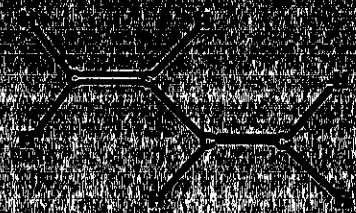
California Environmental Protection Agency

Air Resources Board

Technical Support Document

Proposed Identification of 1,3-Butadiene



as a Toxic Air Contaminant

Part B
Health Assessment

Stationary Source Division

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PART B

HEALTH EFFECTS OF 1,3-BUTADIENE

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

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1.0 SUMMARY

1,3-Butadiene (BD) is an important industrial chemical produced as a by-product of ethylene. About three billion pounds were produced domestically in 1987 and an additional half-billion pounds were imported. BD is used in the production of styrene-butadiene copolymers, polybutadiene, and chloroprene/neoprene. The U.S. Environmental Protection Agency (EPA) has estimated total emissions of BD from industrial production and major uses at 10 million pounds per year in 1987 with approximately 70% attributable to equipment leaks. BD has also been found in automobile exhaust, gasoline vapor, incineration products of fossil fuels, cigarette smoke, and drinking water. The California Air Resources Board (ARB) has measured urban ambient concentrations of BD ranging from <0.1 to 1.8 ppb in a dozen California communities.

Although there are no human data on the metabolism of butadiene, studies in rodents indicate that BD is taken up rapidly by the inhalation route and is distributed to all tissues. At low concentrations (75 ppb) in air about 16-17% of BD was retained following inhalation. BD is metabolized, via cytochrome P450 monooxygenase mediated epoxidation, to 1,2-epoxy-3-butene or butene monoxide (BMO). This key metabolite is further metabolized to 3-butene-1,2-diol and to 1,2:3,4-diepoxybutane. Further conversion of the diol to 3,4-epoxy-1,2 butanediol by monooxygenase has also been observed.

1,3-Butadiene has only mild acute toxicity and produces neurological effects at high concentrations. The lowest concentration associated with neurological effects following subchronic exposure in animals is 13.5 ppm. Only minor noncarcinogenic adverse effects have been observed following subchronic exposures of 0.5 ppm. This level is more than 200-fold greater than ambient levels, thus significant adverse noncarcinogenic effects are not expected to occur at the ambient levels of up to approximately 0.002 ppm (2 ppb). However, due to the unavailability of data, hotspots have not been evaluated.

While a No Observed Adverse Effect Level (NOAEL) for reproductive effects has not been determined for BD, a chronic exposure Lowest Observed Adverse Effect Level (LOAEL) for reproductive toxicity is 6.25 ppm. This level produced gonadal atrophy in female mice. In developmental toxicity studies BD has been shown to be fetotoxic in the absence of producing maternal toxicity. At 40 ppm, BD exposure resulted in reduced fetal weight and in reduced ossifications in the offspring. The LOAEL is approximately 3000-fold greater than the upper range of ambient levels. Thus, these adverse reproductive effects are not expected to occur at ambient levels. However, the findings of a dominant lethal assay in mice may signal a mutagenic effect in male germ cells that might not be characterized by a dose threshold. Thus, reproductive or developmental effects cannot be ruled out at any level of BD exposure, although at ambient levels the incidence of such effects (which in humans might include undetected or very early-term spontaneous abortion) would be expected to be extremely low. Due to the unavailability of data, hot spots have not been evaluated.

A number of lines of evidence indicate that the mechanism of the oncogenic activity shown by BD in rodent bioassays is related to its genetic toxicity. These include: 1) the mutagenicity of BD in Salmonella in vitro with exogenous activation; 2) clastogenic/DNA damaging activities in mammals in vivo; 3) the

direct mutagenic action in Salmonella of the two principal metabolites of BD, namely BMO and 1,2:3,4-diexpoxybutane (DEB), in vitro and their detection in blood of experimental animals exposed to BD via inhalation; 4) the binding of BD (metabolites) to DNA; and 5) the isolation and identification of a DNA adduct from the liver DNA of mice exposed to BD, namely 7-(1-hydroxy-3-buten-2-yl)guanine. These studies clearly indicate that BD is genotoxic. In view of BD's probable mode of action in carcinogenicity (genetic toxicity), the effective dose of the carcinogen may be so low as to be indistinguishable from zero. No threshold mechanisms have been shown to specifically affect the action of BD. No practical threshold is thought to exist.

Epidemiological studies of the effects of BD on human populations have been reviewed and evaluated by the EPA, the International Agency for Research on Cancer (IARC) and the U.S. Occupational Safety and Health Administration (OSHA). EPA (1985) and IARC (1987) concluded that the evidence of human carcinogenicity was inadequate. However, more recent evaluations have considered more recently published studies. Based on a more recent review of epidemiologic studies, OSHA (1990) is of the opinion that exposure to BD is associated with an increased risk of lymphohematopoietic cancer. An IARC committee has concluded that there is limited evidence of human carcinogenicity of BD based on the studies of Downs et al. (1987), Divine (1990) and Matanoski et al (1990). OEHHA staff expect that IARC will soon formally concur with this conclusion. Among the recent epidemiologic studies are two that were published in 1990.

Divine (1990) found a significantly elevated Standardized Mortality Ratio (SMR) for lymphosarcoma among 2,582 workers in a butadiene manufacturing facility. The SMR for lymphosarcoma was even higher in those with routine exposure to butadiene. Matanoski et al. (1990) observed an excess of leukemia and lymphatic and hematopoietic cancers in black production workers and an elevated SMR for residual cancers of the lymphohematopoietic system for all production workers in several styrene-butadiene rubber (SBR) plants in the United States and Canada. Presumably, in SBR plants, production workers had the highest likelihood of exposure to butadiene, although they may have been exposed to other substances that are, or may be, linked to some of these cancers. While it is difficult to establish a causal relation with BD exposure, the fact that cancers of the lymphohematopoietic system were reported in mice suggests that the association deserves close attention in future studies. With respect to quantitative risk assessment, the epidemiological data base is still considered inadequate for predicting risks of community exposure to BD. Thus, the quantitative risk assessment presented in this document relies on data from animal bioassays rather than epidemiologic studies.

Butadiene was identified as a chemical known to the State of California to cause cancer on April 1, 1988. OSHA (1990) has classified 1,3-butadiene as a "potential occupational carcinogen." EPA (1985) and IARC (1987) have concluded that the evidence for carcinogenicity of BD in animals is sufficient. These organizations have classified the chemical as Group B2 and 2B respectively in their schemes of ranking potential human carcinogens. Inhalation of BD induced tumors in mice and rats. The animal bioassays for carcinogenicity indicate that BD can induce cancer in multiple sites. These sites include the heart, lung, mammary gland, ovaries, forestomach, liver, pancreas, Zymbal gland, thyroid, testes, and hematopoietic system. BD is only

one of two chemicals known to induce cancer of the heart in laboratory animals.

The most detailed evaluations of the carcinogenicity of BD are the mouse inhalation studies sponsored by the National Toxicology Program, mouse I - NTP 1984, and mouse II - Melnick et al., 1990. The nominal doses of study I were 0, 625 or 1,250 ppm administered 6 hours/day, 5 days/week for either 60 weeks (males) or 61 weeks (females). Fifty animals per sex/dose were used. Although the study was designed for 103 weeks, early deaths resulted largely from malignant neoplasms involving multiple organs (heart, hematopoietic lymphomas, lung, mammary gland, ovaries, forestomach, and liver). The incidences of total significant tumor bearing animals at control, middle, and high doses were 2/50, 43/49, and 40/45 in the males and 4/48, 31/48, and 45/49 in the females.

In study II, lower exposure concentrations of 1,3-butadiene (i.e., 0, 6.25, 20, 62.5, 200, and 625 ppm) were used than had been employed in the first study. Interim sacrifices at 40 and 65 weeks of exposure were also added to the original study design in order to follow progression of lesions. As in the previous study, hemangiosarcomas of the heart, hematopoietic lymphomas, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, and/or adenocarcinomas of the mammary gland were frequently observed in mice which died between weeks 40 and 65 of the study. In female mice exposed to 6.25 ppm BD, the incidence of alveolar-bronchiolar neoplasms was increased (15/60, 25%) vs. control (4/70, 6%). Also as in the previous study, gonadal atrophy was observed in both sexes at 200 ppm and 625 ppm for males and 6.25 ppm and higher for females. Bone marrow toxicity was evident as a poorly regenerative anemia at 62.5 ppm and higher. Thus this study did not establish a no effect level for reproductive endpoints. The 6.25 ppm nominal dose level might be considered a chronic LOAEL for reproductive toxicity. The Melnick et al. (1990) report is reproduced in the Appendix.

A two-year rat inhalation toxicity/carcinogenicity study (Hazleton Europe, 1981) was also evaluated. Groups of 100 per sex/dose were exposed to 0, 1,000, or 8,000 ppm BD for 6 hours/day, 5-days/week for 105-111 weeks. Unlike the mouse I study, survival of treated animals was not adversely affected in the first year of the study, but during the second year there was a statistically significant relationship between mortality and air concentration of BD. The published incidences are slightly different from those given by EPA (1985). The total significant tumor incidences (number of animals bearing one or more significant tumors) in males based on EPA criteria and 1987 published incidences are 4/100, 5/100, and 20/100 for the control, low, and high dose groups (Leydig cell tumors, pancreatic exocrine tumors, and Zymbal gland tumors). Total female significant tumor incidences in the control, low, and high dose groups were: 18/100, 19/100, and 41/100 (mammary carcinoma, thyroid follicular cell tumors, and zymbal gland tumors).

Cancer potency estimates were made for mice and rats using total significant tumor incidences and individual site incidences, three measures of dose, and the linearized multistage model of low dose extrapolation. The most sensitive tumor site was the lung alveolar and bronchiolar neoplasms in female mice (mouse II). The continuous internal dose was considered to be the best measure of dose available. When interspecies equivalent units of mg/m^2 surface area were used the resulting upper range of human cancer potency based

on all rodent assays was 9.8×10^{-6} to 8×10^{-4} $(ppb)^{-1}$ (4.4×10^{-6} to 3.6×10^{-4} $(\mu g/m^3)^{-1}$). The current EPA cancer risk estimate is 6.4×10^{-4} per ppb. OSHA (1990) has estimated a cancer risk for occupational exposure to 1 ppm BD of 0.0027, corresponding to a lifetime exposure risk of 1.4×10^{-5} $(ppb)^{-1}$ or 6.1×10^{-6} $(\mu g/m^3)^{-1}$, with an upper bound corresponding to 1.9×10^{-5} $(ppb)^{-1}$ or 8.4×10^{-6} $(\mu g/m^3)^{-1}$. More recently, staff of the National Institute for Occupational Safety and Health (NIOSH, 1991) have recommended to OSHA potency values based on data from the NTP "mouse II" study. The NIOSH-recommended cancer risk value for occupational exposure to 1 ppm BD is 0.0305, corresponding to a lifetime exposure risk of 1.5×10^{-4} $(ppb)^{-1}$ or 6.9×10^{-5} $(\mu g/m^3)^{-1}$, with an upper bound approximately corresponding to 2.5×10^{-4} $(ppb)^{-1}$ or 1.1×10^{-4} $(\mu g/m^3)^{-1}$.

The range of upper bound risk is based on the two orders of magnitude difference between potency figures for the mouse and the rat. This difference has been the subject of much additional metabolic and kinetic investigation. In addition to a higher metabolic rate for BD in the mouse, limited detoxification and accumulation of the primary reactive genotoxic metabolite (BMO) may be a significant factor in the increased susceptibility of mice to BD-induced carcinogenesis. Clearly work should continue to determine whether the mouse is the most appropriate model for prediction of human susceptibility to BD-induced carcinogenesis. The most detailed evaluation of the carcinogenicity of BD has been conducted in the mouse. The staff of the Office of Environmental Health Hazard Assessment (OEHHA) concludes that, for use in risk assessment, the quality of the mouse II bioassay data is superior to that of the rat data. The primary reasons for this conclusion are: 1) the use of lower, more relevant dose levels in the mouse II study; 2) the use of five dose levels in the mouse II study, compared to two in the rat study; 3) the presence of two mouse studies; 4) the fact that the rat study has not been replicated; 5) the consistency in sites of carcinogenicity between the two mouse studies; 6) the greater detail in the available mouse data which allows in-depth analysis; and 7) suggestions from limited epidemiological observations that BD exposure may be associated in humans with lymphatic and hematopoetic cancers, effects that were seen in mice.

The best value for the upper bound excess cancer risk is based on the mouse II bioassay data of Melnick et al. (1990). The present analysis gives a mouse based cancer potency of 3.4 $(mg/kg\text{-}d)^{-1}$ [0.37 $(ppm)^{-1}$, or 3.7×10^{-4} (ppb)-1], and a unit risk of 1.7×10^{-4} $(\mu g/m^3)^{-1}$ for the best value. Exposure to 6×10^{-3} $\mu g/m^3$ is associated with a lifetime excess cancer risk of 10^{-6}. The difference between the EPA estimate (6.4×10^{-4} $(ppb)^{-1}$) and the OEHHA best value (3.7×10^{-4} $(ppb)^{-1}$) is largely due to the use of more recent butadiene inhalation absorption data and the mouse II data in the current report. The unadjusted experimental dose and the pharmacokinetic dose were considered inappropriate for establishing the range of risks, although most of these estimates are within the range. In particular, the pharmacokinetic dose was not used since that approach was preliminary and the results did not help explain tumor frequencies in target tissues or species differences. Also for key data sets the multistage model gave relatively poor fits to the pharmacokinetic doses. For comparison purposes, cancer potency values obtained using physiologically based pharmacokinetic modeling (PBPK) of dose are provided. The PBPK values and their assumptions and limitations are presented in Section 4.2. However, in view of our limited understanding of the toxicokinetics of BD and its metabolites and the lack of any direct human

metabolic data on butadiene, it is not possible at present to rely on the pharmacokinetic estimates of metabolized dose for estimating human health risks.

The best potency value of 3.7×10^{-4} $(ppb)^{-1}$ (1.7×10^{-4} $(\mu g/m3)^{-1}$) indicates that community exposure to BD at the statewide population-weighted average ambient (outdoor) level (0.37 ppb) could be associated with an upper limit of 140 additional lifetime cancers per million exposed individuals. The EPA has estimated lifetime extra cancer risks for individuals living near hot spot facilities in California to be in the range of 1 in 10 to 1 in 1000. These calculations are for the upper range of plausible excess cancer risks; the actual risk, which cannot be calculated, may be much lower.

Based on the findings of 1,3-butadiene-induced carcinogenicity and the results of the risk assessment, the OEHHA staff find that, at ambient concentrations, 1,3-butadiene is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

1.1 Butadiene Highlights

1.1.1 National and International Evaluation of Carcinogenicity

1.1.1.1 International Agency for Research on Cancer (IARC)

Evidence for carcinogenicity to animals is sufficient. Evidence for carcinogenicity to humans is inadequate. IARC has classified 1,3-butadiene as "possibly carcinogenic to humans," Group 2B.

An IARC committee has recommended that the Agency's evaluation be changed to report limited evidence of carcinogenicity in humans; it is expected that IARC will make this change in the near future. This would change IARC's classification of BD to Group 2A, "probably carcinogenic to humans."

1.1.1.2 U.S. Environmental Protection Agency (EPA)

There is sufficient evidence of carcinogenicity in two species of rodents. There is inadequate evidence for carcinogenicity in humans. According to EPA guidelines, 1,3-butadiene would be classified as a "probable human carcinogen," Group B2.

1.1.1.3 U.S. Occupational Safety and Health Administration (OSHA)

On the basis of consistency of results from several epidemiologic studies, "OSHA is of the opinion that exposure to BD is associated with an increased risk of death from cancer of the lymphohematopoietic system" (OSHA 1990, Section V.B.2(v)). OSHA has classified 1,3-butadiene as a "potential occupational carcinogen," based primarily on positive findings of chronic inhalation studies in rodents. OSHA has proposed to lower its permissible exposure limit (PEL) for BD from 1000 ppm to 2 ppm (OSHA 1990).

1.1.2 Exposure Sources

1.1.2.1 Air

Butadiene is a common contaminant of urban air largely arising from vehicle emissions. Ambient air levels of butadiene were measured at 20 locations in California by the Air Resources Board in 1988 and 1989. Concentrations varied from less than 0.00004 ppm (0.04 ppb, limit of detection) to 0.0018 ppm (1.8 ppb).

1.1.2.1 Water

While butadiene has been detected in U.S. drinking water, human exposure is primarily through the inhalation route.

1.1.3 Quantitative Risk Assessment

1.1.3.1 Range of Risks

The theoretical human risks associated with a continuous lifetime exposure to butadiene in ambient air have been estimated from animal carcinogenicity bioassay data using the linearized multistage model. Unit risks for humans ranged from 9.8×10^{-6} $(ppb)^{-1}$ [0.0098 $(ppm)^{-1}$, 4.5×10^{-6} $(\mu g/m^3)^{-1}$] to 8.0×10^{-4} $(ppb)^{-1}$ [0.8 $(ppm)^{-1}$, 3.5×10^{-4} $(\mu g/m^3)^{-1}$] based on rat and mouse inhalation studies respectively. The best value is that based on the mouse II inhalation study, 3.7×10^{-4} $(ppb)^{-1}$ [0.37 $(ppm)^{-1}$, 1.7×10^{-4} $(\mu g/m^3)^{-1}$].

1.1.3.2 Extrapolations

1.1.3.2.1 Route

The animal bioassays were conducted by the inhalation route. Quantitative risk estimates assume that 16-17% of a low dose is absorbed and retained (based on uptake studies with radiolabeled butadiene) and that dose units of mg/m^2 surface area/d are equivalent in different species.

1.1.3.2.2 Dose

The doses in the recent NTP mouse carcinogenicity bioassay (Melnick et al., 1990) ranged from 6.25 to 625 ppm (6 hours/day, 5 days/week). There was a significant increase in lung neoplasms in female mice at the 6.25 ppm level. This experimental dose is equivalent to a continuous exposure of approximately 1 ppm. The estimated population-weighted average ambient exposure is approximately 0.4 ppb in California. Thus, the range of extrapolation of animal data to human exposure is about 4 orders of magnitude.

2.0 TOXICOKINETICS

2.1 Absorption and Distribution

Male Sprague-Dawley rats exposed to BD concentrations of ≤ 1000 ppm in a closed inhalation chamber exhibited 'linear' pharmacokinetics with respect to 1,3-butadiene disappearance and exposure concentration; however, at concentrations >1000 ppm, the elimination rate was constant and was indicative of saturation of the metabolic capacity (Bolt, Filser and Stormer, 1984). Kreiling et al. (1986) reported similar results for B6C3F1 mice, as well as for Sprague-Dawley rats, and further determined that mice had a much greater uptake rate than rats. The metabolic V_{max} at saturation was 400 μmol/h-kg for mice and 220 μmol/h-kg for rats, which was in concurrence with the *in vitro* results observed by Schmidt and Loeser (1985). Since metabolic elimination constants were approximately equal for both species up to 1000 ppm, it can be shown that, under conditions where first-order elimination applies, the steady state concentration of 1,3-butadiene in the mouse is approximately two times that in the rat.

Induction of rats with Aroclor 1254, a cytochrome P450 inducer, increased their metabolic capacity to the extent that saturation was not observed at 1,3-butadiene exposure concentrations as high as 12000 ppm (Bolt, Filser and Stormer, 1984). The inducibility of BD metabolism in the rat agrees with the results of *in vitro* studies. Expired air from Sprague-Dawley rats exposed to ≈7000 ppm 1,3-butadiene in a closed inhalation chamber contained 1,2-epoxy-3-butene (butene monoxide, BMO) (Bolt et al., 1983), thus confirming another *in vitro* observation.

