3-Butadiene is a reactive material that can form the dimer 4-vinylcyclohexene and is flammable at atmospheric concentrations of 2% or higher. 1,3-Butadiene can form explosive peroxides in air, and therefore is shipped as a liquified gas under pressure with a peroxide inhibitor.

1,3-Butadiene is a coproduct in steam cracking of petroleum fractions for the manufacture of ethylene. The annual production volume of 1,3-butadiene is approximately 12 billion pounds worldwide and 3 billion pounds in the United States (Morrow, 1990; USITC, 1990). The major uses of 1,3-butadiene are in the manufacture of synthetic rubber (such as styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resins. Butadiene elastomers are used in the manufacture of rubber tires, footwear, sponges, hoses and piping, luggage, packaging, and a variety of other molded products.

According to a 1984 survey by the United States Environmental Protection Agency, atmospheric emissions of 1,3-butadiene from facilities that produce or process 1,3-butadiene were approximately 10 million pounds per year; 70% of these emissions were attributed to equipment leaks and 30% to process venting (Mullins, 1990). 1,3-Butadiene has also been identified in automobile exhaust, cigarette smoke, and gasoline formulations; small amounts are released by the burning of plastics or rubber (Miller, 1978). Low levels of 1,3-butadiene (0.5 to 10 ppb) have been detected in ambient air in urban locations in the United States; however, levels of 1,3-butadiene in community air in Port Neches, Texas, a town with a butadiene production facility and two styrene-butadiene production plants, were measured by the Texas Air Control Board to be as high as 2 to 3 ppm (Durchin, 1990). Approximately 52,000 workers are potentially exposed to 1,3-butadiene annually, as estimated from data compiled from the National Occupational Exposure Survey (NIOSH, 1990). In-depth industrial hygiene surveys were conducted by the National Institute for Occupational Safety and Health at four monomer and five polymer manufacturing plants (Fajen *et al.*, 1990). Occupational exposures to 1,3-butadiene in most process areas were less than 10 ppm; however, maximum 8-hour time-weighted average exposures were frequently between 10 and 150 ppm, and in one case the average exposure was as high as 374 ppm. These exposures occurred in operations involving decontaminating and maintaining process equipment, sampling and

analyzing quality control samples, and loading or unloading tank trucks or rail cars. The odor threshold or recognition concentration for 1,3-butadiene in air is approximately 1 to 2 ppm (Amoore and Hautula, 1983).

The 8-hour, time-weighted, average workroom permissible exposure limit for 1,3-butadiene established by the United States Occupational Safety and Health Administration (OSHA) is 1,000 ppm (U.S. Department of Labor, 1981). Results of carcinogenicity studies of 1,3-butadiene in rats and mice prompted the American Conference of Governmental Industrial Hygienists to lower their recommended threshold limit value for 1,3-butadiene in the work environment from 1,000 ppm to 10 ppm (ACGIH, 1986). OSHA has proposed to lower the occupational exposure standard to a permissible exposure limit of 2 ppm with a 15-minute short-term exposure limit of 10 ppm (OSHA, 1990). A final decision on the proposed change is pending. An international symposium on the "Toxicology, Carcinogenesis, and Human Health Aspects of 1,3-Butadiene" was held at the National Institute of Environmental Health Sciences in 1988. The proceedings of that symposium were published in Environmental Health Perspectives (Melnick *et al.*, 1990a).

TOXICITY IN ANIMALS

1,3-Butadiene has long been considered to have a low, noncumulative toxicity in animals and humans. For rats, the median lethal concentration (LC_{50}) for a 4-hour exposure was 285 mg/L, equivalent to 129,000 ppm or 12.9%; for mice, the LC_{50} for a 2-hour exposure was 270 mg/L, equivalent to 123,000 ppm or 12.3% (Shugaev, 1969). Carpenter *et al.* (1944) exposed groups of 24 rats, 12 guinea pigs, 4 rabbits, and 1 dog to atmospheres containing 600, 2,300, or 6,700 ppm 1,3-butadiene for 7.5 hours a day, 6 days a week, for 8 months. The highest exposure concentration caused slight growth retardation and, in some animals, a mild reversible degeneration in the liver. This degeneration was reported as light cloudy swelling. There were no reported treatment-related effects in hematologic parameters or blood or urine chemistries, nor were there pathologic changes in the eye, adrenal gland, heart, kidney, skeletal muscle, pancreas, spleen, testis, or ovary. Exposure of rabbits to 250,000 ppm (25%) 1,3-butadiene for 2 minutes induced light anesthesia, while exposure for 8 to 10 minutes induced deep anesthesia. Death due to respiratory paralysis occurred after a 25- to 35-minute exposure to this concentration of 1,3-butadiene (Carpenter *et al.*, 1944).

No treatment-related gross or microscopic changes or effects on growth, survival, hematologic or blood biochemical parameters, urinary measurements, or neuromuscular functions were observed in male or female Sprague-Dawley rats exposed to 1,000, 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6 hours a day, 5 days a week, for 13 weeks (Crouch *et al.*, 1979).

Nonneoplastic lesions associated with exposure of B6C3F$_1$ mice to 625 or 1,250 ppm 1,3-butadiene for up to 61 weeks included epithelial hyperplasia of the forestomach, endothelial hyperplasia of the heart, alveolar epithelial hyperplasia, hepatocellular necrosis, testicular atrophy, ovarian atrophy, and lesions in nasal tissues including chronic inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium (NTP, 1984; Melnick *et al.*, 1988). The proliferative lesions in the forestomach, heart, and lung may represent early preneoplastic changes in the development of neoplasms induced by 1,3-butadiene. The nasal lesions were seen only in male mice exposed to 1,250 ppm 1,3-butadiene.

Exposure of male B6C3F$_1$ mice or NIH Swiss mice to 1,250 ppm 1,3-butadiene for 6 weeks caused decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit, and an increase in mean erythrocyte volume (Irons *et al.*, 1986a,b). Anemia due to exposure to 1,3-butadiene was not accompanied by increases in reticulocyte counts or in the frequency of nucleated erythrocytes in peripheral blood. These changes were considered to represent a macrocytic-megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells. In related studies, Tice *et al.* (1987) reported that exposure of male B6C3F$_1$ mice to 1,3-butadiene for 10 days caused decreases in the number and rate of dividing cells in the bone marrow. These findings established the bone marrow as a site of toxicity for 1,3-butadiene in mice. Exposure of male B6C3F$_1$ mice to 1,250 ppm 1,3-butadiene for 6 hours a day, 5 days a week, for 6 or 12 weeks did not produce any persistent defects in humoral or cell-mediated immunity (Thurmond *et al.*, 1986).

METABOLISM AND DISPOSITION

Malvoisin *et al.* (1979) identified 1,2-epoxy-3-butene as the first metabolite in 1,3-butadiene metabolism; this intermediate is formed by an inducible rat liver microsomal cytochrome P-450 monooxygenase (Figure 1; Bolt *et al.*, 1983). 1,2-Epoxy-3-butene was also detected in the expired air of Sprague-Dawley rats (Bolt *et al.*, 1983; Filser and Bolt, 1984) and of B6C3F$_1$ mice (Kreiling *et al.*, 1987) exposed to 1,3-butadiene, indicating that this epoxide intermediate is systemically available in exposed animals. Further metabolic transformation of 1,2-epoxy-3-butene involves conjugation with glutathione by glutathione-*S*-transferase, oxidation to 1,2:3,4-diepoxybutane, or hydrolysis by epoxide hydrolase and further oxidation to 3,4-epoxy-1,2-butanediol (Malvoisin and Roberfroid, 1982).

In studies by Laib *et al.* (1990), the metabolic elimination of 1,3-butadiene or 1,2-epoxy-3-butene was evaluated by measuring the decline in concentration of these chemicals in the gas phase of desiccator jars containing Sprague-Dawley rats or B6C3F$_1$ mice. Saturation of 1,3-butadiene metabolism in each species was reported at atmospheric concentrations between 1,000 and 2,000 ppm. At concentrations below 1,000 ppm, where first-order kinetics apply, the metabolic clearance was 1.6 times higher in mice (7,300 mL/kg per hour) than in rats (4,500 mL/kg per hour) (Bolt *et al.*, 1984; Kreiling *et al.*, 1986). The slightly higher metabolic elimination rate in mice is probably due to the higher respiratory frequency by this strain and species. This conclusion is based on the fact that the metabolic elimination rate constants of 7.6 hour^{-1} for mice (Kreiling *et al.*, 1986) and 8.8 hour^{-1} for rats (Bolt *et al.*, 1984) are nearly equivalent, and the exhalation rate constants are also similar for these species (Kreiling *et al.*, 1986), whereas the rate constant for the uptake of 1,3-butadiene (Kreiling *et al.*, 1986) and the minute air volume per body weight (Bond *et al.*, 1986) are about 2 to 2.5 times higher in mice than in rats.

Bond *et al.* (1986) exposed Sprague-Dawley rats and B6C3F$_1$ mice to various airborne concentrations of 1-[^{14}C]-1,3-butadiene and determined the uptake, distribution, and elimination of ^{14}C after specific periods of exposure. Respiratory measurements were also made to determine the uptake of inhaled 1,3-butadiene. However, because 1,3-butadiene and its metabolites eliminated during the exposure were not collected, it was not possible to determine the actual percentage of 1,3-butadiene absorbed. Instead, only the percentage of inhaled [^{14}C]-butadiene equivalents that was retained at the end of the exposure was reported. After 6 hours of exposure, the percentage of ^{14}C retained ranged from 1.5% to 17% in rats and 4% to 20% in mice; for each species, the percentage retained decreased as the exposure concentration of 1,3-butadiene increased, yet the total amount of 1,3-butadiene inhaled and retained was increased.