Bond et al. (1986) conducted experiments in adult male Sprague-Dawley rats and B6C3F1 mice to: 1) determine the uptake and retention of 1,3[^{14}C]-butadiene over a wide range of exposure concentrations, 2) to identify routes and half-times for excretion of parent compound and metabolites, and 3) to identify BD metabolites in blood. The animals were exposed for a single 6-hour exposure period to 1,3[^{14}C]-butadiene concentrations (nose-only exposures) ranging from 0.14 to 13,000 μg/L (0.08 to 7100 ppm). Mice were not exposed to the highest concentration. The percentage of ^{14}C absorbed and retained at 6 hours ranged from 1.5 to 17% in rats and from 4 to 20% in mice (Table 2-1 and Table 2-2). During the exposure period, respiratory measurements were made and blood samples were obtained for determination of BD, BMO, and 1,2:3,4-diepoxybutane (DEB) concentrations. Immediately post-exposure four animals of each species were placed in metabolism cages and urine and feces samples were collected at suitable time points for up to 65 hours. Expired air was also pulled from the metabolism cages and analyzed for parent compound and metabolites.

Analysis of blood for metabolites revealed an increase in metabolite concentration with increasing exposure concentration for both species; however, the increases in metabolite concentrations were not directly proportional to the increases in exposure concentration (Table 2-3). Mice had higher blood levels of the metabolite BMO than rats at equivalent exposure concentrations, although rats had higher concentrations of $^{14}CO_2$. Levels of 1,2:3,4-diepoxybutane were similar in the two species.

Bond et al. (1987) further examined species differences in the distribution of 1,3-butadiene between rats and mice by exposing young adult male Sprague-

Table 2-1 Respiratory Tract Retention of Inhaled Butadiene in Rats and Mice[a]

Exposure Concentration (ug/l)	Total Volume Inhaled/Animal (l)[b]		Butadiene Inhaled/Animal (umol)		[14C]Butadiene Equivalents Retained at 6 hr/Animal (umol)[c]		Percentage of Inhaled [14C]Butadiene Equivalents Retained at 6 hr	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
0.14	73 ± 5	14 ± 2	0.2 ± 0.001	0.04 ± 0.0002	0.03 ± 0.0002	0.006 ± 0.001	17 ± 2[h]	16 ± 1[h]
1.4	90 ± 4	12 ± 2	2 ± 0.1	0.3 ± 0.06	0.13 ± 0.003	0.06 ± 0.01	6 ± 0.3[g]	20 ± 5
13	83 ± 7	12 ± 1[d]	19 ± 2	3 ± 0.4[d]	0.8 ± 0.1	0.4 ± 0.09[d]	4 ± 0.5[g]	20 ± 5[d]
130	70 ± 8[e]	13 ± 2	170 ± 20[e]	35 ± 5	17 ± 2[e]	3.2 ± 0.1	8 ± 1[e]	8 ± 0.7
1800	100 ± 10	12 ± 2	3,100 ± 360	440 ± 80	65 ± 5	19 ± 0.5	2.5 ± 0.2[g]	4 ± 0.3
13,000	70 ± 15	*f*	17,000 ± 3100	*f*	240 ± 40	*f*	1.5 ± 01	*f*

a Values are the mean ± SE.

b Values corrected for rat mouse body temperature, barometric pressure, inspired gas temperature, and relative humidity.

c Data obtained from radioanalysis of rats and mice which were maintained in plethysmograph tubes during the 6 hr exposure.

d Individual values pooled from three separate experiments.

e Individual values pooled from two separate experiments.

f Mice were not exposed to this concentration of butadiene.

g Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's t test.

h a linear regression analysis of the log transformed data vs the log of the exposure concentration indicated that there was a significant ($p < 0.001$) concentration-related decrease in the percent retained at 6 hr with increasing exposure concentration.

(Table taken directly from Bond et al. 1986)

Table 2-2 Inhaled "Dose" of Butadiene in Rats and Mice[a]

Exposure Concentration (μg/l)	Dose (μmol/kg)[b]		Dose (nmol/cm^2)	
	Rats[c]	Mice[c]	Rats[d]	Mice[d]
0.14	0.08 ± 0.01[h]	0.2 ± 0.02	0.6 ± 0.04	0.6 ± 0.07
1.4	0.3 ± 0.01	2 ± 0.2	3 ± 0.1[h]	6 ± 0.8
13	2 ± 0.1[h]	22 ± 4[e]	14 ± 1[h]	49 ± 10[e]
130	40 ± 3[fh]	110 ± 11	310 ± 20[f]	320 ± 40
1800	160 ± 10[h]	650 ± 50	1200 ± 80[h]	2000 ± 150
13000	680 ± 40	g	4900 ± 290	g

[a] Values are the mean ± SE.

[b] Calculated using μmol butadiene retained at 6 hr (Table 2, Col. 4).

[c] the mean body weights (±SE) for rats and mice for all exposures were 398 ± 4 and 28 ± 0.3 g, respectively.

[d] Mean body surface areas (±SE) for rats and mice for all exposures were 54 ± 0.3 and 9.2 ± 0.07 cm^2, respectively.

Body surface areas were calculated as follows:

surface area (cm^2) = body weight (g)$^{2/3}$.

[e] Individual values pooled from three separate experiments.

[f] Individual values pooled from two separate experiments.

[g] Mice were not exposed to the concentration of butadiene.

[h] Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's test.

(Table taken directly from Bond et al., 1986)

Table 2-3 Distribution of ^{14}C in Blood of Rats and Mice Exposed to Different Concentrations of Butadiene[a]

	Exposure Concentration (ug/l)	Residue[b]			Butadiene diepoxide · 45°C trap[c]			1,2-Epoxy-3-butene · 95°C trap[d]			Butadiene · 130°C trap[e]			CO_2 ·195°C trap[f]		
		2[g]	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Rats	130	5±0.2 (87)	7±0.4 (86)	8±1[h] (77)	0.04±0.02[h] (0.7)	0.1±0.02 (1)	0.1±0.04 (1)	0.4±0.04[h] (6)	0.4±0.08[h] (5)	0.4±0.04 (3)	0.1±0.02 (3)	0.2±0.4 (2)	0.1±0.02[h] (1)	0.3±0.02[h] (5)	0.5±0.02[h] (6)	0.6±0.04[h] (5)
	1800	14±3[h] (66)	40±7[h] (77)	51±8 (82)	0.4±0.02 (2)	1±0.3 (2)	1±0.04 (2)	2±0.4 (12)	4±1[h] (8)	4±0.7[h] (7)	1±0.08[h] (6)	4±0.6 (7)	4±0.3 (6)	2±0.02 (11)	3±0.5 (6)	4±0.2[h] (6)
Mice	13	2±0.4 (56)	3±0.6 (70)	6±0.4 (83)	0.06±0.01 (2)	0.06±0.02 (1)	0.08±0.001 (1)	0.6±0.1 (19)	0.3±0.2 (8)	0.7±0.1 (11)	0.6±0.02 (19)	0.9±0.5 (21)	0.2±0.06 (3)	0.09±0.01 (3)	0.1±0.01 (3)	0.08±0.01 (1)
	130	10±2 (82)	13±3 (86)	17±1 (89)	0.2±0.03 (1)	0.2±0.03 (1)	0.01±0.01 (0.5)	2±0.05 (14)	2±0.2 (10)	0.9±0.4 (5)	0.3±0.1 (2)	0.2±0.1 (1)	0.8±0.1 (4)	0.2±0.04 (2)	0.2±0.0.1 (1)	0.2±0.02 (1)
	1600	71±12 (32)	110±13 (79)	100±20 (70)	3±1 (3)	3±0.2 (2)	1±0.2 (0.8)	4±0.8 (4)	21±1 (14)	15±1 (10)	7±0.3 (8)	8±2 (5)	3±1 (2)	2±0.4 (2)	3±0.8 (2)	2±0.05 (2)

[a]Data are expressed as the mean nmol/ml blood ±SE; n = 3; numbers in parentheses are present of total ^{14}C/ml blood.

[b]Represents nonvolatile butadiene metabolites.

[c]Diepoxybutane codistills in this trap.

[d]1,2-Epoxy-3-butene codistills in this trap.

[e]Butadiene codistills in this trap.

[f]CO_2 codistills in this trap.

[g]Time (hr) after start of exposure.

[h] significantly different ($p < 0.05$) from mice exposed to the same concentration of butadiene.

Table taken directly from Bond, et al. 1986.

Dawley rats and B6C3F1 mice (nose-only) to 670 and 65 ppm 1,3[^{14}C-] butadiene, respectively, for a single 3.4 hour period. These concentrations were chosen because: 1) they resulted in similar concentrations of BD and its metabolites in the two species, and 2) BD excretion and metabolism rates for both species are linear with respect to exposure concentration in this range (Bond et al., 1986).

Results of these studies indicated that 1,3-butadiene and its metabolites were widely distributed to all tissues in both species within one hour after the start of exposure (Table 2-4). Several tissues in both species contained high concentrations of ^{14}C within 1 hour postexposure. Tissues with the three highest ^{14}C-concentrations at 1 hour postexposure were, in rats, the bladder, the thyroid, and the kidney, and, in mice, the bladder, the small intestine, and the kidney. The concentration of ^{14}C in the blood was low compared to other tissues in both species. Although rats absorbed a greater amount of BD on a μmol/kg body weight basis, the concentrations of ^{14}C radiolabel/μmol 1,3-butadiene inhaled were 15 to 100 times greater in the tissues of mice than in rats.

2.2. Metabolism and Excretion

BD was shown to be metabolized to 1,2-epoxy-3-butene (BMO) in the presence of rat liver microsomes and NADPH (Malvoisin et al. 1979; Bolt et al., 1983). The participation of cytochrome P450 in this epoxidation reaction was indicated by 1) the enhancement of the epoxidation reaction following pretreatment of the rats with the P450-inducer, phenobarbital, and 2) the inhibition of the formation of BMO with the known cytochrome P450 inhibitors, SKF-525A and dithiocarb. 3-Methylcholanthrene (3-MC) was also reported by Bolt et al. (1983) to induce the epoxidation of 1,3-butadiene; however, Malvoisin et al. (1979) reported that pretreatment of rats with 3-MC did not result in induction. Bolt et al. (1983) further confirmed the occurrence of this metabolic pathway *in vivo* by identifying BMO in the expired air of rats exposed to BD.

Malvoisin and Roberfroid (1982) showed that BMO was metabolized to both 3-butene-1,2-diol and to 1,2:3,4-diepoxybutane (DEB) by rat liver microsomes, and that the 3-butene-1,2-diol was further oxidized to 3,4-epoxy-1,2-butanediol (EBD) (Figure 1). The metabolism of BMO to 3-butene-1,2-diol presumably occurred via epoxide hydrolase since inhibition of the epoxide hydrolase with 1,1,1-trichloropropene oxide increased the levels of BMO (Bolt et al., 1983). The next metabolic step, formation of two isomers of EBD, occurs via the cytochrome P450-dependent monooxygenase system. This latter reaction is inducible by pretreatment with phenobarbital, and is inhibited by the presence of SKF-525A. Since the P450-system has a greater affinity for BMO than epoxide hydrolase does, the balance lies in favor of the formation of DEB (Malvoisin and Roberfroid, 1982). Bolt et al. (1983) also demonstrated that the presence of glutathione in the medium causes a decrease in the BMO concentration. This decrease probably occurs when the diol, formed by the epoxide hydrolase, is conjugated with glutathione. Removal of the diol as it is formed would shift the reaction equilibrium to the right and decrease the steady state concentration of BMO.

Jelitto et al. (1989) exposed male Sprague-Dawley rats and male B6C3F1 mice for 7 h to 250, 500, or 1000 ppm BD. After exposure cell nuclei of liver and lung tissues were isolated and subjected to alkaline elution. Analysis of the

Table 2-4 Concentration of ^{14}C (nmol/g) in Tissues of Rats and Mice Exposed to ^{14}C-Butadiene[a] at Selected Time Points

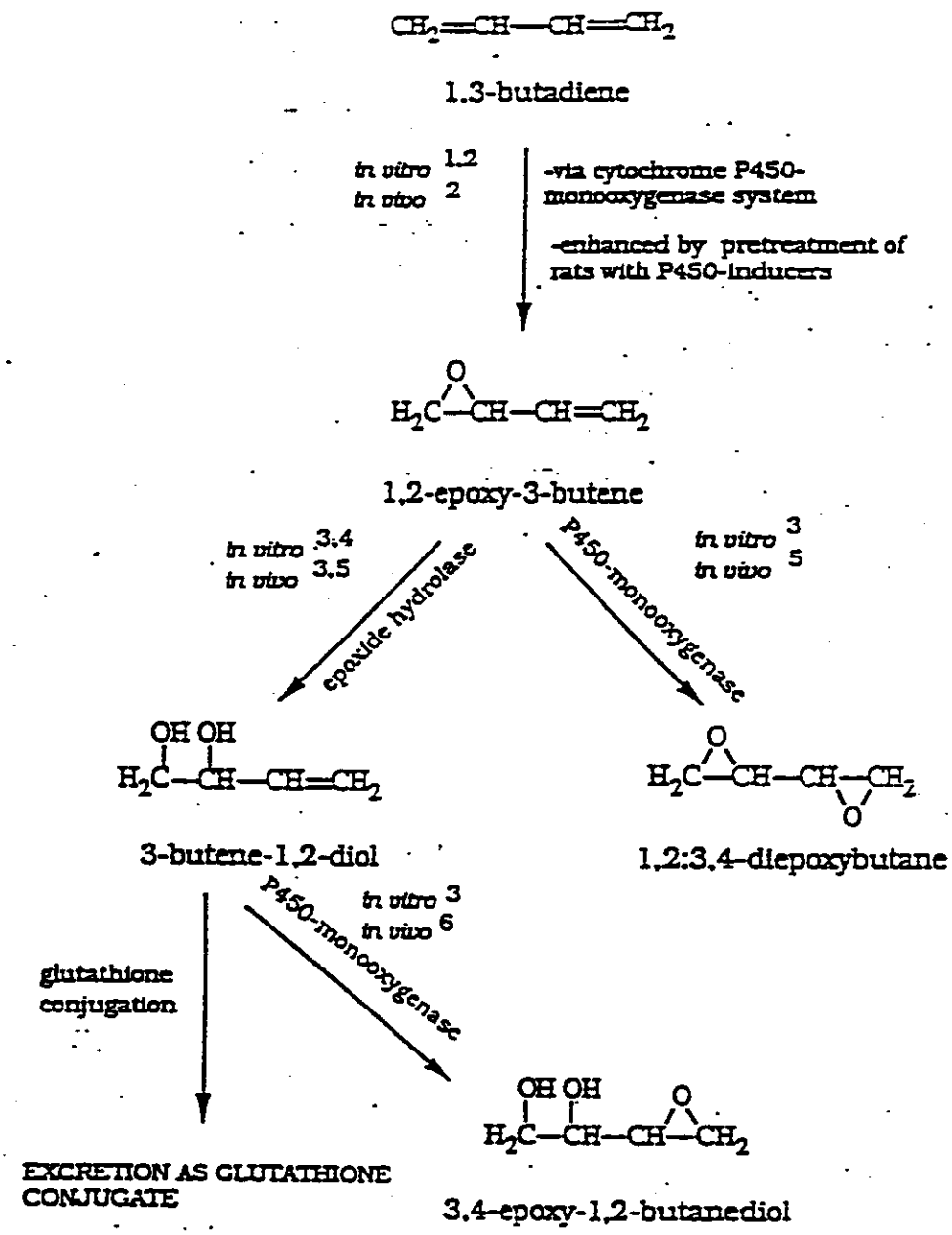
	1 hr[b]		8 hr		67 hr	
	Rats	Mice	Rats	Mice	Rats	Mice
Adrenal Glands	210 ± 24 (0.1)	76 + 6 (6)	120 + 11 (0.07)	204 + 133 (17)	45 + 10 (0.03)	9 + 2 (0.8)
Bladder	950 ± 380 (0.6)	1300 + 660 (19)	63 ± 5 (0.04)	1900 ± 1200 (1)	22 ± 6 (0.0-1)	4 ± 0.6 (0.3)
Blood	124 ± 24 (0.07)	49 ± 3 (4)	116 ± 20 (0.7)	43 ± 17 (4)	20 ± 4 (0.01)	4 ± .06 (0.3)
Heart	120 ± 8 (0.07)	47 ± 1 (4)	83 ± 4 (0.05)	43 ± 17 (4)	20 ± 5 (0.01)	6 ± 0.3 (0.5)
Kidney	390 ± 25 (0.2)	180 ± 18 (15)	190 ± 5 (0.)	110 ± 34 (9)	39 ± 8 (0.02)	8 ± 0.5 (0.7)
Large Intestine	170 ± 20 (0.1)	130 ± 70 (11)	150 ± 20 (0.08)	160 ± 53 (13)	24 ± 6 (0.01)	5 ± 0.3 (0.4)
Liver	330 ± 55 (0.2)	120 ± 9 (10)	240 ± 7 (0.1)	120 ± 45 (10)	47 ± 8 (0.03)	9 ± 0.6 (0.8)
Lung	200 ± 18 (0.1)	96 ± 7 (8)	100 ± 1 (0.06)	95 ± 45 (8)	23 ± 5 (0.01)	4 ± 1 (0.3)
Mammary Glands	140 ± 29 (0.08)	30 ± 7 (2)	48 ± 11 (0.03)	150 ± 82 (13)	12 ± 4 (0.007)	4 ± 0.3 (0.3)
Turbinates	300 ± 35 (0.2)	67 ± 4 (6)	180 ± 7 (0.1)	23 ± 4 (2)1	56 ± 12 (0.03)	9 ± 0.8 (0.7)
Pancreas	320 ± 31 (0.2)	160 ± 25 (13)	200 ± 18 (0.1)	220 ± 110 (18)	20 ± 5 (0.01)	4 ± 0.5 (0.4)
Small Intestine	320 ± 67 (0.2)	300 ± 120 (24)	180 ± 7 (0.1)	130 ± 52 (10)	21 ± 3 (0.01)	4 ± 0.2 (0.3)
Subcutaneous Fat	220 ± 97 (0.1)	31 ± 4 (3)	41 ± 9 (0.01)	170 ± 83 (14)	9 ± 2 (0.005)	4 ± 0.6 (0.3)
Thyroid Gland	490 ± 190 (0.3)	60 ± 11 (5)	86 ± 0.8 (0.05)	62 ± 22 (5)	37 ± 10 (0.02)	12 ± 6 (1)
Trachea	260 ± 130 (0.2)	59 ± 17 (5)	77 ± 6 (0.04)	67 ± 37 (6)	24 ± 6 (0.01)	6 ± 0.6 (0.5)
Bone Marrow	10 ± 2	0.5 ± 0.2	10 ± 3	0.2 ± 0.02	3 ± 0.8	0.05 ± 0.0086

a Values are the mean nmole ^{14}C-butadiene equivalents/g±SE. Values in parentheses are the mean nmole ^{14}C-butadiene equivalents/g tissue/ umole butadiene inhaled. Values for blood are per mL.

b Time after end of exposure.

Values are mean nmole ± SE

Table taken directly from Bond et al. 1987.



1. Malvoisin et al. 1979
2. Bolt et al. 1983
3. Malvoisin and Roberfroid 1982
4. Schmidt and Loeser 1985
5. Bond et al.1986
6. Kreiling et al. 1987

Figure 1. Metabolism scheme for 1,3-butadiene.

mouse liver DNA showed the formation of 7-N-(1-hydroxy-3-buten-2-yl)guanine and 7-N-(2,3,4-trihydroxybutyl)guanine the reaction products of BMO and DEB with guanosine. Analysis of rat liver DNA did not show the trihydroxy butyl-guanine adduct. At exposure concentrations of 250 ppm and above, mouse tissues showed the occurrence of protein-DNA and DNA-DNA crosslinks. These crosslinks were not observed in rat tissues. Contrary to this latter finding in mice Ristau et al. (1990) found no evidence of DEB-induced DNA-DNA crosslinks fomation in purified DNA from the livers of BD-treated mice or rats.

Schmidt and Loeser (1985; 1986) compared the ability of liver microsomes from several species to metabolize BD to BMO. Microsomes from male and female B6C3F1 mice, Sprague-Dawley rats, human (one sex only, not specified), and the rhesus monkey were all capable of forming the epoxide. However, the rates differed considerably. Hepatic microsomes from female mice had the highest activity, followed by male mice, male rats, female rats, human (n=1), and the rhesus monkey. (Male and female activities were the same for the monkey.) The ratio of mice to monkeys was approximately 7:1. The ability of lung tissue microsomes from these species to form the epoxide was also tested, but no measurable activity was found for either human or monkeys and the rodent values were very low (mouse lung was 1/30 mouse liver).

Elfarra et al. (1991) have observed that NADPH - dependent oxidation of 1,3-butadiene by mouse liver microsomes or H_2O_2-dependent oxidation by chloro peroxidase produced both butadiene monoxide and crotonaldehyde. The ratio of BMO to crotonaldehyde was about 50 in microsomal preparations.

Bond et al., (1988) examined the effect of repeated inhalation exposure of rats and mice to 1,3-butadiene on the metabolism of BD by lung and liver microsomes in these species. Male Sprague-Dawley rats and $B6C3F_1$ mice were exposed by nose-only to 0 (air) or 7600 ppm BD and 740 ppm BD respectively for 6 hours/day for 5 days after which the tissues were removed from the animals and microsomes prepared. The authors observed a statistically significant ($p<.05$) depression relative to control preparations in the rate of 1,3-butadiene metabolism in microsomes from lungs of both rats and mice. There was no effect noted on liver or nasal tissue (rat only) metabolism of BD. The data indicate that interspecies differences in carcinogenic susceptability are unlikely to be the result of an inductive or inhibitory effect of BD on its own metabolism, at least in the tissues studied.

The total amount of ^{14}C accumulated on a normalized body-weight or surface-area basis (Bond et al., 1986) indicated that mice accumulated a larger amount of BD and metabolites than rats, in agreement with the predictions of Kreiling et al. (1986). Urine and exhaled air were the major elimination routes for ^{14}C in both species. In mice, exhalation of CO_2 accounted for ≈20% of the total ^{14}C eliminated or retained in the carcass, and about 12% remained in the carcass 65 hours postexposure. At lower concentrations analysis of bubbler traps indicated that all ^{14}C present in traps was accounted for as metabolites including BMO and DEB. In rats as in mice, urine and exhaled air were the major routes of elimination although the percentage of exhaled radioactivity as CO_2 increased considerably at 13,000 μg/L (7100 ppm) (Table 2-5).

Table 2-5 Distribution of ^{14}C in Urine, Feces, Exchaled Air, and Carcass of Rats and Mice Expposed to Different Concentrations of Butadiene at 65 hr after a 6-hr Exposure[a]

	Exposure concentration (ug/l)	Urine	Feces	Exhaled Air		Carcass
				CO_2	Butadiene and Other Volatile Metabolites	
Rats	130	8 ± 0.4 (49)	0.8 ± 0.2 (5)	2 ± 0.5 (12)	3 ± 0.3 (15)	3 ± 0.4 (20)
	1,800	21 ± 4 (31)	1 ± 0.2 (2)	16 ± 0.2 (24)	15 ± 1 (23)	13 ± 0.4 (19)
	13,000	19 ± 2 (8)	4 ± 0.8 (1)	124 ± 4 (51)	62 ± 12 (26)	35 ± 1 (14)
Mice	13	0.4 ± 0.1 (39)	0.06 ± 0.01 (7)	0.2 ± 0.01 (22)	0.2 ± 0.02 (22)	0.09 ± 0.02 (10)
	130	1.6 ± 0.3 (50)	0.4 ± 0.05 (11)	0.1 ± 0.01 (4)	0.6 ± 0.2 (19)	0.5 ± 0.05 (16)
	1,800	7 ± 0.4 (38)	0.6 ± 0.2 (3)	3 ± 0.1 (17)	6 ± 1 (31)	2 ± 0.1 (11)

[a] Values are the mean umol ± SE. Numbers in parentheses are percentage of total ^{14}C absorbed and retained at the end of the 6-hr exposure.

Table taken directly from bond et al. 1986.

There were no differences in the $t_{1/2}$'s for urinary or fecal excretion regardless of exposure concentration for either species. The $t_{1/2}$ for urinary excretion in rats and mice was similar, 5.6 ± 1.6 hours and 4.6 ± 1.5 hours, respectively; however, the $t_{1/2}$ for fecal excretion was much longer in rats than mice, 22 ± 2.6 hours vs 8.6 ± 2.6 hours, respectively.

Sun et al. (1989) and Dahl et al. (1990) have reported on the comparative metabolism and disposition of BD in monkeys (Macaca fasciculanis) and rodents. The normalized uptake and retention values for BD were 3.3, 0.46, and 0.52 μmole/hr/10 ppm/kg body weight in mouse, rat, and monkey respectively. When these values were expressed in terms of body weight to the 2/3 power, a typical method of interspecies scaling, the resulting values were 0.99, 0.35, and 0.94 respectively.

In summary, ^{14}C radiolabel was widely and rapidly distributed to all tissues examined following exposure of rats or mice to [^{14}C] BD and species differences in specific tissue accumulations of ^{14}C were not observed. Consequently, these data do not explain the differences between rats and mice with respect to the target tissues of 1,3-butadiene induced carcinogenicity, or in its carcinogenic potency. (See Carcinogenicity Section 3.5 below for details.)

A first-order elimination pattern was observed for BMO in Sprague-Dawley rats following exposure to the epoxide in a closed inhalation chamber at concentrations up to 5000 ppm; thus saturation of BMO metabolism was not observed (Filser and Bolt, 1984; Kreiling et al., 1987). Mice were found to reach metabolic saturation at a much lower concentration of BMO, ≈500ppm (Kreiling et al., 1987). The estimated metabolic V_{max} at saturation levels of BMO was 350 μmol/h-kg for mice and was > 2600 μmol/h-kg for rats. At the lower levels where first order elimination kinetics applied, mice had a lower metabolic elimination rate than rats by approximately a factor of five. The key finding in their study was that the steady state concentration of BMO in the mouse was about 10-fold greater than in the rat.

When BD metabolism was saturated, BMO was exhaled by both species (Bolt et al., 1983; Kreiling et al., 1987). In addition, when exposed to concentrations of butadiene above 2000 ppm, the hepatic, non-protein sulfhydryl compounds (NPSH, e.g. glutathione) rapidly became depleted in the mouse falling to 20% after 7 h and 4% after 15 h. In the rat depletion was much less, 65-80% of control value after 7 h, and appeared to stabilize (Kreiling et al., 1987, 1988). A further camparison of both species at several concentrations and tissues showed that a dose dependent NPSH depletion was observed in mouse lung, heart, and liver. In rats NPSH depletion is only seen at high exposure concentrations (Deutschmann and Laib, 1989).

The two main routes for detoxification of BMO *in vivo* were conjugation with glutathione-S-transferase and cleavage of the epoxide with epoxide hydrolase to form 3-butene-1,2-diol. (Both pathways have also been shown to occur *in vitro*.) Although these enzyme systems were present in both species, the major route of epoxide detoxification may differ between rats and mice. Mouse liver glutathione-S-transferase had a high affinity for BMO while mouse epoxide hydrolase levels were low and rats had high epoxide hydrolase levels (Schmidt and Loeser, 1985). These differences may have accounted for the fact that BD and BMO metabolic capacities were saturable at lower exposure concentrations

in the mouse than in the rat, and that glutathione depletion was observed in the mouse.

The rat appeared to readily metabolize BMO as was shown by Bolt et al. (1984). This assumption was based on the fact that the actual production rate of the epoxide metabolite (63 μmol/h-kg) was only 29% of the predicted production rate based on the V_{max} for BD metabolism (220 μmol/h-kg). However, since no reference is made to other BD metabolites in this study, the rapid disappearance of BMO may not be due solely to a detoxification reaction.

3.0 HAZARD EVALUATION

3.1 Acute Toxicity

1,3- Butadiene is only mildly acutely toxic. For rats and mice, the median lethal concentrations (LC_{50}) of 1,3-butadiene inhalation are above 100,000 ppm for 2 to 4 hours inhalation. The oral LD_{50} values for rats and mice are 5.48 g/kg and 3.21 g/kg, respectively. Acutely toxic effects of exposure in animals progressed from light anesthesia, to running movements and tremors, to deep anesthesia and death (NIOSH, 1984). Acute toxicity is not expected to occur at ambient levels of butadiene.

3.2 Subchronic and Chronic Toxicity

The subchronic and chronic studies indicate that minor adverse effects can result from prolonged exposure to 0.45 ppm (1 mg/m^3). This concentration is more than 200-fold greater than the ambient levels. Thus, noncarcinogenic effects are not expected to occur at ambient levels of approximately 0.002 ppm.

The following information is taken directly from EPA's 1985 review of butadiene: "A 3-month toxicity study in rats preceded the 2-year chronic inhalation toxicity study conducted at Hazleton Laboratories Europe, Ltd. in England (1981a), and sponsored by the International Institute of Synthetic Rubber Producers, Inc. (IISRP). Further details of the chronic investigation as well as the results with regard to the carcinogenicity of BD are presented in the carcinogenicity section (3.5) of this document. The airborne concentrations of 1,3-butadiene used in the 3-month study were 1,000, 2,000, 4,000, and 8,000 ppm; a group exposed to filtered air (0 ppm) served as controls. The authors considered that there were no effects attributable to exposure to the test chemical on growth rate, food consumption, hemograms, blood biochemical investigations, or pathological evaluation. The only effect the investigators considered to be related to BD exposure was a moderate increase in salivation, particularly among female rats during the last 6 to 8 weeks of exposure at the higher airborne concentrations (Crouch et al., 1979). These results are consistent with an earlier study by Carpenter et al. (1944) in which the investigators found only a slight reduction in body weight gain among rats and guinea pigs exposed for 8 months, 7.5 hours/day, 6 days/week, to a airborne concentration of 8,000 ppm. These authors noted no effects among animals exposed at 600 and 2,300 ppm.

"Preliminary inhalation toxicity studies in mice were used as a basis for dose selection for the chronic studies of B6C3F1 mice, conducted at Battelle Pacific Northwest Laboratories and sponsored by the NTP (1984). Further details of the chronic exposure and the results with regard to the carcinogenicity of 1,3-butadiene are presented in the section 3.5 of this document. Two range-finding studies, a 15-day and a 14-week study, were conducted at International Bio-Test Laboratories. In the 15-day study, weight loss at airborne concentrations of 1,250 ppm was observed. Even the mice exposed to 8,000 ppm, the highest airborne concentration, survived the exposure period. In the 14-week study, reduced body weight and death were observed among mice treated at 2,500 ppm or more. Necropsy findings were not reported (NTP 1984).

"Miller (1978) reviewed a series of papers from Russian investigators, particularly Ripp (1967), and summarized the subchronic toxic effects in rats. Ripp (1967) exposed rats to airborne 1,3-butadiene concentrations of 0.45 ppm, 1.4 ppm, 13.5 ppm (1, 3, and 30 mg/m^3 where 1 mg/m^3 = 0.45 ppm). The highest concentration in this study (equivalent to 13.5 ppm) is only about 1/50 of the lowest concentration (600 ppm) in any of the other studies reported in this section. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor activity was decreased to 60% of the pre-exposure rate. Histopathological evaluation revealed no changes at 0.45 ppm except for congestion in the spleen and hyperemia and leukocyte infiltration in the cardiac tissue. The changes in the cardiac tissues were more marked at the higher levels with hemorrhage and reduced cellular RNA reported at the highest concentration. At 1.4 and 13.5 ppm, atelectasis, interstitial pneumonia, and emphysema were noted in the lung tissue. These results, showing an adverse response at such low levels, may indicate the continuous exposure is more hazardous than intermittent exposure, the regimen used in all other investigations. An alternative explanation for these findings at such low levels is that Ripp (1967) examined more sensitive indicators of toxicity than other investigators.

"A 2-year chronic inhalation toxicity study of the effects of airborne concentrations (1,000 and 8,000 ppm) of 1,3 butadiene on rats was conducted at Hazleton Laboratories Europe, Ltd. in England (HLE, 1981a) and was sponsored by the IISRP. Among rats exposed to 8,000 ppm, clinical signs, consisting of excessive secretion from the eyes and nose plus slight ataxia, were observed between months 2 and 5 of the study. Variations in mean body weight suggested no consistent adverse effect. Review of the hemograms, blood chemistry, urine analysis, and behavioral testing likewise did not indicate an adverse effect. In female rats exposed to either 1,000 or 8,000 ppm, subcutaneous masses appeared earlier and at higher incidences than in the control. A dose-related increase in liver weights was observed among rats at the necropsy performed at 52 weeks and among those killed at the termination of the study. This could indicate that the chemical induces liver enzymes. Otherwise, no significant changes were noted at the 52-week kill. Increased alveolar metaplasia and nephropathy were observed among males of the 8,000 ppm treatment groups at the termination of the study. Marked or severe nephropathy occurred in 27% of the male rats in the high-dose group as compared with 9% to 10% in the control and the low-dose groups. The author considered nephropathy to be the cause of some of the early deaths in this study.

"A lifetime chronic inhalation study in B6C3F1 mice at 1,3-butadiene concentrations of 625 and 1,250 ppm administered for 6 hours/day, 5 days per week, was sponsored by the NTP (1984). The exposures were prematurely terminated after 61 weeks due to deaths resulting largely from the development of cancer. No increases in clinical signs could be associated with exposure to the test chemical. In addition to the neoplastic changes described in the carcinogenicity chapter, testicular atrophy (control-0/50, 625 ppm-19/47, 1,250 ppm-11/48) and ovarian atrophy (2/49, 40/45, 40/48) were elevated among mice at both doses. Furthermore, among male mice, there was a significant increase in liver necrosis at both doses. In female mice, liver necrosis was significantly elevated only at the higher concentration. While neoplastic lesions of the nasal cavity were not found at any dose, there was an increase in non-neoplastic changes at the high-dose. At 1,250 ppm, chronic inflammation of the nasal cavity (male, 33/50; female, 2/49), fibrosis (male

35/50; female, 2/44), cartilaginous metaplasia (male, 16/50; female 1/49), osseous metaplasia (male, 11/50; female, 2/49), and atrophy of the sensory epithelium (male, 32/50) were observed. No non-neoplastic lesions of the nasal cavity were found in the controls (NTP, 1984). Huff et al. (1985) have suggested that the lack of neoplasms in the nasal cavity as compared to the lungs may reflect a requirement for biotransformation of 1,3-butadiene to a reactive epoxide metabolite. The nasal cavity changes then suggest that the intact molecule may have some adverse effects at 1,250 ppm. However, as discussed in the metabolism and carcinogenicity chapters, exposure to 1,3-butadiene did not decrease minute volume, as occurs with other respiratory irritants.

"In summary, since a no-effect dose has not been established for the non-carcinogenic chronic toxicity, further investigations are warranted. These investigations should focus on the liver, testes, and ovaries because in mice these tissues are adversely altered at the lowest concentrations. In addition, the minimum effect dose for cardiac and respiratory tract changes needs to be further explored. For a complete understanding of the toxicity, non-rodent species might be used."

3.3 Reproductive and Developmental Toxicity

3.3.1 Reproductive Effects

An NTP-sponsored sperm morphology study was conducted in B6C3F1 mice following a 5-day inhalation exposure to 1,3-butadiene at 0, 200, 1000, or 5000 ppm, 6 hours/day (Hackett et al., 1988b; Morrissey et al., 1990). Mice were sacrificed 5 weeks after the exposure period and sperm suspensions obtained from the cauda epididymis. The 5-day exposure period to 1,3-butadiene increased the percentage of abnormal sperm heads in a dose-related fashion: 0 ppm, 1.61 $\pm$ 0.38; 200 ppm, 1.95 $\pm$ 0.11; 1000 ppm, 2.79 $\pm$ 0.18; and 5000 ppm, 3.69 $\pm$ 0.42. The values were significantly greater than the control group for both the 1000 and 5000 ppm groups ($p<0.05$). These abnormalities occurred in the absence of any effect on body weight during the 5-week post-exposure period. The collection of sperm at only one post-exposure time period precludes any statements regarding the temporal effects of 1,3-butadiene on sperm development. However, results at 5-weeks post-exposure indicate that late spermatogonia and/or early spermatocytes were sensitive to the effects of 1,3-butadiene. A conclusion as to the reproductive consequences of these abnormalities cannot be made from this study.

The NTP also sponsored a dominant lethal assay in Swiss (CD-1) mice (Hackett et al., 1988a; Morrissey et al., 1990). Males were exposed to 0, 200, 1000 or 5000 ppm BD for 5 days, 6 h/day, then mated to several pairs of females, a new pair each week for 8 weeks. No mortality or changes in body weight were observed in the males. Statistically significant adverse effects were seen in matings from the first 2 weeks postexposure; none were seen thereafter. With the week 1 matings, there was a significant increase in the ratio of dead implants (resorptions) to total implantations in the 1000 ppm group; increases in the other dose groups were not statistically significant. The number of females with 2 or more dead implants was significantly higher in the 200 and 1000 ppm groups, and the number of dead implants per pregnancy was significantly elevated in the 1,000 and 5,000 ppm groups. With the week 2 matings, the number of dead implants per pregnancy was significantly elevated in the 200 and 1000 ppm groups.

BD's effects in this assay were most pronounced with 1000 ppm exposure; no steadily increasing dose-response relationship was observed. This may reflect a biological phenomenon, such as induction of detoxifying enzymes at the high dose level, or random variation within the experiment. These findings signal that BD may have a mutagenic effect in male germ cells, particularly more mature cells (spermatozoa and spermatids) (Morrissey et al., 1990). It is important to note that such a mutagenic effect might not be characterized by a dose threshold below which we would not expect the effect to occur. No NOEL was established by this assay.

In 2-year inhalation studies mice were exposed to 0, 6.25, 20, 62.5, 200, and 625 ppm BD (Huff et al., 1985; Melnick et al., 1988, 1989; Melnick, Huff and Miller, 1989; Miller, 1989; Miller, Melnick and Boorman, 1989). The studies were designed as cancer bioassays and are reported in greater detail in section 3.5. Gonadal atrophy was observed at a high incidence in exposed animals of both sexes at levels of 200 ppm and above, but not in any of the control animals. In the later study, using the entire dose range, levels of 6.25 ppm and higher also produced gonadal atrophy in females. Thus, a NOAEL was not established in these studies, but a LOAEL of 6.25 ppm was observed. In contrast, the Hazleton rat bioassay (HLE, 1981a) did not report any reproductive effects even at the 8000 ppm level.

3.3.2 Developmental Toxicity

The earliest reproductive study reported on BD was conducted by Carpenter et al. (1944). In this study male and female rats were exposed to 600, 2300, or 6700 ppm 1,3-butadiene, 7.5 hours/day, 6 days/week, for an 8-month period. Although this study was not specifically designed as a reproductive study, the fertility and the number of progeny were recorded for the rats. No significant effects due to BD exposure were noted on either the number of litters per female or on the number of pups per litter.