In a follow-up study (Dahl *et al.*, 1991), the respiratory data of Bond *et al.* (1986) were combined with the metabolic elimination rate data of Laib *et al.* (1990) to obtain values for the percentage of inhaled 1,3-butadiene that was eliminated as metabolites. The calculated values were 15% for rats and 12% for mice exposed to either 10 or 300 ppm 1,3-butadiene. The latter data reflect the constancy of uptake as the exposure concentration of 1,3-butadiene is increased in the range of first-order kinetics and also reflect the striking similarity between rats and mice after adjustment for species differences in breathing patterns.

In rats and mice exposed to [^{14}C]-1,3-butadiene, measurements of tissue concentrations of ^{14}C did not reveal any apparent species differences (Bond *et al.*, 1987). ^{14}C was distributed to all tissues examined without any noticeably higher accumulations in the target organs for carcinogenicity of either species. These studies did not identify the metabolites in the tissues of exposed rats and mice.

Species differences in the metabolism of 1,3-butadiene have also been examined in *in vitro* studies. Rates of metabolism of 1,3-butadiene were slightly lower in microsomal fractions isolated from the liver or lung of Sprague-Dawley rats than from similar preparations obtained from B6C3F$_1$ mice (Bond *et al.*, 1988). Exposure of rats or mice to 1,3-butadiene for 6 hours a day for 5 days neither induced nor inhibited the microsomal metabolism of this chemical in either species. The rate of formation of 1,2-epoxy-3-butene from 1,3-butadiene was about seven times higher in lung postmitochondrial fractions obtained from mice than in similar fractions obtained from Sprague-Dawley rats (Schmidt and Loeser, 1985). No activity was detected in a human lung sample. In liver postmitochondrial fractions, the rate of formation of 1,2-epoxy-3-butene was only about 50% greater for mice than for rats or for a single human liver sample. Although this study

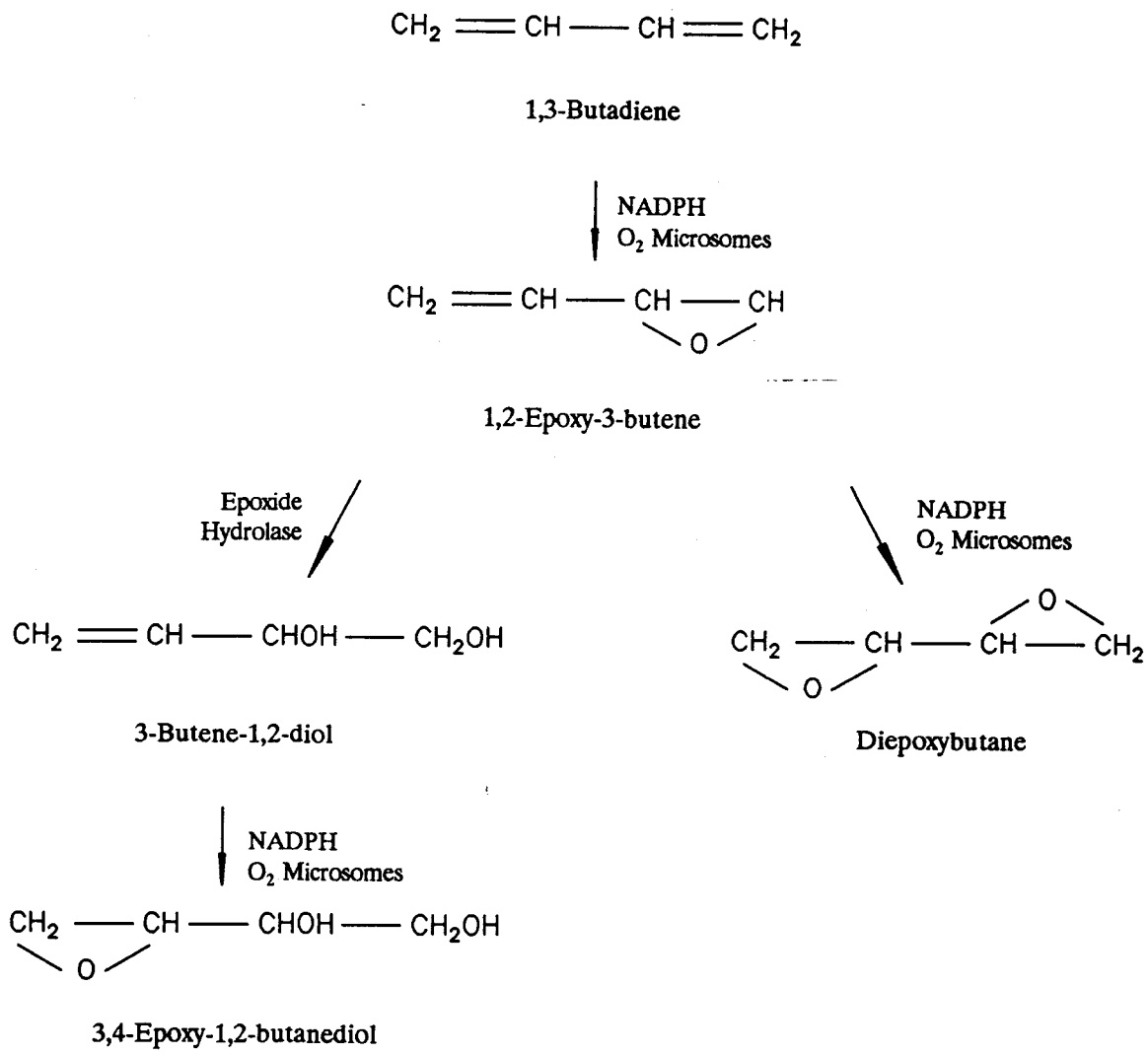


FIGURE 1
Metabolism of 1,3-Butadiene

does not provide data on the variability of this activity in the human population, it does indicate that pathways for 1,3-butadiene metabolism in the liver may be qualitatively similar across species.

CARCINOGENICITY IN ANIMALS

The carcinogenicity of 1,3-butadiene was studied by exposing groups of 100 Sprague-Dawley rats of each sex to 0, 1,000, or 8,000 ppm by inhalation for 6 hours a day, 5 days a week, for 2 years (IISRP, 1981a; Owen *et al.*, 1987). 1,3-Butadiene was carcinogenic at multiple organ sites in rats, as evidenced by increased incidences and dose-response trends for several organ-specific cancers: pancreatic exocrine neoplasms and Leydig cell tumors of the testis in males and uterine stromal sarcomas, Zymbal's gland carcinomas, mammary gland fibroadenomas and carcinomas, and thyroid follicular cell neoplasms in females. Further, the average number of mammary gland fibroadenomas per rat was increased in both exposure groups. The occurrence of nine glial cell neoplasms of the brain in exposed male rats (controls, 1/100; 1,000 ppm, 4/100; 8,000 ppm, 5/100) may also have been related to exposure to 1,3-butadiene because neuroglial neoplasms are uncommon in laboratory rats, occurring at a rate of about 0.2% to 1.0% in untreated male Sprague-Dawley rats (Krinke *et al.*, 1985; Gopinath, 1986).

In long-term inhalation studies of 1,3-butadiene in $B6C3F_1$ mice (NTP, 1984; Huff *et al.*, 1985), groups of 50 male and 50 female mice were exposed for 6 hours a day, 5 days a week, to air containing 0, 625, or 1,250 ppm 1,3-butadiene. These studies, designed to last for 103 weeks, were terminated after 60 to 61 weeks because survival was decreased at both exposure concentrations due to malignant neoplasms occurring in multiple organs of males and females.

Malignant lymphomas, hemangiosarcomas of the heart, and lung neoplasms occurred with positive trends in male and female mice, and the incidences of these neoplasms at both exposure concentrations were higher than those of controls (Table 1). The high incidences of hemangiosarcomas of the heart were particularly unusual findings, because these endothelial cell neoplasms are uncommon in $B6C3F_1$ mice, occurring in none of 573 untreated males and 558 untreated females in recent NTP studies, and they have rarely been induced in long-term studies.

TABLE 1
Incidences of Primary Neoplasms in Mice Exposed to 1,3-Butadiene for 61 Weeks[a]

Neoplasm	Males			Females		
	0 ppm	625 ppm	1,250 ppm	0 ppm	625 ppm	1,250 ppm
Malignant Lymphoma	0/50	23/50	29/50	1/50	10/49	10/49
Heart						
Hemangiosarcoma	0/50	16/49	7/49	0/50	11/48	18/49
Lung						
Alveolar/bronchiolar neoplasm	2/50	14/49	15/49	3/49	12/48	23/49
Forestomach						
Squamous cell neoplasm	0/49	7/40	1/44	0/49	5/42	10/49
Mammary Gland						
Acinar cell neoplasm	0/50	0/50	0/50	0/50	2/49	6/49
Ovary						
Granulosa cell tumor	–	–	–	0/49	6/45	12/48
Liver						
Hepatocellular neoplasm	8/50	6/49	2/49	0/50	2/47	5/49

[a] Incidences are expressed as number of neoplasm-bearing animals/number of animals examined microscopically.