In a teratology study conducted by Hazleton Laboratories, Europe (1981), female Sprague-Dawley rats were exposed for 6 hours/day to 0, 200, 1000, and 8000 ppm 1,3-butadiene from 6 to 15 days of gestation (dg). Dams exhibited a significant exposure-correlated reduction in weight gain during the exposure period, and reductions were significant for the two highest exposure concentrations. The number of fetuses per litter and the number of live fetuses per litter were not affected. Post-implantation loss was increased slightly, but not significantly, and not in an exposure-related fashion. There was a significant reduction in fetal body weight for the 8000 ppm group, and a significant increase in the incidence of fetal variations in the 200 ppm group, while exposure to 8000 ppm increased the incidence of hematomas and major skeletal defects. The authors concluded that the fetal response was not indicative of a teratogenic effect, but was a result of maternal toxicity.

The potential for the inhalation of BD during gestation to cause developmental toxicity was further assessed in Sprague-Dawley rats (Hackett et al. 1987a) and Swiss (CD-1) mice (Hackett et al. 1987b). Dams were exposed to 0, 40, 200, or 1000-ppm 1,3-butadiene on 6-15 dg, 6 hours/day, and killed prior to parturition (e.g., rats on 20 dg and mice on 18 dg). Exposure to these concentrations of BD did not result in significant maternal toxicity in the rat, with the exception of a reduction in the extra-gestational weight gain for the 1000 ppm dams ($p<0.05$). There were no effects on the reproduction

indices (e.g., number of live fetuses per litter or the number of intrauterine deaths), on fetal body weights, or on the incidence of malformations, variations, and reduced ossifications. Under these exposure conditions there was no evidence of developmental toxicity due to BD exposures in the Sprague-Dawley rat. The results of this study are basically in agreement with those reported by Hazleton Laboratories, Europe (1981).

Exposure to these concentrations of BD did not result in significant maternal toxicity in the mouse, with the exception of a reduction in the extra-gestational weight gain for the 200 and 1000 ppm dams ($p<0.05$). There were no effects on the reproduction indices (e.g. number of live fetuses per litter or the number of intrauterine deaths), although there was a concentration-related, statistically significant reduction in fetal body weights for both sexes. There was no exposure-related increase in the incidence of fetal malformations; however, the incidence of reduced ossifications was increased in the 200- and 1000-ppm groups ($p<0.05$). The increased incidence of reduced ossifications and the fetal weight reductions in the absence of apparent maternal toxicity in the 40- and 200-ppm groups is evidence of fetotoxicity, but not teratogenicity, in the Swiss (CD-1) mouse.

3.4 Genetic Toxicity

3.4.1 Mutagenicity

1,3-Butadiene was originally reported to be a direct-acting mutagen to *Salmonella typhimurium* strains TA1530 and TA1535 which had been grown in an atmosphere of 70% BD (de Meester et al., 1978). However, later reports by the same group did not confirm their earlier efforts. BD was later found to be mutagenic to strains TA1530 and TA1538 (strains susceptible to base-pair substitution mutations), but only in the presence of the supernatant (S-9) fraction of rat liver homogenate (de Meester et al,. 1980; de Meester, Mercier and Poncelet, 1981). Furthermore, mutagenic activity was only significant when the S-9 mix was obtained from rats previously exposed to phenobarbital or Arcolor 1254, both cytochrome P450 monooxygenase inducers. BD was not mutagenic in the presence of S-9 mix from uninduced rats, nor was it mutagenic to strains TA100, TA98, or TA1538 (strains susceptible to frame-shift mutations) either in the presence or the absence of S-9 mix. Interestingly, the addition of cytochrome P450-dependent mixed-function-oxidase system inhibitors did not alter the mutagenic activity of BD to TA1530 (de Meester, Mercier, and Poncelet, 1981). This is in contrast to the results of Malvoisin et al. (1979) who found SKF 525A, a cytochrome-P450 inhibitor, to strongly inhibit the formation of BMO, the putative proximate mutagen (see below). The addition of glutathione or N-acetyl cysteine, deactivators of electrophilic intermediates, strongly decreased the mutagenic activity of BD, presumably due to the removal of BMO. Butadiene was also mutagenic when the S-9 preparation was placed in a separate petri dish within the same closed exposure container, thus indicating that the S-9 mix metabolized BD to a volatile, mutagenic metabolite. 1,2-Epoxy-3-butene (BMO), the first metabolite of BD (Malvoisin et al., 1979), was found to be a direct-acting mutagen. *S. typhimurium* strains TA1530 and TA1535 were the most sensitive, followed by strain TA100 (de Meester et al., 1980). The activity of 1,2-epoxy-3-butene in these strains, and the absence of activity in strains TA1537, TA1538 and TA98, indicated that DNA base-pair substitutions were probably involved in the mutagenic action.

Another butadiene metabolite, 1,2:3,4-diepoxybutane (DEB), has been shown to be a potent mutagen in several systems: the *S. typhimurium* mammalian microsome assay (McCann et al., 1975; Gervasi et al., 1985), assays employing the fungi *Neurospora crassa* and *Saccharomyces cerevisiae* (Olzewska and Kilbey, 1975; Polakowska and Putrament, 1979; Zuk et al., 1980), and in Luria and Delbruck's fluctuation test with *Klebsiella pneumoniae* (Voogd, Stel and Jacobs, 1981). Interestingly, in *Saccharomyces cerevisiae* nuclear, but not mitochondrial, DNA was affected (Polakowska and Putrament, 1979). A suitable explanation of this phenomenon was not presented.

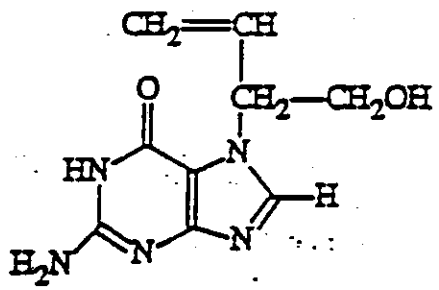
In addition to its mutagenicity, DEB has been shown to activate the enzyme guanylate cyclase which catalyzes the formation of the nucleotide guanosine 3'5'-monophosphate (cyclic GMP) (Vesely and Levey, 1978). Experiments were performed on the supernatant of a centrifuged (37,000 g) homogenate of various male Sprague-Dawley rat tissues and an increase in guanylate cyclase activity was observed in all tissues examined: bladder > kidney > heart > liver > spleen > stomach > pancreas > colon. The activation of guanylate cyclase may play a role in the regulation of cell growth and differentiation.

3.4.2 DNA Alkylation

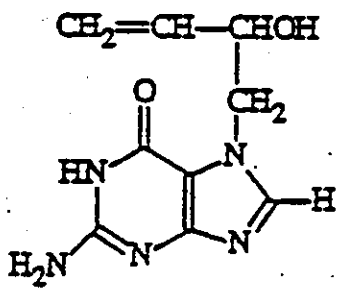
A considerable amount of research has gone into identifying the details of the interaction of these direct-acting mutagenic epoxides with DNA, particularly with respect to the alkylation of the N-7 site of deoxyguanosine. The capabilities of the two BD metabolites, BMO and DEB, to alkylate the N-7 site of the guanine moiety of DNA have long been known and have been studied extensively (Brooks and Lawley, 1961a, 1961b).

In a recent account, Gervasi et al. (1985) examined the correlation between the direct-acting mutagenic activity and the alkylating ability toward the N-heteroatom of nicotinamide of known and possible epoxide intermediates of BD metabolism. Of the three epoxides studied, BMO, DEB, and 1,2-epoxybutane, DEB was the strongest mutagen toward *S. typhimurium* strain TA100 followed by BMO, and finally 1,2-epoxybutane. 1,2-Epoxybutane has not been reported as a metabolite of 1,3-butadiene either *in vivo* or *in vitro*. None of the epoxides demonstrated mutagenic activity in strain TA98, used to detect frame-shift mutations. The relative N-alkylation rates of these compounds toward nicotinamide closely followed the order of their mutagenic activities. These compounds have been shown to alkylate the N-heteroatom through a mixed SN_2/SN_1 nucleophilic substitution mechanism, primarily SN_2 (Ehrenberg and Hussain, 1981).

The *in vitro* interactions of BMO with the N-heteroatom of guanosine (GUO), deoxyguanosine (d-GUO), or DNA were studied by Citti et al. (1983). When BMO was reacted with GUO in acetic acid at 50°C, followed by acid catalyzed hydrolysis to break the N-9 glycosyl bonds, two adducts were identified, 7-(2-hydroxy-3-buten-1-yl) guanine (I) and 7-(1-hydroxy-3-buten-2-yl) guanine (II); the latter was the major product (Figure 2). Reaction of BMO with deoxyguanosine (d-GUO) or with the d-GUO moiety of intact DNA resulted in the formation of the same two adducts; adduct II was the major product in each case. Formation of the adducts appeared to proceed via an SN_2-type mechanism where attack by the nucleophilic N-7 heteroatom occurred at either the C-1 or C-2 carbon. Since a pure SN_2-type mechanism should have resulted in the formation of approximately equal amounts of each adduct, the production



ADDUCT I



ADDUCT II

Figure 2. Guanosine adducts.

of greater amounts of II than I led to the proposal of a mixed SN_1/SN_2-type mechanism (Citti et al., 1984). Studies on the depurination rates of the adducted DNA formed from reaction with 1,2-epoxy-3-butene determined that the rate of spontaneous depurination obeyed first order kinetics with adducts I and II being released at equal rates (Citti et al., 1984). The $t_{1/2}$ for this reaction was ≈50 hours. Thus, the mutagenicity of 1,2-epoxy-3-butene may result from the relative ease of depurination which could subsequently lead to the formation of apurinic sites in the DNA and eventually to strand breaks. These strand breaks are capable of causing *in vivo* replication blocks and provoking error prone repair which could result in mutations (Schaaper, Glickman, and Loeb, 1982).

The *in vivo* alkylation of DNA following inhalation exposure of male Wistar rats or B6C3F1 mice to 1,3-[1,4-^{14}C]-butadiene was shown by Kreiling, Laib, and Bolt (1986). Four rats or 24 mice were exposed to 1,3-butadiene (≈700 ppm maximum concentration) in a closed system until 98% of the radioactivity had been taken up (6.6 hours for rats; 4 hours for mice) and then killed 30 min later. Nucleoproteins and DNA were obtained from purified hepatic nuclei and analyzed for ^{14}C-content. The nucleoproteins of the mice were found to contain approximately two times as much radioactivity as the rat nucleoproteins, values which correlated with the relative metabolic rates for 1,3-butadiene previously observed in the two species (see above). Hepatic DNA was alkylated in both species as shown by the presence of ^{14}C in the DNA fractions; however, unlike alkylation of the nucleoproteins, alkylation levels for DNA were equivalent in both rats and mice. In a subsequent report 7-(1-hydroxy-3-buten-2-yl)guanine, a product of reaction of BMO with guanine, was identified in liver DNA of mice exposed to BD (Laib and Kreiling, 1987).

3.4.3 Sister-Chromatid Exchange and Chromosomal Aberrations

BMO was found to be a powerful inducer of *in vivo* chromosome damage in the C57BL/6 male mouse (Sharief et al., 1986). Thirty minutes after subcutaneous implantation of a 5-bromodeoxyuridine (BrdUrd) tablet to label DNA, mice were administered a single IP injection of 1,2-epoxy-3-butene in corn oil at the following doses: 0 (vehicle control), 25, 50, 100, or 150 mg/kg. Colchicine (2.2 mg/kg) was administered 22 hours after BrdUrd and animals were sacrificed 2 hours later. Three of the four animals in the highest dose group died; however, all animals in the lower dose groups survived and apparently did not exhibit overt symptoms of toxicity. (The 25, 50, and 100 mg/kg doses were referred to as "nontoxic" by the authors.) Bone marrow cells from exposed animals were examined for sister chromatid exchange (SCE) frequency in second division cells and for chromosomal aberrations (CA) in first division cells, and the mitotic indices (MI) were determined. Statistically significant and dose-related increases in the SCE and CA frequencies ($p<0.001$) were observed. The MI was found to be increased for the 25 and 50 mg/kg doses, approximately equal to controls for the 100 mg/kg dose, and extremely low for the one remaining animal in the 150 mg/kg group. Chromosome damage was evident at the lowest dose, 25 mg/kg, as an increase in both CA and SCE frequencies.

BMO has also been shown to be a direct-acting mutagen (see above); however, it cannot be determined from Sharief et al. (1986) whether it was the proximate mutagen, or whether it may have first been metabolized to DEB. DEB has been

reported to induce high levels of SCEs in mice following *in vivo* exposure to this bifunctional alkylating agent (Conner, Luo, and de Gotera 1983), and to induce SCEs and CAs in hamster cells *in vitro* (Dean and Hodson-Walker, 1979; Perry and Evans, 1975).

Marx et al. (1983) studied the ability of DEB to induce chromosomal breakage in cultured human bone marrow cells or lymphocytes. This capability has been used as a diagnostic tool to identify patients either homo- or heterozygous for Fanconi's anemia (FA). Diepoxybutane induces a significant and dose-related increase in the incidence of chromosomal breakage in primary cultures of both human bone marrow cells and lymphocytes (Marx et al., 1983) as well as DNA cross-linking (Lawley and Brooks, 1967). The degree of induction of chromosome breakage by DEB in lymphocytes from patients homozygous for FA was approximately three times greater than it was in control lymphocytes ($p<0.001$). It was also possible to identify patients that were heterozygous for FA with this assay, a finding in contrast to Cohen et al. (1982) who were unable to distinguish between heterozygous FA lymphocytes and control patients in long term lymphoid cell lines. Interestingly, it has been reported that diepoxybutane is ineffective in increasing the SCE frequency in peripheral lymphocytes from patients either homo- or heterozygous for FA, although it did increase SCE frequency in cells from control patients (Porfiro et al., 1983).

Diepoxybutane has also been shown to be genotoxic in the mei-9^a Drosophila chromosome breakage test as evidenced by induced chromosomal loss and partial chromosomal loss in the offspring of both excision repair deficient mei-9^a females and repair proficient females (Zimmering, 1983).

Diepoxybutane tested positive in three in vitro mammalian cell-transformation assays using Balb/3T3 cells, Syrian hamster embryo cells (SHEM), and Fischer 344 rat embryo cells infected with Rauscher murine leukemia virus (Dunkel et al., 1981). No exogenous enzyme-activating systems were added to the cultures and any activation of procarcinogens resulted from the innate metabolic capabilities of the cell lines. The lowest concentration of DEB in the culture medium that induced transformation was 0.008 μg/ml for Balb/3T3 cells, 0.05 μg/ml for SHEM cells, and 0.17 μg/ml for R-MuLV-RE cells. Induction of cell transformation at these low concentrations, plus the fact that it was positive in all three assays, made DEB one of the more potent carcinogens of the ≈45 compounds tested.

3.4.4 In Vivo Sudies Including Hematotoxicity

The potential role of DEB induced DNA cross-links and monoadducts in the induction of SCE in murine bone-marrow and alveolar macrophage cells *in vivo* was investigated by Conner, Luo, and de Gotera (1983). Swiss-Webster mice or C57B16 X DBA/2J (BDF_1) mice were administered 10 to 291 μmol/kg DEB by i.p. injection either just prior to, during, or after infusion with BrdUrd. BrdUrd infusion was followed by administration of 3.3 mg/kg colchicine and bone marrow cells and alveolar macrophages were harvested in 4 hours. The timing of administration of the DEB and BrdUrd was varied in order to evaluate the persistence of SCEs over successive cell cycles.

The dose-response studies, conducted in Swiss Webster mice, produced steep log dose-SCE response curves with significant increases in SCE frequency at all doses for both bone marrow cells and alveolar macrophages (Conner, Luo, and de Gotera, 1983). There were no significant differences between the responses of

hepatectomized and intact mice. Cytotoxicity, evident by a noticeable shift in the distribution of first, second, and third division treated cells relative to controls, was observed in only the two highest dose groups, Cellular SCE responses were similar in Swiss Webster and the BDF_1 mice. Studies on the persistence of SCEs over three cell cycles indicated that repair was rapid and that complete repair of SCE-inducing lesions (e.g. DNA adducts) occurred between the first and second post-treatment cell cycles. The presence or absence of DNA interstrand cross-links could not be determined from this study due to the rapid repair of the DEB induced lesions.

The effects on the immune response of male B6C3F1 mice following inhalation exposure to 1250 ppm 1,3-butadiene, 6 hours/day, 5 days/week for 6, 12, or 24 weeks were reported by Thurmond et al. (1986). Immune function assessment included immunopathology, antibody plaque-forming cell (PFC) response, cell surface marker determination, mitogen-stimulated lymphocyte proliferation, lymphocyte proliferation and cytotoxicity to alloantigens, and spontaneous cytotoxicity. The dose chosen did not cause changes in body weight with respect to controls during the exposure period; however, the same exposure regimen had been shown to result in macrocytic-megaloblastic anemia (Irons et al., 1986a) and in alterations in hematopoietic stem cell development (Liederman et al., 1986).

Thurmond et al. (1986) reported that after 6 weeks of exposure there was a decrease in the spleen to body weight ratio relative to control animals (20%; $p<0.05$), and assumed that the decrease was due to a 29% decrease in spleen cellularity ($p<0.01$). There was also evidence of significant extramedullary hematopoiesis in the spleens of BD-exposed mice, but the number of IgM antibody PFC/10^6 splenocytes remained unchanged. The number of T lymphocytes was reduced, and the numbers of cytotoxic and suppressor T cells appeared to be marginally reduced, although there was no reduction in the percentage of T-helper lymphocytes. These changes may not be biologically significant, but may only reflect a shift in the proportion of mature lymphocytes relative to hematopoietic precursor cells in the spleen. Extramedullary hematopoiesis may also be encountered following exposure to compounds that induce either hemolytic or megaloblastic changes and may have no direct correlation with leukemogenesis. The Ig-bearing B-lymphocyte population was unaffected by BD exposure.

There was an increase in spontaneous lymphocyte proliferation in both the mitogen assay and in mixed lymphocyte cultures, but mitogen responsiveness was reduced at both 6 and 12 weeks. These changes may have resulted from increased hematopoiesis since unpurified splenocytes were used in the assays, thereby reducing the relative proportion of mitogen-responsive lymphocytes. B-lymphocyte proliferation in response to lipopolysaccharide was also decreased. After 12 weeks of exposure there was a slight decrease in bone marrow cellularity which recovered to normal levels after 18 weeks of exposure. BD exposure did not alter the expression of natural killer (NK) cell activity.

Moderate histopathological changes were observed in the spleen and thymus after 24 weeks of exposure, the only time point examined. In the spleen there was evidence of erythroid hyperplasia and extramedullary hematopoiesis, and in the thymus a decrease in the number of cortical lymphocytes.

In summary, Thurmond et al. (1986) state that 1,3-butadiene does not appear to exert a direct or persistent immunomodulatory effect on functional end-stage cells, but may affect early hematopoietic or lymphoid precursor cells. No important functional deficits were detected in the immunological defense of exposed mice.

The ability of 1,3-butadiene exposure to induce genotoxic and cytotoxic damage in mouse bone marrow was evaluated in male B6C3F1 mice (4 to 6 weeks old) following inhalation exposure to 6.25, 62.5, and 625 ppm 1,3-butadiene, 6 hours/day, 5 days/week, for a total of 10 exposure days, 15 days total elapsed time (Tice et al., 1987). The endpoints examined and their respective responses were as follows: CA (chromosomal aberrations), increased; SCE (sister-chromatid exchanged frequencies), increased; AGT (average cell generation time), increased; MI (mitotic index), decreased; %PCE (percent polychromatic erythrocytes), increased; MN-PCE (number of micronucleated PCE), increased; MN-NCE (micronucleated normochromatic erythrocytes), increased.