Irons and coworkers confirmed by cytofluorometric analysis of cell surface markers that the type of lymphoma caused by 1,3-butadiene in $B6C3F_1$ mice is a T-cell lymphoma (Irons *et al.*, 1989; Irons, 1990). Early induction and increased incidences of forestomach neoplasms were also observed in females and low-dose males, and increased incidences of neoplasms of the mammary gland, ovary, and liver were observed in females. The rate of malignant lymphomas was lower in females than in males and the dose-responses for other neoplasms were better characterized in females. These studies demonstrated that 1,3-butadiene is a potent multiple-organ carcinogen in mice.

Irons and coworkers compared the induction of thymic lymphomas and the expression of murine leukemia retrovirus in $B6C3F_1$ mice and NIH Swiss mice exposed to 1,250 ppm 1,3-butadiene for 52 weeks (Irons *et al.*, 1987, 1989; Irons, 1990). The NIH Swiss mouse was used because it does not express the ecotropic murine leukemia viruses expressed in $B6C3F_1$ mice and it has a background rate of nearly zero for thymic lymphoma. The finding that exposure to 1,3-butadiene caused a 14% incidence of thymic lymphomas in NIH Swiss mice clearly shows that 1,3-butadiene induces this neoplasm independently of these activated retroviruses. Irons *et al.* (1989) suggested that ecotropic viruses were involved in the induction of lymphomas by 1,3-butadiene based on the higher incidence of thymic lymphoma in exposed $B6C3F_1$ mice than in exposed NIH Swiss mice.

Reproductive and Developmental Toxicity

Testicular and ovarian atrophy occurred in $B6C3F_1$ mice exposed to 625 or 1,250 ppm 1,3-butadiene for up to 61 weeks (NTP, 1984). Also, a concentration-related increase in sperm head abnormalities was observed in $B6C3F_1$ mice exposed to 200, 1,000, or 5,000 ppm 1,3-butadiene for 6 hours a day for 5 days (Morrissey *et al.*, 1990). Exposure of male Swiss CD-1 mice to 200, 1,000, or 5,000 ppm 1,3-butadiene for 6 hours a day for 5 days followed by cohabitation with untreated female mice for 1 week did not affect male fertility but did cause an increase in the percentage of female mice with two or more dead implantations. Because intrauterine deaths were not increased at 3 weeks or more after exposure, it was concluded that the more mature spermatozoa and spermatids were adversely altered by exposure to 1,3-butadiene (Morrissey *et al.*, 1990).

Exposure of pregnant female Sprague-Dawley rats to 200, 1,000, or 8,000 ppm of 1,3-butadiene for 6 hours a day, from day 6 through day 15 of gestation, caused a dose-related increase in the incidence of major skeletal defects of pups (IISRP, 1981b). "Wavy" ribs was the most common major skeletal defect in the 1,000 ppm and 8,000 ppm exposure groups. Other major skeletal defects, particularly in the high-dose group, included abnormalities of the skull, spine, sternum, ribs, and ilium. An exposure-related retardation of maternal body weight gain was not accompanied by any observable effects on pregnancy incidence, implantation loss, or gravid uterine weight. Mean fetal weights and crown-to-rump lengths were lower in the high-dose group than in the controls; embryonic growth retardation may have been a consequence of reduced maternal weight gain at the 8,000 ppm exposure level.

In another teratogenicity study, pregnant Sprague-Dawley rats and Swiss CD-1 mice were exposed to 40, 200, or 1,000 ppm of 1,3-butadiene for 6 hours a day on gestation days 6 through 15 (Morrissey *et al.*, 1990). There was no evidence of developmental toxicity in rats, although maternal body weight gain was decreased in the 1,000 ppm group. In mice, maternal body weight gain was decreased at the 200 ppm and 1,000 ppm exposure levels, whereas body weights of male fetuses were reduced at concentrations of 40 ppm and above. Thus, the male fetus is more susceptible than the dam to inhaled 1,3-butadiene. Malformations were not increased in rats or mice.

Genetic Toxicity

1,3-Butadiene, a potent *in vivo* clastogen, is mutagenic *in vitro* when tested in the presence of induced liver S9 activation systems. An overview of the mutagenicity of 1,3-butadiene and its oxidative intermediates is presented below.

1,3-Butadiene was mutagenic in *Salmonella typhimurium* TA1530 and TA1535, strains that are sensitive to base-pair substitutions, in the presence of induced liver S9 fractions (de Meester *et al.*, 1980; Arce *et al.*, 1990). Butadiene monoxide (1,2-epoxy-3-butene), *dl*-1,2:3,4-diepoxybutane, and 1,2:3,4-diepoxybutane, oxidative intermediates of

1,3-butadiene biotransformation, were also mutagenic to base-pair substitution strains of *S. typhimurium*, but without S9 (de Meester *et al.*, 1978; Simmon, 1979; Wade *et al.*, 1979; Dunkel *et al.*, 1984; Canter *et al.*, 1986; Zeiger and Pagano, 1989). 1,2:3,4-Diepoxybutane has been reported to induce sex-linked recessive lethal mutations and chromosomal translocations in *Drosophila melanogaster* (Watson, 1972; Shukla and Auerbach, 1980; Olsen and Green, 1982; Sankaranarayanan *et al.*, 1983).

In one study, 1,3-butadiene was negative for induction of sister chromatid exchanges in human lymphocyte cultures in the presence of rat, mouse, or noninduced human S9 (Arce *et al.*, 1990); however, in another study, 1,3-butadiene was positive for the induction of sister chromatid exchanges, with or without S9, in human lymphocytes (Sasiadek *et al.*, 1991). Increases in sister chromatid exchanges were reported in Chinese hamster ovary cells (Perry and Evans, 1975; Nishi *et al.*, 1984) and human lymphocytes and fibroblasts (Friedman *et al.*, 1982; Obe *et al.*, 1982; Porfirio *et al.*, 1983; Sasiadek *et al.*, 1991) treated with the metabolite 1,2:3,4-diepoxybutane. In addition, 1,2:3,4-diepoxybutane induced chromosomal aberrations in human lymphocytes and fibroblasts obtained from patients with chromosome breakage disorders (Auerbach and Wolman, 1979; Auerbach *et al.*, 1982; Marx *et al.*, 1983; Porfirio *et al.*, 1983).

1,3-Butadiene is a potent *in vivo* genotoxic agent to mouse bone marrow cells. Exposure of male B6C3F$_1$ mice to 6.25, 62.5, or 625 ppm 1,3-butadiene for 6 hours a day for 10 exposure days produced increases in chromosomal aberrations and sister chromatid exchanges in bone marrow cells and micronuclei in erythrocytes obtained from peripheral blood samples (Tice *et al.*, 1987). The lowest effective doses for each of these endpoints were 625 ppm for sister chromatid exchanges, 6.25 ppm for chromosomal aberrations, and 62.5 ppm for micronuclei. Exposure to 6.25 to 625 ppm 1,3-butadiene for 5 days a week for 13 weeks resulted in significant increases in micronucleated normochromatic erythrocytes isolated from peripheral blood of male and female B6C3F$_1$ mice (Shelby, 1990). The metabolite butadiene monoxide was also shown to be a strong inducer of sister chromatid exchanges and chromosomal aberrations in bone marrow cells of male C57Bl/6 mice given a single intraperitoneal injection of this chemical (Sharief *et al.*, 1986).

Comparative genotoxicity studies were performed with male B6C3F$_1$ mice and male Sprague-Dawley rats (Cunningham *et al.*, 1986). In these studies, bone marrow cells of mice exposed to 100 to 10,000 ppm 1,3-butadiene for 6 hours a day for 2 days showed significant increases in micronuclei and sister chromatid exchanges; no increase in either endpoint was seen in similarly exposed rats. No induction of unscheduled DNA synthesis was noted in hepatocytes isolated from mice and rats exposed to 10,000 ppm 1,3-butadiene for 2 days (Arce *et al.*, 1990).

In conclusion, 1,3-butadiene is mutagenic *in vitro*, inducing gene mutations in *S. typhimurium*, and *in vivo*, inducing sister chromatid exchanges, chromosomal aberrations, and micronuclei in mice.

Human Effects

Early toxicology studies on 1,3-butadiene indicated that this chemical only caused irritation to mucous membranes, skin, and eyes or caused narcosis at high concentrations (Carpenter *et al.*, 1944). Human volunteers exposed to 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6 to 8 hours experienced minor irritation to the eyes and difficulty in visual focusing.

In tank farm workers at a styrene-butadiene synthetic rubber plant, erythrocyte counts, hemoglobin concentrations, and packed red cell volumes were slightly, but not significantly, lower, and mean erythrocyte volumes were slightly, but not significantly, higher than those of workers in other departments (Checkoway and Williams, 1982). These changes are similar to those observed in mice exposed to 1,3-butadiene.

Associations between occupational exposure to 1,3-butadiene and increased cancer risk have been evaluated in retrospective mortality studies of workers employed at facilities which produce 1,3-butadiene (Downs *et al.*, 1987; Divine, 1990) and at facilities which produce styrene-butadiene rubber (Meinhardt *et al.*, 1982; Matanoski and Schwartz, 1987; Matanoski *et al.*, 1990). Increased mortalities from lymphatic and hematopoietic cancers were detected among subgroups of occupationally exposed workers in each of these studies. Mortality rates for lymphosarcoma and reticulum cell sarcoma were increased by as much as 5.6-fold among workers in a 1,3-butadiene manufacturing plant (Downs *et al.*,

1987), while 5- to 6.6-fold increases in mortality from all lymphopoietic cancers and leukemia were reported among black workers in production areas of styrene-butadiene-rubber plants (Matanoski *et al.*, 1990). In a nested-case control study comparing lymphopoietic cancer cases to an internal population of workers who did not have cancer, Matanoski *et al.* (1989) found that the odds ratio was 9.4 for the association of the leukemia cases with 1,3-butadiene exposure.

STUDY RATIONALE

Because 1,3-butadiene is an important chemical with a large production volume and a potential for exposure, and because the first inhalation studies in mice had been terminated early, additional studies were performed to better characterize exposure-response relationships for neoplasms and nonneoplastic lesions induced by this chemical in mice. Five exposure levels ranging from 625 ppm, corresponding to the lowest concentration used in the previous inhalation studies, down to 6.25 ppm were included to extend the exposure range over two orders of magnitude. Additional studies in which exposure to 1,3-butadiene was stopped after limited periods of time were also included to assess the relationship between concentration and duration of exposure on the outcome of butadiene-induced carcinogenicity.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

1,3-Butadiene was periodically shipped as a liquified gas directly to the study laboratory, Battelle Pacific Northwest (Richland, WA), from Phillips Chemical Company, Philtex Plant (Borger, TX). Although seven lots (J-014, J-025, J-038, J-050, J-149, J-217, and J-375) were used in the 2-year and stop-exposure studies, only one lot was used at any one time. Battelle Pacific Northwest analyzed each lot of 1,3-butadiene and confirmed the identity by infrared spectroscopy and established the purity as ≥99% by gas chromatography. The maximum allowable dimer (4-vinyl-1-cyclohexene) content in the headspace of any lot used in the studies was set at 500 ppm. The dimer content increased with time and was monitored daily. Any cylinder yielding a dimer value greater than 500 ppm was not used.

According to NTP practice, a complete chemical characterization is performed on the chemical lot that will be used in the toxicology study. The bulk chemical is then shipped to the study laboratory. Because the dimer content of 1,3-butadiene increased with time, it was impractical to follow the normal procedure. Therefore, to allow for the instability of the chemical and to determine the appropriate analytical tests, a representative lot (F-850) from the same supplier was subjected to a full characterization. The analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and are described in Appendix G. The study chemical, a clear, colorless gas, was identified as 1,3-butadiene by infrared and nuclear magnetic resonance spectroscopy. Lot F-850 was determined by gas chromatography to be greater than 99% pure, with no impurities with areas of 0.1% or greater relative to the major peak area. Approximation of the concentration of the inhibitor, *t*-butylcatechol, in the liquid phase indicated approximately 4 ppm. The level of 4-vinyl-1-cyclohexene was determined by gas chromatography to be 35 ± 1 ppm for the liquid phase and less than 1 ppm for the headspace.

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

1,3-Butadiene was delivered from the headspace of the cylinders through stainless steel tubing to a distribution manifold and flow control system. Six metering valves and flow meters controlled the gas flow to each chamber (Figure G3). The gas entered the airstream near the top of the study chambers (Hazleton 2000, Lab Products, Inc.).

The concentration of 1,3-butadiene in the chambers and room air was monitored with an automated sampling system coupled to a gas chromatograph. The system automatically cycled through all ports once every 30 minutes. Calibration was performed by analyzing volumetrically prepared standards of 1,3-butadiene. At least 93% of all concentration measurements were within 10% of the target concentrations. Monthly mean exposure concentrations are presented in Figures G4 through G8. A summary of chamber concentrations is presented in Table G1.

CHAMBER ATMOSPHERE CHARACTERIZATION

Uniformity of concentration of 1,3-butadiene in each exposure chamber with animals present was checked at approximately 3-month intervals from 12 chamber positions by the same system used for daily concentration monitoring. The chamber concentration was considered to be uniform if the variability was less than 5% relative standard deviation. During the studies, the variability did not exceed 4.8%.

The time (T_{90}) following the start of generation for the concentration to build to 90% of the final stable concentration in the chamber and the times (T_{10} and T_1) following cessation of generation for the concentration to decay to 10% and 1% of the stable concentration were determined with animals present. The T_{90}, T_{10}, and T_1 values were 15 minutes, 12 minutes, and 35 minutes, respectively.

The major 1,3-butadiene degradation product expected in the studies was the dimer, 4-vinyl-1-cyclohexene, formed from the condensation of two molecules of 1,3-butadiene. Other potential degradation products were the oxidation products 3,4-epoxy-1-butene and 1,3-butadiene diepoxide. As discussed previously, the dimer content increased with time. Once the headspace concentration in a cylinder reached 500 ppm, the cylinder was no longer used. To monitor for the oxidation products, a fraction of the chamber atmosphere was bubbled through dimethyl formamide; the dimethyl formamide was then analyzed by gas chromatography. No evidence of oxidative degradation products at the 1% level was observed.

2-YEAR STUDIES

Study Design

Groups of 70 male and 70 female mice were administered 0 (chamber control), 6.25, 20, 62.5, or 200 ppm 1,3-butadiene by inhalation for 6 hours a day, 5 days a week, for up to 103 weeks; groups of 90 male and 90 female mice were administered 625 ppm 1,3-butadiene on the same schedule. After 9 months and again after 15 months of 1,3-butadiene administration, up to 10 male and 10 female mice were randomly selected from each group for interim evaluations.

2-YEAR STOP-EXPOSURE STUDY

Study Design

Groups of 50 male mice were administered 1,3-butadiene by inhalation at concentrations of 200 ppm for 40 weeks, 312 ppm for 52 weeks, or 625 ppm for 13 or 26 weeks. After the exposures were stopped, animals were placed in control chambers and were evaluated at 103 weeks. Mice were exposed for 6 hours a day, 5 days a week.

The stop-exposure study was designed to test the hypothesis that carcinogenic responses due to exposure to 1,3-butadiene were directly related to the product of the exposure concentration times the duration of exposure. The exposure condition of 625 ppm for 26 weeks was selected because in the previous study, lymphomas were induced by that time in male B6C3F$_1$ mice exposed to this concentration of 1,3-butadiene (NTP, 1984). By reducing the exposure concentration by 50% (312 ppm) and doubling the duration of exposure (52 weeks), the 1,3-butadiene concentration times exposure duration was kept constant. Similarly, the total exposure at 200 ppm for 40 weeks was nearly equivalent to the total exposure at 625 ppm for 13 weeks and equal to approximately half the total exposure given to the group exposed to 625 ppm for 26 weeks. Thus the total exposure to 1,3-butadiene was approximately 487,000 ppm-hr for the groups of mice exposed to 625 ppm for 26 weeks or to 312 ppm for 52 weeks, and approximately 242,000 ppm-hr for the groups of mice exposed to 625 ppm for 13 weeks or to 200 ppm for 40 weeks.

Source and Specification of Animals

Male and female B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year chronic and stop-exposure studies. Mice were quarantined 13 or 15 days. Five male and five female mice were randomly selected and killed for parasite evaluation and gross observation of disease. Blood samples were collected for viral screens. Male mice were approximately 6 to 8 weeks old and females were approximately 7 to 8 weeks old when the studies began. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Mice were housed individually during the studies. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Cages were rotated within the exposure chambers weekly during the studies. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix H.

Clinical Examinations and Pathology

All animals were observed twice daily and findings were recorded monthly or as necessary. Animals were weighed at the beginning of the studies, weekly for 13 weeks, and monthly thereafter.

Up to 10 mice from each group in the 2-year chronic studies were evaluated at 9 months and after 15 months of 1,3-butadiene administration. Blood was drawn from the supraorbital sinus for clinical pathology evaluations. Bone marrow was obtained from the right femur of animals evaluated at 15 months for determination of bone marrow

cellularity. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus of each animal selected for the 9- and 15-month interim evaluations were weighed at necropsy. Further details of the interim evaluations are presented in Table 2.

Necropsies were performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. At the 9- and 15-month interim evaluations, complete histopathology was performed on all control mice, all mice in the highest exposure group with survival of at least 60%, and all mice in groups with higher exposure concentrations. All animals that died or were killed moribund, all 2-year core study mice, and all stop-exposure mice also received a complete histopathologic examination. Tissues examined are listed in Table 2.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet-tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The heart, lung, forestomach, liver, ovary, uterus, harderian gland, Zymbal's gland, kidney, small intestine, and skin of male and female mice, the brain, preputial gland, and testis of male mice, and the mammary gland, ovary, and uterus of female mice were reviewed microscopically by the quality assessment pathologist for neoplasms or nonneoplastic lesions.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative examples of lesions from the forestomach, liver, heart, harderian gland, and lung of male and female mice, kidney and brain of male mice, ovary and mammary gland of female mice, and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidence of neoplasms or nonneoplastic lesions is given as the number of animals bearing such lesions at a specific anatomic site and the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin, intestine, mammary gland, or harderian gland neoplasms) prior to histologic sampling, or when lesions had multiple potential sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidence

The large numbers of exposed mice that died or were killed moribund early in these studies were considered to be due primarily to lymphoma or to hemangiosarcoma of the heart. Moreover, carcinomas of the forestomach, lung, preputial gland, mammary gland, and Zymbal's gland and sarcomas of the skin were considered to be lethal neoplasms.

Consequently, for these particular neoplasms, primary emphasis in the analysis of neoplasm incidence was given to the life table test (Cox, 1972; Tarone, 1975), a survival-adjusted procedure appropriate for rapidly lethal neoplasms.