All endpoints, with the exception of MI, were significantly affected by 625 ppm BD. The AGT and MN-PCE were significantly altered at 62.5 ppm BD. Longer term exposure (13 weeks) led to significant induction of MN-NCE in both male and female mice exposed to 6.25 ppm BD (Jauhar et al., 1988). Trend tests with increasing exposure concentration were statistically significant for alterations in all endpoints. The induction of SCE and the type of CA observed (predominantly chromatid-type breaks and exchanges) were consistent with the induction of DNA damage by an S-phase-dependent clastogen. The presence of micronuclei associated with the chromosomal aberrations suggests that the micronuclei result from chromosomal breakage; however, one of the BD metabolites could also be inducing lagging chromosomes in addition to, or instead of, clastogenic damage.

An early depression in the %PCE in bone marrow (after only 2 days of exposure) followed by a significant elevation in circulating %PCE after 15 days (10 days total exposure) suggests a compensatory response in the rate of erythropoiesis, probably extramedullary (Thurmond et al., 1986). However, the number of proliferating cells in the marrow as well as their rate of division remained significantly depressed. There was also a high correlation between the frequency of SCE and the number of MN-PCE.

The most sensitive endpoint for detecting the ability of BD to induce bone marrow toxicity in male B6C3F1 mice was the increase in SCE frequency, which was significantly greater at 6.25 ppm than in the controls. (Note that the current workplace TLV (threshold limit value) for 1,3-butadiene is 10 ppm [ACGIH, 1987]). The next most sensitive indicator was in the number of circulating MN-PCE, which were significantly increased at 62.5 ppm. In summary, Tice et al. (1987) report that 1,3-butadiene is genotoxic and cytotoxic to the bone marrow cells of male B6C3F1 mice subjected to multiple inhalation exposures.

Irons, Oshimura, and Barrett (1987) examined genotoxic endpoints in bone marrow, similar to those described above after exposing male B6C3F1 mice or NIH Swiss mice to a single 6-hour exposure of 1250 ppm BD. In concurrence with Tice et al. (1987), they found BD caused a high frequency of chromosomal aberrations and chromatid breaks as well as increased chromatid and iso-

chromatid gaps in both species. Both mouse strains tested were subject to BD-induced CAs.

The comparison between these two strains of mice with respect to bone marrow toxicity was significant since B6C3F1 male mice exhibited a high incidence of leukemia/lymphoma following 12 months of exposure to 1250 ppm BD (65%) while NIH Swiss mice exhibited a much lower incidence (14%) (Irons et al., 1988). One important difference between the strains is that the B6C3F1 strain, but not the NIH Swiss mouse, has an endogenous ecotropic retrovirus (MuLVL). This retrovirus may play a role in murine leukemogenesis.

Both strains of mice exhibit macrocytic anemia as a result of exposure to BD, a particularly striking feature of which is a large increase in the frequency of micronuclei in circulating peripheral erythrocytes (RBC) and their prominence in precursor cells in bone marrow. These results indicate that BD induces cytogenetic damage in murine bone marrow cells, and that this damage is independent of both endogenous retroviral background and leukemogenesis.

Altered stem cell development was assessed in 6 to 8 week old male B6C3F1 mice following exposure to 1250 ppm BD, 6 hours/day, 5 days/week, for either 6 or 30 weeks (Leiderman et al., 1986). Quantitative assessment of pluripotent stem cells was made using the spleen colony-forming assay (CFU-S). The differentiation of committed myeloid cells was made by enumerating the CFU of granulocyte/macrophage (CFU-GM), and the effects on hematopoiesis were assessed by long-term bone marrow culture. Neither the number of CFU-S nor CFU-GM were altered following 6 weeks of exposure to BD, although the colonies derived from treated animals were smaller in size. Large colonies were assumed to have arisen from mature pluripotent stem cells which differentiated upon stimulus, while the smaller colonies originated from the more primitive stem cells. There were no changes in bone marrow cellularity after 6 weeks of exposure.

The number of both CFU-S and CFU-GM were significantly decreased after 30 weeks of exposure. There was also a significant suppression in the number of CFU-GM in long-term bone marrow cultures after 14 days in culture relative to cultures from control animals; after 28 days of culture the situation was reversed and there was a significant increase in CFU-GM relative to control cultures. This alteration in the kinetics of stem cell proliferation in long-term cultures suggests a profound change in stem cell regulation, a shift in maturation or a delay in differentiation to the granulocyte/macrophage committed cell (Leiderman et al., 1986). A similar delay in stem cell maturation has been described for bone marrow cells cultured from preleukemic patients (Golde and Cline, 1973; Koeffler and Golde, 1978).

Exposure of male B6C3F1 mice to 1250 ppm BD, 6 hours/day, 6 days/week for 3, 6, 12, 18, or 24 weeks resulted in an increased incidence of macrocytic-megaloblastic anemia (Irons et al., 1986a). Peripheral hematology was examined after 6, 12, 18, and 24 weeks of exposure. The numbers of micronuclei and reticulocytes were assayed after 6 and 24 weeks, bone marrow cellularity after 3, 6, 12, and 18 weeks, and bone marrow cell cycle kinetics after 6 weeks of exposure.

Exposure to 1250 ppm BD caused no alteration in body weight gain during the exposure period. No biologically significant effects were observed on bone

marrow cellularity after 3, 6, 12, or 18 weeks of exposure, although there was a 58% increase in the proportion of cells in S phase, and an increase in the overall proliferative index after 6 weeks. Bone marrow cells did exhibit mild megaloblastic changes and numerous micronuclei. Significant differences in peripheral hematology were observed at all time points examined; the number of leukocytes and erythrocytes, the hemoglobin content, and the hematocrit were all reduced. There was no evidence of accelerated erythropoiesis, nor any increase in the number of polychromatic cells or of nucleated erythrocytes. However, there was a marked increase in the number of circulating micronucleated cells. The platelet count was unaffected.

These findings were considered consistent with megaloblastic anemia and were reported to be similar to those encountered in human preleukemic syndrome. The results suggest that 1,3-butadiene exposure interferes with normal bone marrow cell differentiation and/or DNA synthesis in replicating bone marrow cells of the B6C3F1 mouse. Irons and colleagues suggest that the observed changes in bone marrow indicate that, although the thymus may not represent the primary target organ for BD toxicity, alterations in thymocyte precursor cells in the bone marrow may play an essential role in the development of 1,3-butadiene-induced murine thymic lymphoma/leukemia.

An experiment to assess the potential for BD to cause macrocytic-megaloblastic anemia, similar to the study described above, was conducted in NIH Swiss mice (Irons et al., 1986b). The NIH Swiss mouse does not carry the endogenous type-C murine leukemia retrovirus (MuLV1) which may play a role in the induction of murine thymic lymphoma/leukemia in B6C3F1 mice by BD. Male Swiss mice, 4 to 6 weeks old, were exposed to 1250 ppm BD 6 hours/day, 5 days/week, for 6 weeks. This exposure regimen did not result in body weight changes in treated animals during the course of the experiment. Peripheral hematology reports after 6 weeks of exposure demonstrated treatment-related reductions in the number of erythrocytes and decreases in the hemoglobin and hematocrit parameters. There was no evidence of accelerated erythropoiesis nor an increase in the number of polychromatic or nucleated erythrocytes; however, there was a striking increase in the number of micronucleated erythrocytes. Evaluation of the bone marrow indicated a significant decrease in bone marrow cellularity with only mild megaloblastic changes.

The results obtained from NIH Swiss mice (Irons et al., 1986b) are entirely consistent with those described above for B6C3F1 mice (Irons et al., 1986a), thereby indicating that BD-induced bone marrow toxicity is independent of the endogenous ecotropic MuLV.

The potential for BD to induce sister-chromatid exchanges (SCEs) and micronuclei in bone marrow polychromatic erythrocytes (MN-PCEs) was assessed in male B6C3F1 mice and Sprague-Dawley rats exposed to 1,3-butadiene vapor at concentrations of 10 to 10,000 ppm, 6 hours/day on 2 consecutive days (Cunningham et al., 1986). Bone marrow was obtained 24 hours after the last exposure and examined for MN-PCEs and SCEs. Evidence of bone marrow toxicity, present as a decrease in the ratio of polychromatic erythrocytes to normochromatic erythrocytes, was observed in both rats and mice and was related to increasing exposure concentrations. Mortality was present in the 10,000 ppm group for mice, but no mortality was observed in rats. A significant concentration-related increase was observed in MN-PCEs in the mice starting at 100 ppm 1,3-butadiene. The percent MN-PCEs ranged from 0.44% at

100 ppm to 3.0% at 10,000 ppm while the value for control animals was 0.08%. No increases in MN-PCEs were observed at or below 50 ppm. No significant increase in MN-PCEs were found in the rats.

SCE frequencies in bone marrow cells were increased in mice, in a dose-related fashion, from 10 to 100 ppm, with the first significant increase observed at 100 ppm. However, in a second experiment where BD concentrations ranged from 200 to 10,000 ppm, sufficient second-division metaphases were not obtained for adequate analyses although there was evidence of elevated frequencies. SCE frequencies in rats were slightly, but not significantly elevated at 500 ppm. In the second set of experiments at 200 to 10,000 ppm no significant increases were observed in rats.

Results of these studies (Cunningham et al., 1986) indicate that 1,3-butadiene does not cause bone marrow toxicity in the rat, at least when exposures are of a short duration. However, it is curious that the SCE frequencies observed in the mice in this study did not exhibit a better dose-response relationship, especially since this has been demonstrated by others (Tice et al., 1987).

3.5 Carcinogenicity

The earliest reported study on the inhalation toxicology of 1,3-butadiene was conducted by Carpenter et al. (1944). In this study, rats, rabbits, and guinea pigs were exposed to 0, 600, 2300, and 6700 ppm 1,3-butadiene, 7.5 hours/day, 6 days/week, for 8 months. Although the data obtained from this study were limited with respect to blood cytology, fertility evaluation, and gross pathology, the authors reported that these BD exposures did not result in "significant progressive injury to small animals during the 8-month exposure period."

In a more recent inhalation study (Huff et al., 1985) male and female B6C3F1 mice (8 to 9 weeks old) were exposed to 0, 625, or 1250 ppm BD, 6 hours/day, 5 days/week, for 60 or 61 weeks. Although this study was intended to last 103 weeks, it was terminated at 60 or 61 weeks because of reduced survival in the BD exposed groups due to fatal tumors. Data from this publication are provided in Table 3-1.

The early deaths were primarily due to malignant neoplasms involving multiple organs. The total numbers of primary malignant and benign neoplasms per animal were much greater in the BD-exposed groups than in the control group. Primary tumors caused by exposure to BD at both exposure levels in both sexes included malignant lymphoma, hemangiosarcoma of heart, alveolar-bronchial neoplasms, and squamous cell carcinoma of the forestomach. Tumors observed in females only (in both exposure groups) were acinar cell carcinoma of mammary gland, granulosa cell neoplasm of the ovary, and hepatocellular neoplasms. Malignant lymphomas were observed in both sexes as early as week 20 and appeared to originate in the thymus; however, involvement of the spleen, lymph nodes, liver, lung, kidney, heart, pancreas, and stomach was common. Hemangiosarcomas of the heart were also a major cause of death in both exposure groups and both sexes. A significant dose-response relationship was observed for the lesions in females, but not in males. The absence of a dose-response in males was attributed to a lower survival rate for the high-dose males than for the low-dose males. Most heart lesions were encountered in

Table 3-1 Incidence of Primary Tumors in Mice Exposed to Butadiene for 60-61 weeks[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air		
		0	625	1250
Heart/Hemangiosarcoma	M	0/50[d]	16/49[c]	7/49[c]
	F	0/49[b]	11/48[c]	18/49[c]
Hematopoietic System Malignant Lymphoma	M	0/50[b]	23/50[c]	29/50[c]
	F	1/50[b]	10/49[b]	10/49[c]
Lung/Alveolar and Bronchiolar Adenoma	M	2/50	12/49	11/49
	F	3/49	9/48	20/49
Lung/Alveolar and Bronchiolar Neoplasm	M	2/50[b]	14/49[c]	15/49[c]
	F	3/49[b]	12/48[c]	23/49[c]
Mammary/Acinar Cell Carcinoma	F	0/50[b]	2/49	6/49[e]
Ovary/Granulosa Cell Neoplasm	F	0/49[b]	6/45[c]	13/48[c]
Forestomach/Papilloma and Carcinoma	M	0/49	7/40[c]	1/44
	F	0/49[b]	5/42[e]	10/49[c]
Liver/Adenoma	F	0/50	1/47	4/49
Liver/Adenoma and Carcinoma	F	0/50[d]	2/47	5/49[e]

[a] Incidences based on EPA evaluation of NTP (1984) study.
[b] Increasing trend ($p < 0.01$).
[c] Increase compared to control ($p < 0.01$).
[d] Increasing trend ($p < 0.05$).
[e] Increase compared to control ($p < 0.05$).

early death animals after 40 weeks of exposure or in animals killed at the termination of the study. However, one hemangiosarcoma was diagnosed in a low-dose male after only 32 weeks of exposure. 1,3-Butadiene is one of two known animal carcinogens that induce vascular cardiac neoplasms; the other is the fungicide captafol (Ito et al., 1984). A high incidence of these rare neoplasms (23% in males and 30% in females) occurred at a relatively young age in BD exposed mice. The heart was regarded to be the primary site of the lesion and the lesions in liver, lung, and kidney were regarded as metastatic foci (Solleveld et al., 1988). Gonadal atrophy was also observed at a high incidence in exposed animals of both sexes, but not in any of the control animals. The sites of neoplastic development in these B6C3F1 mice were different from those observed for Sprague-Dawley rats (Owen et al., 1987).

Interim and final results have been published on a second 2-year inhalation study in B6C3F1 mice (Melnick et al., 1988, 1989; Melnick, Huff and Miller, 1989; Miller, Melnick and Boorman, 1989; Melnick et al., 1990). In this study, lower exposure concentrations of 1,3-butadiene (i.e. 0, 6.25, 20, 62.5, 200, and 625 ppm) were used than had been employed in the first study. Interim sacrifices at 40 and 65 weeks of exposure were also added to the original study design in order to follow progression of lesions. Four stop-exposure groups were also added; 50 male mice were exposed to 625 ppm for 13 or 26 weeks, 312 ppm for 52 weeks, or 200 ppm for 40 weeks, and then held for the remainder of the 2-year period. Lymphocytic lymphoma was the major cause of death in the 625 ppm group during the first 50 weeks of the study and appeared to limit the expression of neoplasms at other sites. For example, in male mice exposed to 625 ppm BD, the evidence of lymphocytic lymphomas was 70%, whereas, in male mice exposed to 200 ppm BD, the incidence of lymphocytic lymphomas was 3% and of hemangiosarcomas of the heart was 30%. In the 625 ppm, 13-week, stop-exposure group, lymphocytic lymphomas were induced (17/50) and the incidence was doubled (30/50) in mice exposed to 625 ppm for 26 weeks. However, if the exposure concentration was reduced by one-half to 312 ppm and the exposure duration doubled to 52 weeks, the incidence of lymphocytic lymphoma was less than for the 625 ppm, 26-week group. Thus, the dose rate appears to have a significant effect on carcinogenesis of butadiene. This may be important for occupational exposures, and interpretation of epidemiology based on occupational exposures. As in the previous study, hemangiosarcomas of the heart, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, and/or adenocarcinomas of the mammary gland were frequently observed in mice which died between weeks 40 and 65 of the study. In female mice exposed to 6.25 ppm BD, the incidence of alveolar-bronchiolar neoplasms was increased (15/60, 25%) vs. control (4/70, 6%). Also as in the previous study, gonadal atrophy was observed in both sexes at 200 ppm and 625 ppm for males and 6.25 ppm and higher for females. Bone marrow toxicity was evident as a poorly regenerative anemia at 62.5 ppm and higher. Thus this study did not establish a no effect level for reproductive endpoints. The 6.25 ppm nominal dose level might be considered a chronic LOAEL for reproductive toxicity. The published Melnick et al. (1990) study (Mouse II) is reproduced in the Appendix. The tumor incidence used in the quantitative assessment are given in Table 3-2.

Irons et al. (1986c) characterized BD-induced thymic lymphomas in B6C3F1 mice exposed to 1250 ppm BD for 28 to 45 weeks. The lymphomas consisted of a uniform proliferation of poorly differentiated lymphoblastic cells which

Table 3-2 Incidence of Primary Tumors in Mice Exposed to Butadiene for 2-Years (Melnick et al. 1990)[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air					
		0	6.25	20	62.5	200	625
Heart/hemangio-sarcroma	M	0/70	1/49	1/50	5/38	20/35	6/11
	F	0/70	0/50	0/50	1/33	20/31	26/31
Hematopoietic system/ All malignant lymphomas	M	4/50	3/50	8/42	11/44	9/33	69/71
	F	10/50	14/47	18/44	10/38	19/33	43/48
Lymphocytic lymphomas	M	2/50	1/50	2/40	4/40	2/29	62/65
	F	2/50	4/44	6/43	3/38	11/27	36/42
Lung/alveolar and bronchiolar neoplasm	M	22/48	23/48	20/44	33/46	42/48	12/16
	F	4/50	15/44	19/43	27/44	32/40	25/30
Forestomach/papilloma and carcinoma	M	1/70	0/50	1/60	5/38	12/33	13/17
	F	2/70	2/50	3/38	4/33	7/23	28/33
Ovary/granulosa cell neoplasm	F	1/69	0/59	0/59	9/38	11/25	6/14

[a]Figures adjusted for intercurrent mortality.

possessed surface markers indicative of early T lymphocytes and demonstrated variable, but elevated, levels of murine leukemia virus.

In an attempt to define the possible role of the endogenous retrovirus in BD toxicity, Irons et al. (1987) examined the expression and behavior of the retroviruses in male B6C3F1 and NIH Swiss mice during the preleukemic phase of BD exposure. Animals were exposed to 1250 ppm BD, 6 hours/day, 5 days/week, for 3 to 21 weeks. The spleen, thymus, and bone marrow tissues were evaluated for ecotropic murine leukemia virus (eMuLV) content after the first three weeks of exposure.

The results indicated that BD exposure did not qualitatively alter the pattern or types of retroviruses detectable in mouse tissues. However, there was an increase in the frequency of ecotropic virus-producing cells isolated from the bone marrow, thymus, and spleen of the B6C3F1 mouse. The greatest relative increase occurred in the spleen where virus-producing cells were quantifiable following only three weeks of exposure. The frequency of virus-producing cells in the spleen increased logarithmically over the 21-week exposure period. In contrast, no viruses were detected in the control of BD exposed NIH Swiss mice over the 21-week exposure period.

An attempt was then made to characterize the mechanism by which 1,3-butadiene enhanced the eMuLV present in the B6C3F1 mice by: 1) testing for generation of NB-tropic viruses which would allow escape of the virus from the *Fv-1* restriction gene which governs the spread and infection of murine leukemia viruses, and 2) by evaluating the possibility of abrogation of host *Fv-1* restriction (Irons et al., 1987). The authors concluded that the increases in eMuLV expression were probably not associated with intrinsic alterations in the host *Fv-1* restriction, and were not due to the emergence of an eMuLV which exhibited an altered host range. Therefore, the most likely conclusion is the *de novo* activation of endogenous sequences in many individual cells. The activation of the eMuLV may be due to alteration (e.g., methylation) of some gene regulatory site by one of the epoxide metabolites of 1,3-butadiene. Although the role of eMuLV activation in the etiology of BD-induced murine leukemogenesis is not clearly understood, these results provide presumptive evidence for eMuLV involvement. This presumption is supported by the fact that, although target organ toxicity is virtually identical during the preleukemic phase in both mouse strains, NIH Swiss mice, which lack the retrovirus, are not nearly as susceptible to BD-induced lymphomas as are the B6C3F1 mice, who carry the retrovirus (Irons et al., 1986a and 1986b).

Goodrow et al. (1990) examined the tumors generated in mice by inhalation of 6.25 to 625 ppm BD for 1-2 years for the presence of activated oncogenes. Activated K-ras genes were detected in 2 of the 11 lymphomas assayed. These authors concluded that the K-ras activation probably occurred as a result of genotoxic effects of BD or its metabolites. The oncogenes most frequently detected in human lung adenocarcinomas are K-ras genes. Activated K-ras genes have also been detected in some human lymphomas.

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Table 3-3 Incidence of Primary Tumors in Rats Exposed to Butadiene for 2-Years[a]

Site/Lesion	Sex	Nominal Dose (ppm) in Air		
		0	1,000	8,000
Mammary/Fibroadenoma	F	32	64	55
Carcinoma		18	15	26
TOTAL		50[b]	79[c]	81[c]
Thyroid/Follicular cell-Adenoma	F	0	2	10
carcinoma		0	2	1
TOTAL		0[b]	4	11[c]
Uterus/Cervical Stromal Sarcoma	F	1	4	5
Testis/Leydig Cell Adenoma/Carcinoma	M	0[d]	3	8[c]
Pancreas/Exocrine Adenoma	M	3[d]	1	10[e]
Zymbal Gland Adenoma	M	1	1	1
	F	0	0	0
Carcinoma	M	0	0	1
	F	0	0	4
TOTAL	M	1	1	2
	F	0[d]	0	4
Number of rats examined	M	100	100	100
	F	100	100	100

[a] Tumor incidences from Owen et al, 1987.
[b] Increasing trend ($p < 0.01$).
[c] Increase compared to control ($p < 0.01$).
[d] Increasing trend ($p < 0.05$).
[e] Increase compared to control ($p < 0.05$).

detected in human lung adeno carcinomas are K-ras genes. Activated K-ras genes have also been detected in some human lypphomas.

In addition to the mouse studies described above, several inhalation studies on 1,3-butadiene have been conducted in rats. In a study performed at Hazleton Laboratories in Europe, Sprague-Dawley rats (5 weeks old) were exposed to 0, 1000, or 8000 ppm BD, 6 hours/day, 5 days/week for 105 weeks for females and 111 weeks for males (Owen et al., 1987). Tables of data from this publication are summarized in Table 3-3.

A statistically significant relationship was observed between increased mortality and increasing exposure concentration during the second year of the study. There was a significant increase in liver to body weight ratios for low- and high-dose males during both years of the study, for low-dose females during the first year of the study, and for low- and high-dose females during the second year of the study. High-dose males had increased heart, lung, spleen, and kidney weight to body weight ratios during the second year of the study.

Treated rats also exhibited some types of tumors in larger numbers than did the controls animals. For males there was an increased incidence of pancreatic exocrine adenoma and Leydig-cell tumors in the high-dose group, and for females an exposure- related trend in uterine sarcoma and mammary gland tumors. Zymbal's gland carcinoma, confined to the high-dose females, exhibited a significant treatment-related trend. The incidence of mammary gland adenoma and carcinoma and of thyroid follicular-cell adenoma were increased to a similar extent in both treatment groups.

The total number of tumor bearing animals in this study was not greater for exposed animals, male or female, than it was for the 0 ppm group, nor were the number of treated males with multiple tumors greater than for control males. However, both exposure groups of females had a greater proportion (not statistically significant) of animals with multiple tumors than did the control group. A strong dose related trend in fatal multiple mammary tumors was noted for both adenomas ($p<.001$) and carcinomas ($p<.001$). This relationship of tumor incidence to BD exposure was expressed by the authors (Owen et al., 1987) as follows: "although the biological interpretation of the significance of some of the tumor types is equivocal, the evidence suggests that the test article is a weak oncogen to the rat."

Crouch et al. (1979) conducted another relatively short-term study in male and female Sprague-Dawley rats. The rats were exposed to 0, 1000, 2000, 4000, or 8000 ppm BD, 6 hours/day, 5 days/week, for up to 12 weeks. No statistically significant effects on body weight were observed during the course of the study. The authors reported that rats exposed to these concentrations of BD showed no treatment related untoward effects.

3.6 Cancer Epidemiology

Several studies have examined cancer mortality rates among industrial workers who were likely to have been exposed to butadiene. However, these studies generally considered workers likely to have had contemporaneous exposure to other potential carcinogens (most notably styrene). Nevertheless, studies of two work environments are sufficiently specific to butadiene exposure to

provide limited supporting evidence for the carcinogenic effects observed in animal bioassays. These studies are a case-control study of rubber workers by Matanoski et al. (1989) and cohort studies of a butadiene manufacturing plant by Downs et al. (1987) and Divine et al. (1990). In addition, Checkoway and Williams (1982) observed statistical associations of blood parameters with butadiene exposure at a facility where excess leukemia and lymphoma had been reported. Although the review below discusses these studies, the quantitative risk assessment presented in this document is based on bioassays in laboratory animals.

Investigators studying the relationship of 1,3-butadiene and cancer in humans face certain limitations. First, exposure must be measured indirectly for most of the cohort since personal monitoring data of workers did not begin until the mid-1970's. Second, changes in processing in styrene-butadiene rubber (SBR) plants after World War II (WWII) substantially reduced worker exposure such that workers from 1943 to 1945 (the earliest workers) were exposed to much higher levels of 1,3-butadiene than other workers. Studies of the petrochemical industry have found that WWII workers remained at their jobs for a much shorter time than other workers. Although earlier (WWII) butadiene-exposed workers were more heavily exposed, they are likely to have stayed at their jobs for less time than later workers. Thus, studies of earlier workers may be less likely than studies of later workers to detect a dose-response association if the studies use duration of employment as the measure of dose. In such studies, WWII workers should be examined separately. These factors should be considered when reviewing the epidemiological studies presented in this document.

3.6.1 Studies of Cancer Mortality

The first epidemiological study evaluating the possibility of an increased risk of carcinogenicity following occupational exposure to 1,3-butadiene and other compounds was conducted by the National Institute of Occupational Safety and Health (NIOSH). NIOSH was informed of five leukemia cases among workers in a Port Neches, Texas SBR plant in March 1976. That information and a study conducted at the University of North Carolina (Spirtas, 1976) prompted NIOSH to hold a meeting addressing the issue of styrene-butadiene exposures and a possible link to leukemia (NIOSH, 1976). Leukemia rates in the area surrounding the Port Neches plants were found to be above national rates. While the leukemia rate for males per 100,000 from 1950-1969 for the United States was 8.8, it was 9.2 for Texas, 10.7 for Orange County, Texas, 11.1 for Jefferson County, Texas, and 13.2 for Chambers County, Texas (Wagoner, 1976). The Port Neches plant mentioned above as well as several other SBR facilities are located in Orange County, Texas.

At the time of the NIOSH symposium in March, 1976, elevated leukemia rates were reported at two SBR plants in Texas, and elevated leukemia and lymphoma rates were reported at an Ohio SBR plant. There was also another plant where the rate appeared to be in excess of the total U.S. male population (Lloyd, 1976). SBR facilities do not actually produce 1,3-butadiene, but use it as a monomer in the styrene-butadiene polymerization process. Deaths at an adjacent 1,3-butadiene manufacturing facility were reviewed and no cases of leukemia were found (n = 122).

McMichael et al. (1976) examined deaths occurring from 1964 through 1973 in a male population which had been employed at a large tire manufacturing plant in

Akron, Ohio. Standardized mortality ratios (SMRs) for the study population indicated that deaths due to several types of cancer exceeded rates for the 1968 U.S. male population. Statistically significantly elevated SMRs were found for stomach cancers (171) and lymphatic and hematopoietic cancers (136). In order to obtain more detail, worker histories were reviewed and a series of occupational title groups (OTGs) were identified. A study design was used that required work histories for ≈25% of the entire cohort; then each case group of interest (e.g., a specific cancer type) was compared to an age-stratified random sample equivalent to 22% of the total study population. An odds ratio was computed relating risk of disease to work in a particular OTG for more than two or five years. This odds ratio was then used to estimate a relative risk. Results from workers in the synthetic plant OTG, where elastomers of SBR and other rubbers are synthesized, are reviewed here. Other solvents and monomers to which these workers were exposed included styrene, benzene, and toluene. Work for five years or more in the synthetic plant was associated with significantly elevated risk ratios for lymphatic and hematopoietic neoplasms (6.2), lymphatic leukemia (3.9), and stomach cancer (2.2). McMichael et al. also compared odds ratios for those with 2 versus 5 years work in a given OTG. The authors found an effect of job duration in many OTGs including the synthetic plant. The synthetic plant workers used in this analysis represent 2% of the study population.

Andjelkovich et al. (1976) and Andjelkovich et al. (1977) reported the mortality experience of 8,418 white male workers in a large rubber manufacturing plant, also in Akron, Ohio. They included in the analysis any man who was an active worker or a living retiree of the plant as of January 1, 1964, and at least 40 years old. Deaths from January 1964 to December 1973 were included in the analysis. The cohort was initially divided into two age groups, those under 65 and those 65 or older so that the cohort could be compared to the one analyzed by McMichael et al. (1976). SMRs were elevated (but not necessarily significantly) in both age groups in both cohorts for cancers of the stomach, large intestine, and prostate as well as for lymphosarcoma. The SMR for monocytic leukemia (311) was significantly elevated for this entire cohort. An OTG was then determined for each worker, and the data reanalyzed. SMRs (compared to U.S. population for all workers combined across OTGs) were recalculated for each OTG. Workers from the compounding and mixing (479) and milling (369) OTGs accounted for the observed excess in stomach cancer. Excess leukemia was found only in the general service OTG (246). Meinhardt et al. (1982) point out that this OTG probably has little 1,3-butadiene contact. The SMR for lung cancer was high (434) among synthetic latex workers, but not among the cohort as a whole. As in the McMichael cohort, synthetic latex workers had the highest likelihood of butadiene exposure.

Another cohort of 13,570 white males who had worked for ≥ 5 years in a Goodrich plant in Akron, Ohio was examined for mortality outcome from 1940-1976 (Monson and Fine, 1978). External comparisons (SMR) of mortality, based on U.S. white males, and internal comparisons of incidence were performed in this study. Leukemia and lymphatic cancers were elevated in a number of job categories as was the incidence of gastrointestinal cancer. Solvents were suggested by the authors to have been responsible for the increased incidences; the elevation cannot be specifically attributed to 1,3-butadiene. The authors did not divide this cohort into those who worked during World War II and those who did not. However, the report on the Akron cohort makes

reference to the fact that workers employed after 1945 did not have an excess incidence of leukemia.

In an investigation of the health effects of styrene exposure, Ott et al. (1980) studied 2,904 employees of Dow Chemical plants who had worked for at least one year over the years from 1937 to 1970. Plants in four different states were included in this study: Michigan, Texas, Connecticut, and California. SMRs for leukemia (176) and for lymphatic and hematopoietic neoplasms (132) were elevated but not significantly. In this analysis, only 391 of the workers studied were employed in styrene/butadiene latex manufacturing and there were no cases of leukemia observed in this group. OEHHA staff were not able to confirm that this OTG was the only one in which butadiene exposure occurred. Therefore, as with the previous studies (McMichael et al., 1976; Andjelkovich et al., 1976; Andjelkovich et al., 1977; Monson and Fine, 1978), this report is suggestive of an increase in incidence of lymphatic and hematopoietic cancers in a cohort associated with multiple types of chemical exposures. However, the increase cannot be definitely attributed to 1,3-butadiene exposure.

White male workers who had been employed for at least six months in two SBR plants in eastern Texas were studied for an excess of leukemia (Meinhardt et al., 1982) and an attempt was made to correlate the results to occupational chemical exposures. There were 1,662 study subjects in plant A and 1,094 in plant B. Workers were followed from 1943-1976 at plant A and from 1950-1976 at plant B. Since plant B did not include WWII workers, one might expect the SMRs from this cohort to be lower. In fact, there were no significantly elevated SMRs observed from workers in plant B. In plant A, SMRs were elevated but not significantly for lymphatic and hematopoietic neoplasms (155) and for several subcategories within that classification including leukemia (203). Among workers first employed from 1943 to 1945 (all from plant A), there were excesses of leukemia (278) as well as all lymphatic and hematopoietic tumors combined (212). The authors note that the excesses for early workers would have been significant using a one-tailed test. A nonsignificant elevation of lymphopoietic tumors was also observed among all workers when both plants were included in the analysis. All 5 of the qualifying cases and 3 of 6 nonqualifying cases had first been employed between 1943 and 1945, pointing to the possible increased risk of these workers. According to the authors, this study had the power to detect only a 4-fold or greater increase in risk. In a subsequent analysis, mortality was followed up to 1982 in plant A and 1981 in plant B. The same pattern of results was observed (Lemen et al., 1990).

In more recent studies, Matanoski and Schwartz (1987) and Matanoski et al. (1990) examined a cohort which consisted of 12,110 males with at least one year working in the SBR industry in the U.S. or 10 years working in an SBR plant in Canada. Deaths from each plant were originally followed until 1979 (Matanoski and Schwartz, 1987) but were subsequently followed to 1982 (Matanoski et al., 1990) The results from this most recent analysis are presented below. Follow-up began in different years for different plants. Only four plants had complete records back to 1943 and follow-up for one plant did not begin until 1970. All plants were included in the analysis. In this study, 75% of the cohort were white males. An additional nineteen percent of the cohort, in which data on race were missing, were assumed to be white. No SMR, calculated on the basis of U.S. males for specific causes of death for

all males, all plants, or all job types in the cohort, was significantly elevated.

The cohort was also subdivided by major work area and the SMRs recalculated based on the U.S. male population and race-specific rates where appropriate. In black production workers, SMRs for cancers of the hematologic system (507) and leukemia (656) were significantly elevated. There was also a significantly elevated SMR of 260 for other lymphomas among all production workers.

The authors offer two caveats to the findings among production workers. (1) Elevated SMRs in black production workers and the lack of significant SMRs among white production workers could have resulted from the bias of having racial information on all deaths but misclassification of race among living workers. This type of bias would inflate the white expected and deflate the black expected, resulting in higher SMRs for black workers and lower SMRs for white workers. (2) More than 2000 workers with incomplete work history were excluded from the analysis by job areas. Many of these were active workers in 1976 and therefore likely to be alive at the end of the follow-up period. The authors note that the higher percentage of deceased workers in this analysis could result in higher cause-specific ratios by work area than those seen in the total population.

The following factors should also be considered in interpreting the results: (1) there may have been as much as 32 years difference in the length of employment periods among workers (a mean employment period was not given); (2) the authors note that much of the population was still relatively young; (3) the probability of the study to find an effect may have been hampered by the exclusion of employees who worked for less than one year which may have excluded a greater proportion of war time workers. Follow up from four of eight plants did not include WWII workers.

To elucidate the role of 1,3-butadiene versus styrene in the cancers observed in the cohort study, Matanoski et al. (1989) conducted a nested case-control study. Lymphopoietic cancer cases were compared to workers who did not have cancer. A log transformation of exposure data was used to classify workers into high- and low-exposure categories. The leukemia cases were associated with exposure specifically to 1,3-butadiene (OR=9.4, 95% CI=2.1-22.9) but not to styrene. This study's results appear sensitive to the choice of exposure classification scheme. In addition, Acquavella (1989), in a review of butadiene epidemiology studies, notes that, since the original analysis of the cohort did not reveal a leukemia excess overall, the high odds ratio may have resulted from an exceptionally low leukemia rate among unexposed workers. However, since elevated rates of leukemia and other lymphatic and hematopoietic cancers have been observed consistently in other studies of SBR plants (Meinhardt et al., 1982) and in those parts of tire manufacturing plants in which SBR is synthesized, these results cannot be easily dismissed. This case-control study is also important in that it was able to assign different odds ratios for butadiene and styrene exposure.

Cause-specific mortality in a cohort of male workers employed from 1943 for at least six months in a 1,3-butadiene plant (Neches Butane, Port Neches, Texas) was assessed by Downs et al. (1987) and Divine (1990). Deaths were followed initially to 1979 and later to 1985. The results from the most recent studies

are presented below. Since work histories and historical industrial hygiene data were not available for this cohort, a qualitative scale for 1,3-butadiene exposure was constructed based on department codes. Four exposure groups (I-IV) based on the estimated frequency of exposure to 1,3-butadiene were defined for the cohort (n=2582): I - low exposure (very rare; n=433), II - routine exposure (daily; n=715), III - nonroutine exposure (intermittent; n=993), and IV - unknown exposure (varied; n=451).

This cohort was initially analyzed on the basis of race (black or white); however, it was later decided to treat all subjects as white and use death rates for U.S. white males in the final analyses. In the most recent study (Divine, 1990) the SMR for lympho- and reticulosarcoma (229) was significantly greater for the cohort than for U.S. white males. When the cohort was partitioned into a wartime cohort (>6 months employment prior to the end of 1945) and a postwar cohort (>6 months employment after 1945), the SMRs for lympho-hematopoietic cancers for those hired before 1946 were significantly elevated (SMR=269). SMRs were also calculated for the cohort after it had been subdivided into exposure groups I through IV. These calculations showed a significant increase in deaths from lymphosarcoma among those with routine exposure (SMR=561). All of the lymphosarcomas occurred in persons employed for less than ten years. No effect of latency was observed. The SMR for leukemia (SMR=102) in the total cohort was not elevated.

The authors note that the cohort has certain shortcomings, mainly an unreliable designation of race, a lack of worker histories, and a lack of industrial hygiene data; furthermore nearly half of the total cohort worked at the plant for less than 5 years. In addition, many individuals spent significant time working at the neighboring SBR plants, thus confounding the duration and type of chemical exposures. The cohort is also relatively small, especially when split into groups on the basis of exposure category. However, this cohort was employed specifically for the manufacture of butadiene, unlike cohorts in SBR plants and tire manufacturing plants where exposures to styrene were encountered. It is important to note that in this cohort associations with cancer were observed that are similar to those observed in cohorts with mixed exposures.

3.6.2 Cancer-Related Studies

Checkoway and Williams (1982) measured hematologic parameters of male workers employed in the SBR synthesizing plant at the Akron, Ohio, facility studied by McMichael et al. (1976). This study was part of a health and industrial hygiene survey initiated after McMichael et al. reported excess leukemia and lymphoma (see Section 3.6.1, above). Checkoway and Williams sought to quantify workplace exposures to styrene, butadiene, benzene, and toluene, and to relate these exposures to measurable hematologic parameters which may be indicative of bone marrow toxicity. Personal breathing zones of 163 workers were sampled, and blood was drawn from 154 workers. Based on medical history questionnaires, one worker who reported a history of leukemia was excluded from this study. The mean 8-hour time-weighted average concentrations of butadiene, styrene, benzene, and toluene were less than 2 ppm in all departments, with the exception of the "Tank Farm" area where the values were 20.03 ppm and 13.67 ppm for butadiene and styrene, respectively. There were statistically significant correlations of butadiene and styrene levels (considered separately) with red blood cell (negative correlation) and basophil (positive correlation) counts. After stepwise linear regression to

control for age and medical status, the positive association of basophil count with butadiene exposure and the negative association of red blood cell count with styrene exposure persisted, and there were significant findings regarding the two chemicals and corpuscular hemoglobin concentration (positive association for butadiene, negative for styrene). The reported associations explained very little of the variance in the blood parameters, however.