For incidental neoplasms (neoplasms discovered as a result of death from an unrelated cause), the primary statistical method used in these studies was logistic regression, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidence (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Because the increased mortality in the 1,3-butadiene-exposed groups reduced the sensitivity of logistic regression analyses for detecting carcinogenic effects, supplemental analyses were performed by the survival-adjusted "Poly-3" quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989). This procedure, which is currently being evaluated by the NTP, modifies the Cochran-Armitage/Fisher exact test by adjusting the denominators of the neoplasm rates to take into account survival differences. This adjustment was derived by Portier and Bailer (1989), based on their fitting a Weibull model to historical control neoplasm data for a variety of site-specific neoplasms. The use of the power of k=3 in the poly-k adjustment for intercurrent mortality in the 1,3-butadiene study is justified by the following: (1) For liver and lung neoplasms in historical control animals, the observed survival adjusted value of k that best fits the data is approximately 3 in both male and female mice (Portier *et al.*, 1986). For leukemia/lymphoma combined, a slightly higher value was observed (6 in females and 5 in males). In male mice, the value of k for all neoplasms combined was approximately 3 and for females it was approximately 4.5. (2) Simulations have shown that it is better (in terms of preserving false positive error rate) to underestimate the value of k than to overestimate it. Thus, for example, it is better to use k=3 when in truth k=6 than it is to use k=6 when in truth k=3. Because none of the values for k estimated by Portier *et al.* (1986) were significantly less than 3, and few were significantly greater than 3, the choice of 3 seems reasonable. The corresponding survival-adjusted neoplasm rates are also reported.

Determining Dose-Response Shape for 1,3-Butadiene

For those neoplasms showing chemical-related effects, the shape of the dose-response curve was estimated by fitting the following modified Weibull model (Portier *et al.*, 1986) to the data:

$$P(dose) = 1 - e^{-(intercept + scale \cdot dose^{shape})}$$

where $P(dose)$ is the probability of a neoplasm prior to study termination for animals administered dose *dose* of 1,3-butadiene. The parameters *intercept, scale,* and *shape* are estimated via maximum likelihood estimation using the likelihood

$$L = \sum_{i=0}^{5} x_i \log[P(d_i)] + (n_i - x_i)\log[1-P(d_i)]$$

where x_i is the number of animals with neoplasm in dose group d_i, and n_i is the poly-3 adjusted number of animals at risk in dose group d_i, i=0,1,2,...5. A likelihood ratio test is used to test the hypothesis that the shape parameter equals 1. The test statistic

is given as -2 times the differences in the log likelihoods. A one-sided test was used so that the critical values are 2.706 for P=0.05 and 5.410 for P=0.01 (these are the squares of the critical regions from standard normal distribution). The shape parameter was restricted to be less than or equal to 10.

If the estimated shape parameter is greater than 1, the resulting dose-response has more curvature than a linear model and exhibits "threshold-like" behavior. If the estimated shape parameter is less than 1, then the dose-response curve is very steep in the low-dose region.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of neoplasm incidence. Consequently, control neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test).

Quality Assurance Methods

The 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so all had been resolved or were otherwise addressed during the preparation of this Technical Report.

Genetic Toxicity

The genetic toxicity of 1,3-butadiene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, trifluorothymidine resistance in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in mouse bone marrow cells, sex-linked recessive lethal mutations in *Drosophila melanogaster*, and micronuclei in peripheral blood erythrocytes of mice. The protocols for these studies and tabular presentations of their findings are in Appendix D.

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene

2-Year Studies	Stop-Exposure Study
Study Laboratory Battelle Pacific Northwest Laboratories, Richland, WA	Battelle Pacific Northwest Laboratories, Richland, WA
Strain and Species $B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source Frederick Cancer Research Facility, Frederick, MD	Frederick Cancer Research Facility, Frederick, MD
Size of Study Groups 70 males and 70 females. 90 males and 90 females in 625 ppm dose groups	50 males
Doses 0, 6.25, 20, 62.5, 200, or 625 ppm in air	200, 312, or 625 ppm in air
Time Held Before Study Males: 15 days Females: 13 days	15 days
Average Age When Placed on Study Males: 6 to 8 weeks Females: 7 to 8 weeks	6 to 8 weeks
Date of First Exposure 23 January 1986	23 January 1986
Duration of Exposure 6 hours daily, 5 days a week, for up to 103 weeks	6 hours daily, 5 days a week, for 13, 26, 40, or 52 weeks
Date of Last Exposure 9-month interims: 30 October 1986 15-month interims: 23 April 1987 2-year studies: 13 January 1988	13-week stop exposure: 23 April 1986 26-week stop exposure: 23 July 1986 40-week stop exposure: 29 October 1986 52-week stop exposure: 21 January 1987
Average Age When Killed 111 to 113 weeks	111 to 113 weeks
Method of Sacrifice 70% carbon dioxide	70% carbon dioxide
Method of Animal Distribution Animals randomly assigned to control and exposure groups with body weight as the blocking variable, using the XYBION PATH/TOX System.	Animals randomly assigned to control and exposure groups with body weight as the blocking variable, using the XYBION PATH/TOX System.
Animals per Cage 1	1

(continued)

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene (continued)

2-Year Studies	Stop-Exposure Study
Method of Animal Identification Toe clip	Toe clip
Diet NIH-07 open-formula diet, pellets (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*, except during exposure periods	NIH-07 open-formula diet, pellets (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*, except during exposure periods
Water Tap water (City of Richland water supply) via automatic watering system (Systems Engineering, Napa, CA), available *ad libitum*	Tap water (City of Richland water supply) via automatic watering system (Systems Engineering, Napa, CA), available *ad libitum*
Cages Stainless steel wire-bottom cages (Hazleton Systems, Inc., Aberdeen, MD), changed weekly	Stainless steel wire-bottom cages (Hazleton Systems, Inc., Aberdeen, MD), changed weekly
Chambers Stainless steel chambers (Lab Products, Inc., Harford Division, Aberdeen, MD)	Stainless steel chambers (Lab Products, Inc., Harford Division, Aberdeen, MD)
Animal Chamber Environment Average temperature: 23.5° ± 1.5° C Relative humidity: 55% ± 15% Fluorescent light: 12 hours/day Room air changes: 17-21/hour	Average temperature: 23.5° ± 1.5° C Relative humidity: 55% ± 15% Fluorescent light: 12 hours/day Room air changes: 17-21/hour
Type and Frequency of Observation Observed twice daily; weighed initially, weekly for 13 weeks, monthly thereafter; clinical observations recorded monthly	Observed twice daily; weighed initially, weekly for 13 weeks, monthly thereafter; clinical observations recorded monthly
Necropsy Necropsy performed on all animals. The following organs were weighed at the 9- and 15-month interim evaluations: brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus.	Necropsy performed on all animals.
Clinical Pathology Clinical pathology studies were performed at the 9- and 15-month interim evaluations. ***Hematology:*** Packed red cell volume, hemoglobin, erythrocytes, Howell-Jolly bodies, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocytes, and leukocyte count and differential, total bone marrow cellularity (15 months only) ***Clinical chemistry:*** Creatine kinase and lactate dehydrogenase	None

(continued)

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene (continued)

2-Year Studies	Stop-Exposure Study
Histopathology Complete histopathology was performed on all controls, 200, and 625 ppm mice at the 9-month interim evaluation; all controls, all exposed males, and 62.5, 200, and 625 ppm females at the 15-month interim evaluation; all animals dying early or killed moribund; and all 2-year core study mice. The following tissues were routinely examined microscopically: gross lesions and tissue masses with regional lymph nodes, adrenal gland, brain, epididymis, esophagus, gallbladder, harderian gland, heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph node (bronchial, mediastinal, mandibular, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, sternebrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, and uterus.	Complete histopathology was performed on all stop-exposure mice. The following tissues were routinely examined microscopically: gross lesions and tissue masses with regional lymph nodes, adrenal gland, brain, epididymis, esophagus, gallbladder, harderian gland, heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph node (bronchial, mediastinal, mandibular, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, sternebrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, and trachea.

DISCUSSION AND CONCLUSIONS

1,3-Butadiene is produced in large volumes for use mainly in the manufacture of synthetic rubber and thermoplastic resins. Previous long-term inhalation studies have shown that 1,3-butadiene is carcinogenic at multiple organ sites in Sprague-Dawley rats (IISRP, 1981a; Owen *et al.*, 1987) and B6C3F$_1$ mice (NTP, 1984; Huff *et al.*, 1985). The 2-year studies in rats, sponsored by the International Institute of Synthetic Rubber Producers (IISRP), were conducted at exposure concentrations of 1,000 and 8,000 ppm. The highest exposure level was limited by the safety requirement of being below 50% of the explosive limit of 1,3-butadiene in air, while the 1,000 ppm concentration was selected because it represented the occupational exposure standard for this chemical. The NTP usually conducts long-term studies in F344/N rats and B6C3F$_1$ mice; however, because the IISRP studies in rats were in progress at the time of chemical selection, the NTP studies were limited to long-term evaluations of 1,3-butadiene exposure in mice.