Checkoway and Williams (1982) divided their cohort into two populations, Tank Farm workers (n=8) and all others, in order to compare blood parameters between the subgroups with the greatest exposure differences. The investigators reported that the small number of Tank Farm workers precluded reliable statistical tests of significance. Nevertheless, increased band neutrophil and monocyte counts were apparent in the Tank Farm workers. Checkoway and Williams noted other differences, which they characterized as "small" or "slight." They found their results to be suggestive of possible biological effects of butadiene and styrene exposure (including cellular immaturity), with clinical consequences that are not readily apparent. Firm conclusions cannot be drawn from this study, however, as it did not control for smoking or other behavioral factors that may affect blood parameters, and was hampered by a small sample size and limited sampling protocol. It is important to note, however, that Checkoway and Williams (1982) found differing effects of styrene and butadiene exposure.

3.6.3 Interpretation of Human Butadiene Studies

Results from selected studies are presented in Table 3-4. In evaluating epidemiological studies of 1,3-butadiene, the following factors must be considered: (1) Misclassification of exposure; (2) Exclusion of most highly exposed workers; (3) No dose-response effect; (4) Varying health endpoints.

(1) Misclassification of exposure: Personal monitoring data for exposed workers did not begin until the mid-1970's. Therefore exposure must be inferred from job title, function, and duration. Jobs are categorized in different ways in each study. None of the cohort studies reviewed tested their exposure estimate methods against actual exposure data. Misclassification of unexposed as exposed would bias results toward the null.

In those studies with significant results, however, it is possible that the cancers are a result of some other compound which covaries with butadiene. The strongest evidence that the lymphopoietic and hematopoietic cancer excesses observed are from butadiene exposure is provided by the nested case-control study of Matanoski et al. (1989). In this study leukemia was significantly associated with butadiene and not styrene exposure.

(2) Exclusion of most highly exposed workers: Changes in SBR processing after World War II reduced worker exposure such that workers from 1943 to 1945 (the earliest workers) were exposed to much higher levels of 1,3-butadiene than other workers. Some cohorts have excluded WWII workers because of lack of records, or because plants were not yet in operation. If WWII workers are at greater risk, then those studies in which these workers were not included may be less likely to see significant effects. This was observed in the study conducted by Meinhardt et al. (1982) in which SMRs were only elevated in the plant with WWII workers, and it may have affected the results of Matanoski and Schwartz (1987) and Matanoski et al. (1990) where four of eight plants studied did not have WWII workers.

Table 3-4. Human Cancer Epidemiology Cohort Studies Related to Butadiene Occupational Exposures

Study, Size (n)	Cancer Sites	# Observations / population	Measure Used	Value	Notes
McMichael et al., 1976 (n=1,500)	Lymphatic and hematopoietic neoplasms	–	Risk Ratio	6.2 *	Values only reported for those in the synthetic latex plant, 2% of the study population.
	Lymphatic leukemia	–	Risk Ratio	3.9 *	Increases with duration worked.
	Stomach cancer	–	Risk Ratio	2.2 *	No excess of other cancers in this OTG.
Andjelkovich et al. 1977 (n=8,418)	Lung	3	Risk Ratio	4.3 *	Finding in synthetic latex workers.
Meinhardt et al, 1982 (n=1,662 Plant A n= 1,094 Plant B)	Lymphatic and hematopoietic neoplasms	9 (Plant A) 9 (A, early workers)	SMR SMR	155 212	Risks in early workers in plant A are significant when one-tail test is used.
	Leukemia	5 (A) 5 (A, early workers)	SMR SMR	203 278	
Matanoski et al., 1990 (n=12,110)	Lymphatic and hematopoietic neoplasms	48 (all) 7 (black) 6 (black production)	SMR SMR SMR	92 146 507 *	None of the cancer ratios was significant when the total cohort was analyzed nor when it was analyzed by race.
	Leukemia	3 (black production)	SMR	656 *	
	Other lymphomas	9 (all production)	SMR	260 *	
Divine et al, 1990 (n=2,582)	Lymphosarcoma	9	SMR	229 *	Butadiene manufacturing facility.
		7 (early workers) 5 (routine exposure)	SMR SMR	269 * 561 *	No effect of latency or duration.

* $p<0.05$

(3) No dose-response effect: Recent studies conducted at SBR or butadiene manufacturing plants have not detected an effect of duration of butadiene exposure (as measured by job or employment duration). Studies of workers in the petrochemical industry show that WWII workers remained at their jobs for a much shorter time than other workers (Wen et al., 1986). Since earlier workers were more heavily exposed, if (as in the petrochemical industry) their stay at work was much shorter, studies would be less likely to detect a dose-response effect by using duration of employment. There might be a subset of the worker population that quickly develops certain kinds of cancers when exposed to moderate levels of butadiene. This also might represent a real effect. Stop-exposure studies conducted by Melnick et al. (1990) in mice indicated that short-term high exposures to butadiene may result in greater tumor incidences than longer-term, lower exposures. In view of the data in animal studies, it is difficult to conclude that cancer among short-term exposed workers is due to confounding factors. McMichael et al. (1976) did note an increased risk in workers who worked in the synthetic plant for five versus two years.
(4) Varying health endpoints: Divine (1990) questioned the significance of an association of butadiene exposure with different and sometimes inconsistent subtypes of lymphatic and hematopoietic neoplasms. Matanoski et al. (1989) note that this apparent inconsistency may not be real since there are close interrelationships between many of these tumors. Landrigan (1990) observed that the diagnostic categories are overlapping and are also complicated by changes in nomenclature over time. Therefore in his review of the major 1,3-butadiene epidemiological studies, he states that the observed increases in all subtypes are probably related. Furthermore, 1,3-butadiene exposure has consistently caused cancers of the hematopoietic and lymphatic system in mice (although not in rats), thus lending support to the evidence that the lymphatic system may be a target organ for 1,3-butadiene in humans.

The evidence for an association between butadiene exposure and lymphatic and hematopoietic neoplasms is strengthened by several considerations. First, a strong effect was observed in the routinely exposed workers of the butadiene production plant cohort which presumably has the likeliest exposure to butadiene (and not styrene) of all cohorts studied. Second, the cancers observed in cohorts from SBR plants and the synthetic plants of tire manufacturing facilities are consistent with the findings from the cohort in the butadiene production facility. Third, the case-control study conducted by Matanoski et al. (1987) and the cellular study of Checkoway and Williams (1982) both attribute effects specifically to butadiene, independent of styrene exposure. Fourth, the cancers observed are consistent with those seen in mice in butadiene exposure studies.

In conclusion, the epidemiological studies reported to date give limited evidence for increased incidences of leukemia and/or lympho-hematopoietic neoplasms resulting from exposure to vapors in styrene-butadiene rubber plants or butadiene production plants. Butadiene production workers and those with routine exposure to butadiene have been at higher risk for these cancers. High levels of butadiene during WWII in certain facilities may have contributed to this excess risk, although elevated cancer rates specific to WWII workers have not been conclusively demonstrated. The evidence for elevated rates of stomach and lung cancer is not conclusive. Elevated stomach cancer and lung cancer rates were observed in some studies but not

consistently. Further studies could clarify the relationship between butadiene and these types of cancer.

4.0 QUANTITATIVE RISK ASSESSMENT

Bioassays of 1,3-butadiene (BD) in laboratory animals have provided data that may be used in quantitative assessment of BD's cancer risk to humans. Three bioassays have provided satisfactory data: two in mice and one in rats. The bioassay that provided the best data is the most recent of the two mouse assays (Melnick et al. 1990, or "mouse II"). Epidemiologic data adequate for use in quantitative risk assessment are not available.

4.1 Objectives and Limitations

The chief objective of this section is to derive a numerical value for the incremental cancer risk associated with anticipated lifetime exposures to ambient concentrations of BD. Initially an individual lifetime extra risk will be estimated. A unit risk value associated with lifetime exposure to 1 $\mu g/m^3$ of BD will also be derived.

Several subordinate objectives include:

a) defining a rationale for the mechanism of carcinogenicity;

b) evaluating the potency of BD and its metabolites at anticipated exposures in terms of response per unit dose with an appropriate high to low dose extrapolation model(s);

c) evaluating experimental applied doses in terms of the dose which was probably retained by target tissues and organs (internal dose), the dose which was metabolized to genotoxic metabolites (metabolized dose), and the dose which may have reacted with cellular DNA in selected target tissues (molecular dose);

d) extrapolating animal potency values to human values with appropriate scaling factors and/or other appropriate assumptions.

The limitations of these procedures include uncertainties regarding the applicability of the low dose extrapolation model, particularly where individual tumor site data do not readily fit the model parameters. Further uncertainties may be associated with the possibility of low dose thresholds for toxic metabolites, large intercurrent mortality corrections for excessive early deaths on study, excessively high doses in the mouse study which probably saturated metabolic systems, large interspecies differences in toxicokinetics of BD and metabolites (Laib et al., 1988), and possible cooperative or synergistic effects resulting from concurrent exposure to other air toxicants.

4.2 DOSE-RESPONSE RELATIONSHIP

4.2.1 Mechanism of Oncogenicity

A number of findings noted in Section 3 above bear on the mode of action of BD: the mutagenicity of BD in Salmonella *in vitro* with exogenous metabolic activation; clastogenic/DNA damaging activities in mammals *in vivo*; the direct mutagenic action of the two principal metabolites of BD, namely 1,2-epoxybutene (BMO) and 1,2:3,4-diepoxybutane (DEB), *in vitro*, and their detection in blood of experimental animals exposed to BD; the binding of BD (metabolites) to DNA; and the isolation and identification of DNA adducts.

These findings strongly suggest that genetic toxicity via gene mutation, chromosomal aberrations, and less specific damage to DNA represent at least one `mechanism' by which BD exerts its oncogenic action.

4.2.2 Dose Threshold

There are no data suggesting a practical threshold to the carcinogenic action of BD. In view of the probable mode of action (genetic toxicity) the effective dose for this carcinogen may be so low as to be indistinguishable from zero. While threshold mechanisms based on saturation of detoxification enzyme(s), DNA repair, cytotoxicity, and multiple stages in the carcinogenic process have been proposed, none has been convincingly shown to specifically affect the action of BD. For the purposes of this risk assessment it is assumed that no practical threshold exists.

4.2.3 Evaluation of Biological Data for Quantitative Risk Assessment

Quantitative estimations of human risk are usually based on lifetime animal studies and/or human studies showing excess cancer risk resulting from exposure to the agent in question. In the case of BD, while the data base of human cancer epidemiology, largely from occupational exposures, is indicative of possible carcinogenic impact on the lymphatic system, the data are insufficient for quantitative estimation. Therefore, this risk assessment will rely on lifetime cancer bioassays in experimental animals.

The NTP (1984) mouse inhalation study showed significant early increases in hemangiosarcomas of the heart and malignant lymphomas in both sexes (Huff et al., 1985). The Hazleton (1981) (Owen et al., 1987) rat inhalation study showed Leydig cell tumors, pancreatic exocrine tumors, and Zymbal gland carcinomas in males, and mammary gland carcinomas, thyroid follicular tumors, and Zymbal gland carcinomas in females. Both of these studies will be evaluated in the quantitative risk assessment presented below.

4.2.4 Estimation of Delivered Dose

4.2.4.1 Continuous Internal Dose

Both rodent bioassays employed a repetitive chronic exposure regimen of 6 hours/day x 5 days/week at measured external BD concentrations. In this subsection continuous internal dose estimates are derived for the various treatment levels in terms of mg per kg body weight per day. For the purposes of this risk assessment a 6 hours/day exposure results in a daily dose and is not averaged over 24 hours. The most relevant data currently available to estimate internal dose from applied external BD is that of Bond et al. (1986). These investigators exposed rats and mice to [^{14}C]-butadiene at concentrations ranging from 0.14 μg/L (75 ppb) to 13,000 μg/L (6964 ppm) and measured the quantities of inhaled [^{14}C]-BD equivalents retained after 6 hours exposure. These data are reproduced in Table 4-1 and show that mice retained approximately 5 to 10% of inhaled BD whereas rats retained approximately 1.5 to 3% at the doses tested. To estimate doses in mg/kg-d the higher applied doses ($\log_{10}$ ppm) are linearly regressed against retained BD ($\log_{10}$ μg/kg-d). For the mouse data the following relation was obtained:

Table 4-1 Respiratory Tract Retention of Inhaled Butadiene in Rats and Mice[a]

Exposure[b] Concentration (ppm)	Total Volume Inhaled/Animal (L)[c]		Butadiene Inhaled/Animal (umol)		[^{14}C]Butadiene Equivalents Retained at 6 hr/Animal (umol)[d]		Percentage of Inhaled [^{14}C]Butadiene Equivalents Retained at 6 hr	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
0.075	73 ± 5	14 ± 2	0.2 ± 0.01	0.04 ± 0.002	0.03 ± 0.002	0.006 ± 0.001	17 ± 2[i]	16 ± 1[i]
0.75	90 ± 4	12 ± 2	2 ± 0.1	0.3 ± 0.06	0.13 ± 0.003	0.06 ± 0.01	6 ± 0.3[h]	20 ± 5
7	83 ± 7	12 ± 1[e]	19 ± 2	3 ± 0.4[e]	0.8 ± 0.1	0.4 ± 0.009[e]	4 ± 0.5	20 ± 5[e]
70	70 ± 8[f]	13 ± 2	170 ± 20[f]	35 ± 5	17 ± 2[f]	3.2 ± 0.1	8 ± 1[f]	8 ± 0.7
1000	100 ± 10	12 ± 2	3100 ± 360	440 ± 80	65 ± 5	19 ± 0.5	2.5 ± 0.2[h]	4 ± 0.3
7000	70 ± 15	g	17000 ± 3100	g	240 ± 40	g	1.5 ± 0.1	g

[a]Values are the mean ± SE.
[b]Concentration expressed in ppm were calculated from reported exposures of 0.14, 1.4, 13, 130, 1800 and 13,000 ug/L.
[c]Values corrected for rat and mouse body temperature, barometric pressure, inspired gas temperature, and relative humidity.
[d]Data obtained from radioanalysis of rats and mice which were maintained in plethysmograph tubes during the 6 hour exposure.
[e]Individual values pooled from three separate experiments.
[f]Individual values pooled from two separate experiments.
[g]Mice were not exposed to this concentration of butadiene.
[h]Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's t test.
[i]A linear regression analysis of the log transformed data vs the log of the exposure concentration indicated that there was a significant ($p < 0.001$) concentration-related decrease in the precent retained at 6 hour with increasing exposure concentration.

Source: Bond et al., 1986

$$\log_{10}(\mu g/kg\text{-}d) = 0.68 \log_{10} (ppm) + 2.51, R = 1.00$$

Using this relation the bioassay measured applied doses are converted to internal doses:

627 ppm = 25800 μg/kg-d = 25.8 mg/kg-d
1236 ppm = 41000 μg/kg-d = 41.0 mg/kg-d

These doses were then adjusted from 5 days/week to a continuous (7 days/week) scenario.

$$25.8 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 18.4 \ \mu g/kg\text{-}d$$

$$41.0 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 29.3 \ \mu g/kg\text{-}d$$

Similarly for the rat:

$$\log_{10}(\mu g/kg\text{-}d) = 0.60 \log_{10} (ppm) + 2.22, R = 1.00$$

999 ppm = 10470 μg/kg-d = 10.47 mg/kg-d
7886 ppm = 36150 μg/kg-d = 36.15 mg/kg-d

$$10.47 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 7.5 \text{ mg/kg-d}$$

$$36.15 \text{ mg/kg-d} \times \frac{5 \text{ days}}{7 \text{ days}} = 25.8 \text{ mg/kg-d}$$

These continuous internal doses, as well as the measured experimental doses, will be employed in low dose extrapolation models to assess the BD potencies or slope factors at environmentally relevant exposures for various oncogenic endpoints.

4.2.4.2 Metabolized Dose

Since the internal doses calculated from the data of Bond et al. (1986) are based only on the amount of ^{14}C-radiolabel retained at the end of the 6-hour exposure and do not take into account any butadiene metabolites that were absorbed and exhaled during the exposure period, Hattis and Wasson (1987) have employed a pharmacokinetic modeling approach to estimate internal exposure to the chief metabolite of butadiene, namely BMO. The modeling, based largely on partition coefficients and Michaelis-Menten enzyme kinetics, including many measured parameters for BD, does take several additional factors into account and estimates delivered or metabolized doses for the applied doses used in the cancer bioassay studies. Of course, only BMO is considered and the possible influence of DEB or other epoxide metabolites is not addressed. Also, it is important to point out that the pharmacokinetic modeling is not extended to predict human doses, since no appropriate human pharmacokinetic data on BD were available to serve as a guide. Of course the model can be used to estimate human exposure if one employs various scaled parameters and makes assumptions about BD metabolism and ambient exposure.

The model employed by Hattis and Wasson (1987) was based somewhat on that of Ramsey and Andersen (1984) and Fiserova-Bergerova (1983). The body is divided into a number of compartments representing organs with similar ratios of blood flow to tissue volume. Each compartment is represented as a well mixed pool. Three compartments are considered:

- o Liver and Vessel-Rich Group (LVRG) containing liver, brain, kidney, heart, adrenal, and thyroid tissues as well as additional small viscera,
- o Muscle Group (MG) containing the lean body tissue: muscle, skin, and tongue; and
- o Fat Group (FG) containing perirenal and subcutaneous fat and the bone marrow.

The concentration of BD in blood leaving each compartment is assumed to have reached equilibrium with the concentration of BD in the compartment. The following expression relates the increase or decrease in BD stored in each compartment:

$$\frac{d(BD)}{dt} \text{ (moles/min)} = Q_{tissue} \; [BD]_{art} - \frac{Q_{tissue} \; [BD]_{tissue}}{V_{tissue} \; L_{tissue/blood}} - M$$

where:

Q_{tissue} = rate of blood flow to the compartment in L/min
$[BD]_{art}$ and $[BD]_{tissue}$ = butadiene concentrations in arterial blood and tissue

e.g., $= Q_{tissue} \; [BD]_{art} - \frac{Q_{tissue} \; [BD]_{tissue}}{V_{tissue} \; L_{tissue/blood}} - M$

$Q_{tissue} \; [BD]_{art}$ = input from arterial circulation

V_{tissue} = volume of the compartment
$L_{tissue/blood}$ = equilibrium ratio of tissue and blood butadiene concentrations
$Q_{tissue} \; [BD]_{tissue}/V_{tissue} \; L_{tissue/blood}$ = output via venous blood
M = metabolism

The values for tissue volumes and flows were based on 400 g rats and 28 g mice. The metabolism term which was included only for LVRG is a simple Michaelis-Menten expression:

$$M = V_{max} \; [BD]_{tissue}/(K_m + [BD]_{tissue}).$$

V_{max} is the maximum reaction velocity in moles/min and K_m, the Michaelis constant, is the BD concentration at which the reaction proceeds at half its maximum velocity. This is the key nonlinear relation that makes the model as a whole respond nonlinearly at high external exposures.

A possible weakness of this pharmacokinetic approach was that the various partition coefficients for BD and BMO (octanol/water, blood/air, oil/air, tissue/blood) were not determined experimentally but rather were estimated

based on regression analyses or the use of empirically derived atomic or group fragment constants and structural factors (Lyman et al., 1982).

Hattis & Wasson (1987) employ several values of blood/air partition coefficient and K_m to determine various "best estimate" and "alternate" model predictions of metabolized dose of BD (BMO) for rats and mice. The results of the model runs are summarized in Tables 4-2 and 4-3. These 'best estimate' model simulations were verified using STELLA 2.10 (High Performance Systems, Inc. 1988) run on an Apple Macintosh computer. Model simulations were also carried out for the measured applied doses and these are also given in Table 4-2 and 4-3. The published doses were used to derive comparative estimates of cancer risk.