The exposure concentrations selected for the NTP studies, 625 and 1,250 ppm, were based on increased mortality and decreased body weight gains in mice exposed to concentrations of 2,500 ppm or higher for 14 weeks (NTP, 1984). The carcinogenicity studies in mice, designed to last for 103 weeks, were terminated after 61 weeks because of reduced survival due to malignant neoplasms involving multiple organs at both exposure concentrations. Malignant lymphomas, which appeared to originate in the thymus and were observed as early as week 20, were considered to be the major cause of early death, while hemangiosarcomas of the heart, an uncommon neoplasm in untreated B6C3F$_1$ mice (none occurred in 573 control males or 558 control females in recent NTP studies), were the second major cause of death. The incidences of primary neoplasms caused by exposure to 1,3-butadiene are shown in Table 1. Because these studies had been terminated early and dose-response relationships for various lesions were sometimes unclear (e.g., hemangiosarcomas of the heart in male mice), a second set of long-term inhalation studies of 1,3-butadiene in mice was performed to better characterize the carcinogenicity of this important industrial chemical. The latter studies, which are presented in this Technical Report, were conducted at concentrations ranging from 6.25 to 625 ppm 1,3-butadiene. The exposure level of 625 ppm corresponds to the low-exposure level in the previous inhalation studies in mice, and 6.25 ppm is two orders of magnitude lower. A preliminary account of the results of these studies has been reported (Melnick *et al.*, 1990 b,c).

Exposure to 1,3-butadiene for up to 2 years had no apparent adverse effect on body weight gains for male or female mice; however, survival was reduced in all groups exposed to concentrations of 20 ppm or higher. As in the previous studies, lymphomas that occurred early, prior to 15 months, were the major cause of death for male and female mice exposed to 625 ppm 1,3-butadiene. T-cell lymphoma is caused by exposure to 1,3-butadiene (Irons *et al.*, 1989; Irons, 1990). In the present studies, most butadiene-induced lymphomas were well differentiated and lymphocytic, and appeared to originate in the thymus. After month 15, there was a marginal but statistically significant increase in histiocytic sarcomas. Additionally, other histological types of lymphoma (malignant mixed and malignant undifferentiated) commonly associated with the spontaneous lymphoma of aging B6C3F$_1$ mice were observed in all remaining groups. Lymphocytic lymphomas were analyzed separately from histiocytic sarcomas and all lymphomas, because they provide a clearer response of 1,3-butadiene-induced hematopoietic cancers.

The incidence of hemangiosarcomas of the heart was increased in male mice exposed to 62.5, 200, or 625 ppm and in female mice exposed to 200 or 625 ppm. In addition, one male exposed to 20 ppm and one female exposed to 62.5 ppm were observed to have this uncommon endothelial cell neoplasm; the occurrence of these rare sarcomas at the lower concentrations was also likely due to 1,3-butadiene exposure. Increased incidences of endothelial hyperplasia in the heart at all exposure concentrations probably represent preneoplastic changes caused by 1,3-butadiene.

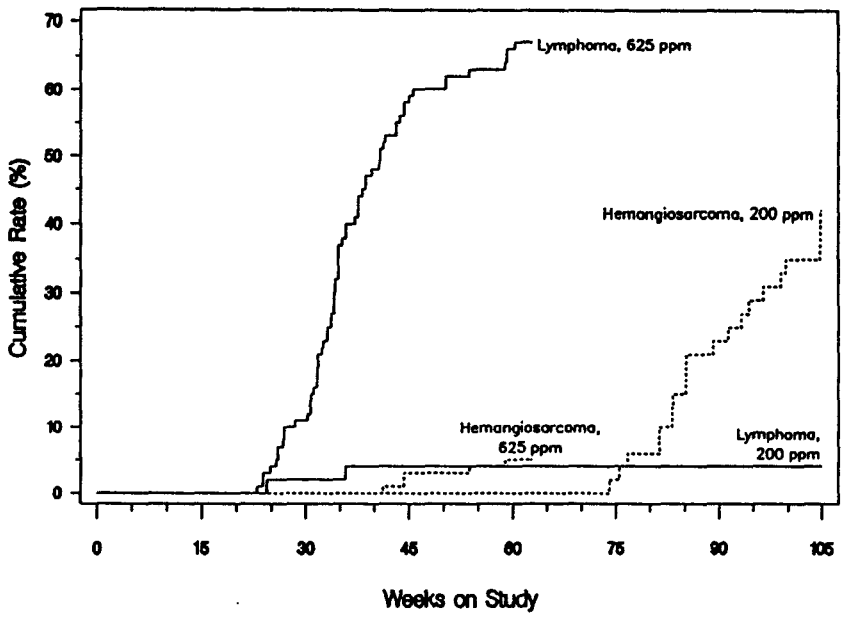
The incidence of hemangiosarcomas of the heart was greater in male mice exposed to 200 ppm than in

male mice exposed to 625 ppm. The lower incidence in males receiving 625 ppm was probably due to the early and extensive induction of lymphocytic lymphoma, which resulted in a significantly reduced number of mice at risk for the later-developing heart hemangiosarcomas. The median survival time was about 40 weeks for male mice exposed to 625 ppm and about 70 weeks for male mice exposed to 200 ppm. The effect of competing risks of early occurring lethal thymic lymphomas on the development of hemangiosarcomas of the heart is evident from the plots of the cumulative death-with-neoplasm rates of these neoplasms against the number of weeks on study for male mice exposed to 200 or 625 ppm 1,3-butadiene (Figure 8). In the 625 ppm group, the incidence of early lymphocytic lymphoma was very high (67%) and the incidence of hemangiosarcomas of the heart was low (5%); however, in the 200 ppm group, the incidence of early lymphocytic lymphoma was low (4%) and the incidence of hemangiosarcomas of the heart (42%) was much higher than that in the 625 ppm group. Furthermore, in male mice that died early after exposure to 200 or 625 ppm 1,3-butadiene, the incidences of hemangiosarcomas of the heart were nearly equivalent for the first 65 weeks of the study. After that time, a high incidence of hemangiosarcomas of the heart (approximately 50%) was observed in the 200 ppm group, whereas there were no surviving animals in the 625 ppm group. Thus, for male mice exposed to 1,3-butadiene concentrations below 625 ppm, the dose response for hemangiosarcomas of the heart is more clearly demonstrated. The impact of early mortality on the expression of later-developing neoplasms is largely accounted for in the mortality-adjusted neoplasm rates shown in Tables 30 and 31 for each neoplasm induced by exposure to 1,3-butadiene.

The incidence of alveolar/bronchiolar neoplasms in male mice was increased at concentrations of 62.5 and 200 ppm compared to that of the controls. In female mice, the incidence of alveolar/bronchiolar neoplasms was significantly increased in all exposure groups compared to that of the controls. Thus, even at a concentration of 6.25 ppm, 1,3-butadiene is carcinogenic to $B6C3F_1$ mice. Furthermore, in control female mice, all of the alveolar/bronchiolar neoplasms were adenomas, whereas in female mice exposed to 1,3-butadiene, including the 6.25 ppm exposure level, alveolar/bronchiolar carcinomas were observed. Because there was no exposure level at which a carcinogenic response was not induced, it is likely that exposure concentrations below 6.25 ppm would also cause cancers in mice. The reduced incidence of lung neoplasms in mice exposed to 625 ppm compared with the incidence in mice exposed to 200 ppm is attributed to the high rate of early deaths due to competing risks of lymphocytic lymphoma in female mice exposed to 625 ppm (Figure 8). The time-to-neoplasm detection of alveolar/bronchiolar neoplasms was slightly shorter for animals exposed to 625 ppm than for animals exposed to 200 ppm; however, because all female mice exposed to 625 ppm 1,3-butadiene died by 65 weeks, the final incidence of this later developing and rarely lethal neoplasm was lower than that for female mice exposed to 200 ppm. Increased incidences of alveolar epithelial hyperplasia in exposed male and female mice probably represent preneoplastic changes caused by 1,3-butadiene in the lung.

Increased incidences of neoplasms of the forestomach (squamous cell papillomas or carcinomas), mammary gland (carcinomas, adenoacanthomas, and malignant mixed tumors), ovary (benign or malignant granulosa cell tumors), liver (hepatocellular adenomas or carcinomas), and other organ sites identified in the first studies were again observed in mice exposed to 1,3-butadiene. Additionally, the harderian gland and preputial gland were identified as sites of 1,3-butadiene-induced neoplasia. Increased incidences of proliferative nonneoplastic lesions in these organs, including epithelial hyperplasia of the forestomach, mammary gland hyperplasia, germinal epithelium and granulosa cell hyperplasia of the ovary, and hyperplasia of the harderian gland, probably represent preneoplastic changes at these sites. In control female mice, no neoplasms, or only benign neoplasms, were observed in the harderian gland, forestomach, and ovary; however, in female mice exposed to 1,3-butadiene, malignant neoplasms were observed at each of these sites. The greater tendency to malignant neoplasia in mice exposed to 1,3-butadiene further demonstrates the strong carcinogenic potency of this chemical.

The conclusion that the marginally increased incidences of hepatocellular neoplasms in male and female mice were related to chemical administration is strengthened by the detection of activated K-*ras* oncogenes with a specific codon 13 mutation in liver neoplasms obtained from mice exposed to 1,3-butadiene (Goodrow *et al.*, 1990). Activated



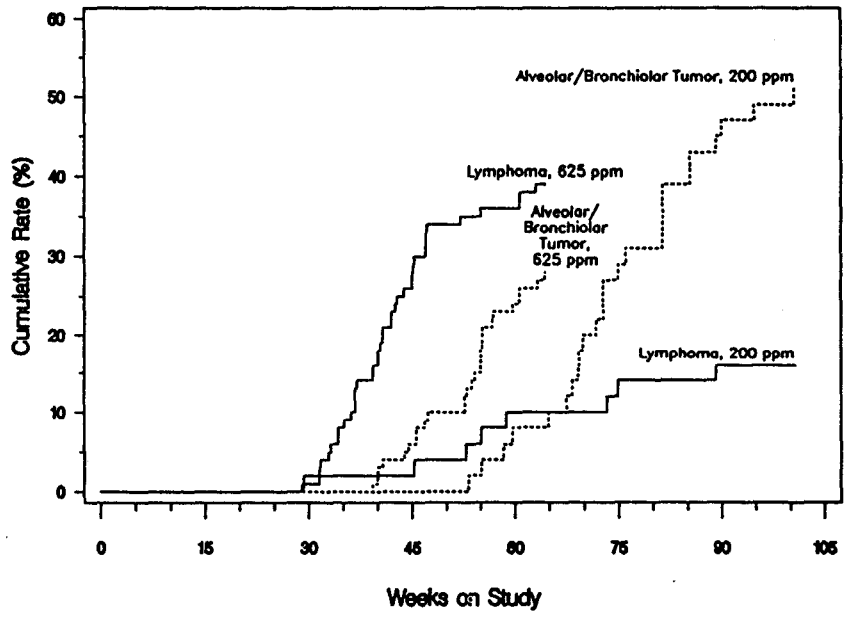


FIGURE 8
Cumulative Death-With-Neoplasm Rates of Selected Neoplasms in Mice Exposed to 1,3-Butadiene for 2 Years

K-*ras* oncogenes have never been detected in liver neoplasms from untreated $B6C3F_1$ mice (Reynolds *et al.*, 1987). Activated K-*ras* genes with codon 13 mutations were also found in lung neoplasms and in some of the lymphomas induced by exposure to 1,3-butadiene.

In the stop-exposure study, groups of male mice were exposed to one of the following regimens: 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 terminated, these groups of animals were placed in control chambers for the remainder of the 2-year study. For the first two groups, the total exposure to 1,3-butadiene (concentration times duration of exposure) was approximately equivalent (8,000 ppm · weeks for regimens A and B) and was equal to about half the total exposure given to the latter two groups (16,000 ppm · weeks for regimens C and D).

Survival was markedly reduced in all of the stop-exposure groups due to the development of compound-related malignant neoplasms. The neoplasm incidence profiles in the stop-exposure groups show that the incidences of lymphocytic lymphomas, histiocytic sarcomas, hemangiosarcomas of the heart, alveolar/bronchiolar adenomas or carcinomas, squamous cell papillomas or carcinomas of the forestomach, hepatocellular adenomas or carcinomas, adenomas or adenocarcinomas of the harderian gland, and preputial gland carcinomas were increased even after only 13 weeks of exposure to 625 ppm 1,3-butadiene compared with the control males. It is likely that shorter exposure durations would also produce a positive multiple-organ carcinogenic response.

At similar total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for a longer time. This is evident by comparing the incidence of lymphocytic lymphoma in the 625 ppm 13-week stop-exposure group (34%) with that in the 200 ppm 40-week stop-exposure group (12%), or more notably by comparing the incidence in the 625 ppm 26-week stop-exposure group (60%) with that in the 312 ppm 52-week stop-exposure group (8%). Doubling the duration of exposure to 625 ppm from 13 weeks to 26 weeks resulted in less than a two-fold increase in the incidence of lymphocytic lymphoma. Thus, for the induction of thymic lymphomas, the concentration of 1,3-butadiene is a much greater contributing factor than is the length of exposure.

Renal tubule cell adenomas were observed in 9 of the 200 male mice in the stop-exposure groups; the highest incidence was 4 in the 200 ppm 40-week stop-exposure group. The increased incidences of kidney neoplasms are particularly noteworthy, because these lesions rarely occur in untreated $B6C3F_1$ mice (historical incidence less than 0.2% in recent NTP studies). The detection of late-developing renal tubule cell adenomas in the stop-exposure groups is possibly due to the increased survival of these animals compared to those groups of male mice that were exposed to similar concentrations of 1,3-butadiene throughout their lifetimes or for up to 2 years.

Brain neoplasms including two neuroblastomas and three gliomas were observed in mice exposed to 625 ppm 1,3-butadiene for 13 or 26 weeks in the stop-exposure study. Because brain neoplasms are extremely rare in untreated $B6C3F_1$ mice, occurring in no historical control animals in the NTP database, their occurrence in this study was probably due to exposure to 1,3-butadiene. Furthermore, in the previous NTP inhalation studies of 1,3-butadiene in $B6C3F_1$ mice, gliomas were observed in one male mouse exposed to 1,250 ppm and two male mice exposed to 625 ppm (NTP, 1984). Although the incidence of brain neoplasms is low, the consistency between the previous studies and the present study is indicative of an exposure-related effect. It is also likely that the early and extensive development of lymphomas in mice exposed to 625 ppm 1,3-butadiene substantially reduced the number of mice at risk for later-developing brain neoplasms. Interestingly, glial cell neoplasms of the brain were also observed in male Sprague-Dawley rats exposed to 1,000 or 8,000 ppm 1,3-butadiene for up to 2 years (IISRP, 1981a).

In addition to the carcinogenic effects noted above, exposure to 1,3-butadiene caused a poorly regenerative anemia and gonadal toxicity in male and female mice (Melnick *et al.*, 1990b). Hematologic changes after 9 months of exposure to 1,3-butadiene included concentration-dependent decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume at exposure levels from 62.5 to 625 ppm

in males and at levels of 200 or 625 ppm in females. These changes were not accompanied by significant increases in reticulocyte counts or in the frequency of polychromatic erythrocytes in peripheral blood; however, there was a statistically significant increase in the percentage of erythrocytes with Howell-Jolly body inclusions. Other hematologic changes caused by exposure to 625 ppm 1,3-butadiene were an increase in mean erythrocyte volume and an increase in mean erythrocyte hemoglobin. Additionally, changes at other organ sites, bone marrow atrophy, and increases in splenic and hepatic extramedullary hematopoiesis were observed in mice exposed to 625 ppm 1,3-butadiene. These findings indicate partial or poorly regenerative, macrocytic anemia. The mechanism of the anemia cannot be determined from the data available from these studies; however, a mild megaloblastic anemia resulting from ineffective erythropoiesis in the bone marrow cannot be excluded. Tice *et al.* (1987) reported that exposure of male $B6C3F_1$ mice to 1,3-butadiene for 2 weeks caused a decrease in the number and rate of dividing cells in the bone marrow. Thus, in mice exposed to 1,3-butadiene, hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites. Consistent with this explanation, Thurmond *et al.* (1986) observed extramedullary hematopoiesis in spleens of male $B6C3F_1$ mice exposed to 1,250 ppm 1,3-butadiene for approximately 6 months.

Testicular atrophy was induced in male $B6C3F_1$ mice exposed to 1,3-butadiene concentrations of 625 ppm or above in the current studies and in previous studies (NTP, 1984). In female mice exposed to 1,3-butadiene for 9 months, ovarian atrophy of moderate severity was observed in the 200 and 625 ppm groups; the ovaries of mice exposed to 62.5 ppm for 9 months appeared normal. The atrophic ovaries had no identifiable oocytes, follicles, or corpora lutea. After 15 months of exposure to 1,3-butadiene, ovarian atrophy was observed at exposure levels of 20 ppm and above. In female mice exposed to 1,3-butadiene for up to 2 years, the incidence of ovarian atrophy was increased at all exposure concentrations (6.25 to 625 ppm) compared with controls. Even though ovarian atrophy in the 6.25 ppm group was not observed until late in the study, when reproductive senescence was probably occurring, the dose-response data clearly establish the ovary as a target organ of 1,3-butadiene toxicity at concentrations as low as 6.25 ppm, the lowest concentration studied.

The mechanism of butadiene-induced carcinogenicity is not known; however, oxidative intermediates of 1,3-butadiene biotransformation, 1,2-epoxy-3-butene, and diepoxybutane, or a combination of these (Malvoisin and Roberfroid, 1982), are likely involved. These metabolites are direct-acting mutagens in *Salmonella typhimurium* (de Meester *et al.*, 1978; Wade *et al.*, 1979), whereas the elicitation of *in vitro* mutagenicity of 1,3-butadiene appears to require metabolic activation (de Meester *et al.*, 1980). Furthermore, these epoxides have been shown to induce local (application site) neoplasms when applied to the skin of Swiss mice or when administered to Swiss mice or Sprague-Dawley rats by subcutaneous injection (Van Duuren *et al.*, 1963; 1966).

The carcinogenicity studies of 1,3-butadiene in Sprague-Dawley rats (IISRP, 1981a; Owen *et al.*, 1987) and $B6C3F_1$ mice (NTP, 1984; Huff *et al.*, 1985), including the current studies, demonstrate a species difference in the sites of neoplasm induction and the magnitude of the dose-dependent responses. In addition, in *in vivo* genotoxicity studies, 1,3-butadiene induced chromosomal aberrations, sister chromatid exchanges, and micronuclei in mice (Cunningham *et al.*, 1986; Tice *et al.*, 1987), but not in rats (Cunningham *et al.*, 1986). Biochemical and pharmacokinetic studies have been performed to determine the mechanism of neoplasm induction by 1,3-butadiene and to provide an explanation for the different toxic and carcinogenic responses between rats and mice. The possibility that the induction of thymic lymphomas in $B6C3F_1$ mice was a consequence of the expression of a murine leukemia retrovirus has been considered (Irons *et al.*, 1987, 1989; Irons, 1990). However, the finding that thymic lymphomas were induced by 1,3-butadiene in NIH Swiss mice, a strain that does not express the ecotropic murine leukemia viruses expressed in $B6C3F_1$ mice, demonstrates that these neoplasms were produced independently of these activated retroviruses.

In vivo alkylation of liver DNA was equivalent in $B6C3F_1$ mice and Wistar rats exposed to 1,3-butadiene (Kreiling *et al.*, 1986); however, expected reaction products between guanine and

1,2-epoxy-3-butene or diepoxybutane were detected in liver DNA from exposed mice, but not from exposed rats (Jelitto *et al.*, 1989). Further studies are needed on the dose-responses for DNA adduct formation in the major target organs of 1,3-butadiene-induced carcinogenicity in rats and mice and on the nature of the butadiene-derived material bound to rat liver DNA.

Differences in nonprotein sulfhydryl depletion in the liver, lung, and heart of rats and mice exposed to 1,3-butadiene were suggested as a basis for species differences in 1,3-butadiene-induced cytotoxicity and carcinogenicity (Kreiling *et al.*, 1988; Deutschmann and Laib, 1989). However, a causal relationship between decreases in nonprotein sulfhydryl levels in these organs and 1,3-butadiene-induced neoplasia after long-term exposure has not been established.

Studies on the pharmacokinetics of 1,3-butadiene in Sprague-Dawley rats and $B6C3F_1$ mice were designed to determine and compare the patterns of 1,3-butadiene metabolism in those species for which carcinogenicity data are available. At exposure concentrations below 1,000 ppm, where first-order kinetics apply, the metabolic elimination rate is nearly two times faster in $B6C3F_1$ mice than in Sprague-Dawley rats (Bolt *et al.*, 1984; Kreiling *et al.*, 1986). This difference has been attributed to the higher respiratory frequency of mice compared to rats (Kreiling *et al.*, 1986). The steady-state concentration of the epoxide metabolite, 1,2-epoxy-3-butene, is higher in mice than in rats exposed to similar atmospheric concentrations of 1,3-butadiene, largely because the metabolic elimination rate constant for this compound is five times higher in rats than in mice (Kreiling *et al.*, 1987; Laib *et al.*, 1990). Although quantitative differences in 1,3-butadiene metabolism have been observed among species, the differences are not of sufficient magnitude to account for the different dose-dependent toxic or carcinogenic responses seen for 1,3-butadiene in rats and mice. This is illustrated by a comparison of the carcinogenicity of 1,3-butadiene in mice exposed to 62.5 or 200 ppm 1,3-butadiene (as shown in Tables 8 through 17) to the carcinogenicity in rats exposed to 1,000 ppm (Owen *et al.*, 1987). Additional factors, such as steady-state levels of diepoxybutane, target organ levels and DNA reactivity of 1,3-butadiene intermediates, and differences in repair mechanisms, must be involved in distinguishing the site specificity of 1,3-butadiene-induced carcinogenicity between species.

Dahl *et al.* (1991) reported that the blood concentrations of total 1,3-butadiene metabolites were lower in monkeys than in rats or mice exposed to equivalent concentrations of this gas. Based on these results, humans may be at lower risk for cancer than rodents following equivalent inhalation exposures to 1,3-butadiene. A number of important factors impact on this interpretation. First, a large part of the species differences observed by Dahl *et al.* (1991) is accounted for in risk assessment models that adjust for breathing rate differences between species. Second, because metabolic intermediates may vary greatly in their carcinogenic potential, measurement of total 1,3-butadiene metabolites in blood is not a good indication of cancer risk. In related studies, Bond *et al.* (1987) found that the accumulation of ^{14}C in rats and mice exposed to ^{14}C-labeled 1,3-butadiene was not greater in target organs of 1,3-butadiene carcinogenicity than in nontarget organs. A preliminary identification of 1,3-butadiene metabolites in the blood of rats, mice, and monkeys exposed to ^{14}C-labeled 1,3-butadiene was made by a vacuum line-cryogenic distillation procedure (Dahl *et al.*, 1991). In the one instance in which the material in the "1,2-epoxy-3-butene trap" was analyzed by high-performance liquid chromatography, it was found that only 5% to 15% of the trapped radioactivity was 1,2-epoxy-3-butene. Thus, there is uncertainty in the identification of specific metabolites by the procedures used. Third, only three monkeys of unmatched age were used in the studies of Dahl *et al.* (1991). Fourth, the results in monkeys are clouded because, unlike the rats or mice, the monkeys were anesthetized during their exposure to 1,3-butadiene. Alterations in respiratory rates and cardiac output caused by anesthesia likely influenced the inhalation pharmacokinetics of 1,3-butadiene. Finally, because metabolic differences are not of sufficient magnitude to account for the different target site carcinogenic responses of 1,3-butadiene in rats compared to mice, it is unreasonable to assume that the kinetic data obtained in monkeys are predictive of human risk.

The finding of increased mortalities from lymphatic and hematopoietic cancers among subgroups of occupationally exposed workers (Meinhardt *et al.*, 1982; Downs *et al.*, 1987; Matanoski and Schwartz, 1987; Divine, 1990; Matanoski *et al.*, 1990) raises

additional concern for the carcinogenicity of 1,3-butadiene to humans, particularly because these results correspond to increased incidences of lymphoma observed in mice exposed to 1,3-butadiene. The detection of K-*ras* oncogenes in neoplasms induced by 1,3-butadiene adds further relevance to the potential carcinogenicity of 1,3-butadiene in humans, because K-*ras* is the most commonly detected oncogene in human cancers (Bos, 1989).

CONCLUSIONS

The previous inhalation studies of 1,3-butadiene in male and female B6C3F$_1$ mice provided *clear evidence of carcinogenicity** at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies — 2-year exposures of 6.25, 20, 62.5, 200, or 625 ppm or shorter duration exposures of 200, 312, or 625 ppm — provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male B6C3F$_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, preputial gland, brain, and kidney. There was *clear evidence of carcinogenicity* of 1,3-butadiene in female B6C3F$_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, ovary, and mammary gland.

Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also have been related to administration of 1,3-butadiene.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of peer review comments and the public discussion on this Technical Report appears on page 13.

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APPENDIX C

Description of Online Searches for 1,3-Butadiene

DESCRIPTION OF ONLINE SEARCHES FOR 1,3-BUTADIENE

Searches were limited to 1991 [the year before the IARC Monograph (1992), which has an extensive literature review, and the year before the ATSDR (1992) Toxicological Profile] through July 1997.

Online searches for 1,3-butadiene [CASRN 106-99-0] were performed in databases on the systems of STN International, DIALOG, NLM's TOXNET, and the Chemical Information System from 1980 to date. Toxicology information was sought in the EMIC, EMICBACK, TSCATS (epidemiology, chromosomal aberration, gene toxicity, mutagenicity), the Toxic Chemicals Release Inventory 1995 (online availability 1997), RTECS, TOXLINE (reviews as well as MESH heading for all neoplasms), CANCERLIT, EMBASE, BIOSIS, and MEDLINE (name and CASRNs combined with terms for metabolism and the MESH heading for all neoplasms). Occupational safety and health information was obtained from NIOSHTIC. HSDB provided a general review. The Chemical Abstracts files were searched by appropriate section codes (59, air pollution and industrial hygiene; 60, waste treatment and disposal; 61, water). The Chemical Abstracts Service Registry file and SANSS provided chemical identification information.

The MSDS database of Material Safety Data Sheets was searched via the Internet (URL = gopher.//ecosys.drdr.Virginia.EDU:70/00/library/gen/toxics/1%2C3-Butadiene).

Market information, including production, shipments, sales and consumption, labor use, and workers by type was sought in PROMT, *The Predicasts Overview of Markets and Technology,* and The Chemical Economics Handbook.

Regulatory information was obtained from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to the titles21 (FDA), 29 (OSHA), and 40 (EPA) regulations.

Also, the review of 1200 life sciences journals was accomplished using Current Contents on Diskette® for current awareness.

APPENDIX D

Report on Carcinogens (RoC), 9th Edition
Review Summary

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Review Summary

1,3-Butadiene

NOMINATION
Review for possible upgrading of current listing for 1,3-Butadiene from *reasonably anticipated to be a human carcinogen* to *known to be human carcinogen* based on recent epidemiology reports.

DISCUSSION
Butadiene, used primarily as a chemical intermediate and polymer component in the manufacture of synthetic rubber, is currently listed in the RoC as *reasonably anticipated to be a human carcinogen.* Epidemiology studies of butadiene workers have found excess mortality from lymphatic and hematopoietic cancers associated with occupational exposure to butadiene. The more recent epidemiology studies addressed many of the limitations of earlier studies, including use of modeling efforts that quantitatively estimated exposure to butadiene. Industry representatives have pointed out that the predominant types of human cancers differ in workers exposed in the butadiene monomer industry (lymphosarcoma) from those in the butadiene/styrene industry (leukemia), and that this discrepancy may be associated with co-exposure to dithiocarbamates in the butadiene/styrene industry. Butadiene is metabolized to mutagenic and carcinogenic epoxides (epoxybutene and diepoxybutane) in all mammalian species studied, including humans. The mechanism of tumor induction by butadiene in rodents and humans appears to be due to its metabolism to DNA reactive intermediates resulting in genetic alterations in protooncogenes and/or tumor suppressor genes. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	upgrade and list as known human carcinogen	9 yes/0 no
NTP EC Working Group (RG2)	upgrade and list as known human carcinogen	8 yes/0 no
NTP Board RoC Subcommittee	upgrade and list as known human carcinogen	4 yes/1 no/1 a*

*a-abstentions

Public Comments Received
A total of 6 public comments were received:

- 5 against upgrading current listing to a known to be human carcinogen
- 1 providing comments on the content of the background document prepared for the review of this nomination