The risk estimates from this metabolized dose approach for mice fall within the range of risks presented here. The approach was not considered reliable for risk assessment purposes, primarily due to its consideration of only BMO and not DEB or other epoxide metabolites. Furthermore, numerous input values used to derive the estimates were not validated experimentally. As indicated in Section 2.2, accumulation of ^{14}C labeled BD and the rate of elimination of ^{14}C from tissues are not different for rats and mice. And finally, estimates of metabolites in various tissues (as described below) do not provide an explanation of the tumor frequencies in rats and mice. Consequently, cancer risk estimates using this approach were presented for comparative purposes only.

4.2.4.3 Target Tissue Doses

In this subsection the concentrations of the mutagenic metabolites, BMO and DEB, were estimated in various tissues of rodents at doses similar to those employed in the carcinogenicity bioassays. OEHHA staff consider these estimates to be highly uncertain. The data of Bond et al. (1986, 1987) on [^{14}C]-BD absorption, tissue distribution, and metabolite profile in blood were employed. The blood metabolite data of Bond et al. (1986) is reproduced in Table 4-4. In order to estimate tissue concentrations at the bioassay doses (625 or 1250 ppm) or at doses other than those employed in the tissue distribution studies (65 ppm for mouse and 1200 for rat), it was necessary to assume that these were proportional to the uptake of ^{14}C radiolabel into the animal carcass (Table 4-5). Uptake values were estimated graphically for exposure concentrations employed in the cancer bioassays. Distribution data are available for periods of 1, 8, and 65 to 67 hours after a 3.4 hour exposure to [^{14}C]-BD. The results of converting 1 hour data are shown in Tables 4-6 and 4-7.

The first column in the tables gives the tissue distribution of ^{14}C radiolabel in nmol BD equivalents/g tissue. The second column gives an estimate of tissue distribution at the higher bioassay dose based on carcass uptake. The columns to the right give estimates of how this tissue distribution of ^{14}C radiolabel would break down if the relative proportions of metabolites were the same as observed in blood. The table headings in parentheses give the percentage values of each metabolite as found in blood.

The sum of the estimated concentrations of mutagenic metabolites BMO and DEB are given in the right hand column of these tables. Naturally these

Table 4-2 Predictions of Metabolized Dose (BMO) by Pharmacokinetic Modeling of Mouse Inhalation Data (Hattis and Wasson, 1987)

Blood/Air Partition Coefficient	K_m 5 days	Exposure 6h/d	BD Metabolized 5 days		7days	
		ppm	µmol/animal	µmol/kg	µmol/kg	mg/kg-d
Best Estimate Model 0.35	2×10^{-5}	625	28.1	1003	716	38.8
		1250	45.8	1634	1167	63.2
		627	28.2	1007	719	38.9
		1236	45.5	1625	1161	62.8
Alternate Model 0.2552	2×10^{-5}	625	23.8	850	607	32.8
		1250	41.4	1479	1056	57.2
		627	22.2	793	566	30.6
		1236	38.1	1361	972	52.6
EPA, 1985 Continuous Internal		625	13.3	474	339	18.3
		1250	20.1	719	514	27.8

NOTE:

Alveolar Ventilation = 0.0233 L/min.

$V_{max} = 1.87 \times 10^{-7}$ for all runs.

EPA projections of continuous internal doses based on Bond et al. (1986). Net absorption data were 25.7 mg/kg (474 µmol/kg) at 625 ppm and 38.9 mg/kg (719 µmol/kg) at 1250 ppm. Mouse weight of 0.028 kg assumed.

Table 4-3 Predictions of Metabolized Dose (BMO) by Pharmacokinetic Modeling of Rat Inhalation Data (Hattis and Wasson, 1987)

Blood/Air Partition Coefficient	K_m	Exposure 6h/d 5 days	BD Metabolized 5 days		7days	
		ppm	µmol/animal	µmol/kg	µmol/kg	mg/kg-d
Best Estimate Model 0.35	5×10^{-6}	1000	359.6	899	642	34.7
		8000	607.7	1519	1085	58.7
		999	359	898	641	34.7
		7886	603	1508	1077	58.2
Alternate Model 0.2552	1×10^{-5}	1000	274.7	686.8	490	26.5
		8000	347.1	867.8	620	33.5
		999	263	658	470	25.4
		7886	559	1398	998	54.0
EPA, 1985 Continuous Internal		1000	78	195	139	7.5
		8000	274	685	489	26.5

NOTE:

Alveolar Ventilation = 0.15 L/min.

$V_{max} = 1.47 \times 10^{-6}$ for all runs.

EPA projections of continuous internal doses based on Bond et al (1986), net absorption data were 10.5 mg/kg (195 µmol/kg) at 1000 ppm and 37.1 mg/kg (685 µmol/kg) at 8000 ppm. Rat body weight of 0.4 kg assumed.

Table 4-4 Blood Distribution of BD and Metabolites (Bond et al., 1986)[a]

Species	Dose[b] ppm	Metabolites and Parent nmol/ml				
		nonvolatile[c] (conj)	DEB	BMO	BD	CO_2
Rat	70	8	0.1	0.4	0.1	0.6
	1000	51	1.0	4.0	4.0	4.0
Mouse	7	6	0.08	0.7	0.2	0.08
	70	17	0.1	0.9	0.8	0.2
	1000	100	1.0	15.0	3.0	2.0

[a] Metabolites measured 6 hours after exposure.
[b] Concentrations exposed in ppm were calculated from reported exposure of 13, 130 and 1000 μg/L.
[c] Nonvolatile metabolites thought to be largely conjugates (conj) of diol metabolites.

Table 4-5 Incorporation of ^{14}C Radiolabel from ^{14}C-BD into Carcass[a]

Species	Dose[b] ppm	μmol/kg	mg/kg	μmol ^{14}C/carcass
Rat	70	18.8	1.0	3
	1000	167	9.0	13
	7000	860	46.5	35
	(1000)[c]			(14)
Mouse	7	12.8	0.7	0.09
	70	90.5	4.9	0.5
	1000	845	45.7	2.0
	(625)[c]			(1.7)

[a] Radiolabel measured 65 hours after 6 hours exposure.
[b] Concentrations expressed in ppm were calculated from reported exposures of 13, 130, 1000 and 13,000 μg/L.
[c] Values in parentheses were estimated graphically and correspond to 1000 ppm - rat and 625 ppm - mouse bioassay doses.

Table 4-6 Estimated Concentrations of Metabolites in Mouse Tissues After Inhalation Exposure to [^{14}C]BD

Tissue	65 ppm[1]	625 ppm[2]	nmol BD equiv/g tissue				
	Test Dose	Est. Dose	BD(5)[3]	BMO(14)	DEB(2)	CONJ(79)	BMO+DEB
	Total ^{14}C						
Adrenal	76	258	13.0	36.1	5.2	204	41.3
Blood	49	167	8.4	12.3	3.3	132	26.7
Heart	47	160	8.0	22.4	3.2	126	25.6
Kidney	180	612	30.6	85.7	12.2	483	97.9
Liver	120	408	20.4	57.1	8.2	322	65.3
Lung	96	326	16.3	45.6	6.5	258	52.1
Mammary	30	102	5.1	14.3	2.0	80.6	16.3
Pancreas	160	544	272	76.2	10.9	430	87.1
Thyroid	60	204	10.2	28.6	4.1	161	32.7

1 3.6 hour exposure to 65 ppm [^{14}C]BD, values measured at 1 hour after exposure (Bond et al., 1987). Figures are nmol/g tissue.

2 625 ppm estimates based on carcass uptake vs. applied dose (Graphical Interpolation Table 4-5). Figures are nmol/g tissue.

3 Relative metabolite values in percent based on blood values (Table 4-4).

Table 4-7 Estimated Concentrations of Metabolites in Rat Tissues After Inhalation Exposure to [^{14}C]BD

Tissue	1200 ppm[1]	1000 ppm[2]	nmol BD equiv/g tissue				
	Test Dose	Est. Dose	BD(7)[3]	BMO(8)	DEB(2)	CONJ(77)	BMO+DEB
	Total ^{14}C						
Adrenal	210	190	13.0	15.2	3.8	146	19
Blood	124	112	7.8	9.2	2.2	86	11.4
Heart	120	108	7.6	8.6	2.2	83	10.8
Kidney	390	352	24.6	28.2	7.0	271	35.8
Liver	330	269	18.8	21.5	5.4	207	26.9
Lung	200	181	12.7	14.5	3.6	139	18.1
Mammary	140	126	8.8	10.1	2.5	97	12.6
Pancreas	320	289	20.2	23.1	5.8	222	28.9
Thyroid	490	442	30.9	35.4	8.8	340	44.2

1 3.4 hour exposure to 1220 ppm [^{14}C]BD values measured at 1 hour after exposure (Bond et al., 1987). Figures are nmol/g tissue.

2 1000 ppm estimates based on carcass uptake vs. applied dose (Graphical Interpolation Table 4-5). Figures are nmol/g tissue.

3 Relative metabolite values in percent based on blood values (Table 4-4).

figures rest on many assumptions: that carcass ^{14}C concentrations can be used to extrapolate average tissue concentrations with varying dose; that the metabolite pattern of blood reflects that of the various tissues; and that the differences between 3.4 hours exposure and 6 hours exposure are relatively minor. While admittedly crude these estimates may be improved by considering target tissue/blood partition coefficients as calculated by Hattis and Wasson (1987) or Fiserova-Bergerova (1986). Using oil/water partition coefficients estimated by the method of Hansch and Leo (Lyman et al., 1982), the tissue/blood partition coefficients are as shown in Table 4-8. These figures indicate that tissue metabolite patterns are likely to show higher concentrations of BMO and lower concentrations of DEB than blood. BD tissue concentrations would probably have been much higher than blood concentrations. In Table 4-9 are shown the relative proportions of BD and its epoxide metabolites in selected tissues as a result of blood/tissue partitioning.

Since the blood/tissue partition coefficients of the chief toxic epoxide metabolites of BD are close to 1.0 (within a factor of 2), blood patterns of the compounds may give a reasonable estimate of tissue concentrations in most cases. From Tables 4-6 and 4-9 it appears that at 625 ppm applied dose target tissue concentrations of BMO and DEB in the mouse could vary from 3.2 x 0.75 = 2.4 nmol/g for DEB in heart tissue to 45.6 x 9/8 = 51.3 nmol/g for BMO in the lung. In the rat target tissues, at 1000 ppm doses could vary from (5.8 x 0.75 =) 4.35 nmol DEB/g pancreas to (35.4 x 8.5/7 =) 43.0 nmol BMO/g thyroid (Table 4-10). These estimates of concentrations of mutagenic epoxides do not appear unusually high with respect to non-target (e.g., mammary) tissues, with the possible exception of rat thyroid.

The target tissue dose estimates were not considered to be reliable for risk assessment purposes for several reasons. The target tissue dose estimates were qualitative and not quantitative. Tissue concentrations for the bioassay studies had to be considered proportional to those measured in the distribution studies. The tissue dose estimates did not correspond with tumor incidences. For example, the kidney exhibited the greatest concentration of all metabolites in the mouse, but was not a site of increased tumor incidence. Consequently, cancer risk estimates using this approach were presented for comparative purpose only.

4.2.4.4 Molecular Tissue Doses

When B6C3F1 mice or Wistar rats were exposed to 1,3-[1,4-^{14}C] butadiene in a closed system, the mice metabolized the test compound at twice the rate observed in rats based on body weight (Kreiling et al., 1986). Covalent binding of [^{14}C]BD derived radiolabel was observed in both rat and mouse liver DNA at comparable amounts. Covalent binding to mouse-liver nucleoproteins was twice as high as seen in rats (Table 4-11). After exposure of mice to butadiene, 7-(1-hydroxy-3-buten-2-yl)guanine, a product of the reaction of epoxybutene (BMO) with deoxyguanosine and DNA (Citti et al., 1984) was identified in liver DNA (Laib and Kreiling, 1987). The doses applied to the rats and mice (ca. 800 ppm peak) approximate 1 to 2 hours of the low dose bioassay level. For mice, this corresponded to roughly 1,300 times the dose employed for the DNA binding study and resulted in 100% incidence of fatal tumors.

Table 4-8 Estimated Tissue/Blood Partition Coefficients of BD and Epoxide Metabolites

Tissue	Coefficient	BD	BMO	DEB
	Oil/Water (K_{ow})[1]	125.9	27.5	6.02
Lung	Tissue/Blood[2]	1.65	1.20	1.10
Kidney/Vessel Rich Group	Tissue/Blood	5.34	1.22	0.77
Muscle	Tissue/Blood	4.20	1.39	0.77
Liver	Tissue/Blood	5.93	1.74	0.83

NOTE:

[1] Determined by the method of Hansch & Leo: $\log K_{ow} = \Sigma f + \Sigma F$ where f are atomic or group fragment constants and F are structural parameters such as molecular flexibility, unsaturation, halogenation, branching, etc.

For BD: $\log_{10} K_{ow} = 4fC + 10fH\ 1 + (3\text{-}1)Fb + 2f =$
$4(.2) + 10(.23) + 2(-.12) + 2(-.38) = 2.10 \pm 0.1$ SD.
$K_{ow} = 125.9(100\text{-}158)$.

[2] Tissue/blood partition coefficients estimated by the regressions of Fiserova-Bergerova, 1986: $L_{T/B} = a(L_{F/B} + b)$ where the fat/blood coefficient is assumed to be similar to K_{ow}.

Table 4-9 Estimated BD and Epoxide Metabolite Proportions in Selected Tissues

	Rat				Mouse			
	Blood	Lung	Kid/VRG	Muscle	Blood	Lung	Kid/VRG	Muscle
BD	7	12	37	29	2	3	10	8
BMO	7	8	8.5	10	8	9	10	11
DEB	2	2	1.5	1.5	1	1	0.75	0.75

Table 4-10 Estimate of Mutagenic Epoxide Concentrations in Rodent Target Tissues[1]

	RAT		MOUSE	
Metabolite	Pancreas	Thyroid	Heart	Lung
BMO	28.0[2]	43.0	30.8	51.3
DEB	4.4	6.6	2.4	4.9
Total	32.4	49.6	33.2	56.2

1 Based on tissue distribution data for 3.4 hour exposures to [^{14}C]BD measured 1 hour after exposure.

2 Values in nmol BD equivalent/g tissue at interpolated applied doses of 625 ppm (mouse) and 1000 ppm (rat) and adjusted for blood/tissue partition.

Table 4-11 Radioactivity in Nucleoproteins and DNA of Mouse and Rat Liver After Exposure to [1,4 -^{14}C]1,3-Butadiene[a]

Number of Animals		pmol/mg ($\bar{X}$ ± SD)[d]	
RATS[b]	MICE[c]	RATS[e]	MICE[f]
		(a) Early Eluting Nucleoproteins	
4	4 x 6	15.6 ± 2.6	29.7 ± 3.5
		(b) Late Eluting Nucleoproteins	
4	4 x 6	7.6 ± 0.6	13.2 ± 1.7
		(c) Liver DNA	
4	4 x 6	34.9 ± 10.2	30.4 ± 7.2

[a] Source: Kreiling et al., 1986.

[b] Dose of 2.3 mCi/mmol = 0.205 mmol/4 rats/6.4 L air.

[c] Dose = 0.205 mmol/24 mice/6.4 L air.

[d] Covalent Binding; pmol BD equivalents/mg DNA or nucleoproteins.

[e] Uptake = 5.95 x 10^9 DPM/kg

[f] Uptake = 6.15 x 10^9 DPM/kg.

While the total radiolabel content of rat liver DNA is virtually the same as for the mouse, the pattern of metabolites could be quite different due to the more rapid metabolism of BMO in the rat. Conceivably, mouse DNA and nucleoprotein radiolabel could contain adducts from subsequent alkylating metabolites, such as diepoxybutane and 3,4-epoxy-1,2-butanediol. Since the liver is not a major target for BD carcinogenesis in either species, heavily affected target tissues may have greater covalent binding.

Alternatively, as indicated by nucleoprotein binding in the mouse, molecular doses may need to include more than DNA adducts. Also, measurements based on whole tissues may miss sensitive subpopulations of cells with high affinity for adduct formation, proliferation, etc. (Belinsky et al., 1987). Clearly more work needs to be done on the molecular aspects of BD carcinogenesis before one can relate molecular doses in target tissues with tumor incidences seen in those tissues.

Risk estimates were not derived using molecular tissue doses due to the limited amount of information available. Molecular tissue doses are only available for the liver which is not a major tumor site. Furthermore, the molecular tumor dose results do not shed any light on the different sensitivity of mice and rats to BD, since molecular doses were equivalent in the two species. Consequently, this information was presented only to provide a complete perspective of the risk assessment of BD.

4.2.5 Intercurrent Mortality Correction

For animal carcinogenicity studies where the duration of the experiment Le was less than the experimental lifetime of the animal L, the dose response is corrected according to the assumption that the cumulative tumor incidence would be expected to increase by (at least) the third power of age. In this report, the correction was applied to the carcinogenic potency or 95% upper confidence limit of the slope of the dose response relationship, q_1*:

$$q_1^* \text{ animal} = q_1^*(L/Le)^3 = q_1^*(104/Le)^3; \text{ for } Le < L.$$

4.2.6 Models For Low Dose Extrapolation

4.2.6.1 Linearized Multistage Model

In order to extrapolate from high experimental doses in animal studies to low doses of relevance to human environmental exposures, a mathematical extrapolation model was employed. With the type of data available on BD, the model of greatest relevance and flexibility was the linearized multistage. The program employed was GLOBAL 86 (K.S. Crump & Co., Ruston, LA, May 1986) which fits the multistage model to quantal animal tumor incidence data and calculates maximum likelihood estimates and statistical confidence limits for the extra risk over background at a given dose and for the dose corresponding to a given value of extra risk. The multistage model is given by

$$P(d) = 1 - \exp - (q_0 + q_1 d + q_2 d^2 +, \ldots, + q_k d^k),$$

$q_i \geq 0$, $i = 1, \ldots k$, where d is the dose, P(d) is the lifetime probability of a health effect at a dose d, and k and q_i are parameters. The extra risk is defined as

$$[P(d) - P(0)]/[1 - P(0)].$$

The parameter q_0 represents the background lifetime incidence of tumor(s). The parameter q_1 is the maximum likelihood estimate of the slope or cancer potency, and q_1* the 95% upper confidence limit of that estimate. The GLOBAL 86 algorithm selects the number of stages in the multistage model k, $1 \leq k \leq 6$, that minimizes q_1* subject to the condition that the model fit is adequate ($p \geq 0.01$). When the dose is expressed in units mg/kg-d, the parameters q_1 and q_1* are given in units $(mg/kg\text{-}d)^{-1}$. At low environmental doses, the risk is equal to q_1*d. A variant of the linearized multistage model, Weibull 82 (K.S. Crump and Co., 1985) was also employed. This program fits a model which is multistage in dose and Weibull in time to time-to-tumor dose response data:

$$P(d,t) = 1 - \exp - [(q_0 + q_1 d + \ldots + q_k d^k)(t-t_0)^j]$$

where $q_i \geq 0$, $i = 0, \ldots, k$, $j \geq 1$, t, the time t_0 tumor is $\geq t_0$, the latency period. The value of t_0 can be estimated by the program or set to zero. The value of k, which determines the degree of the polynominal, is chosen by the user. In this case the extra risk is defined as:

$$[P(d,t) - P(0,t)]/[1-P(0,t)].$$

Additional description of the multistage-Weibull model and its comparison with other models can be found in Krewski et al. (1983).

4.2.6.2 Other Models

The BD animal carcinogenicity data and various assay dose estimates were also evaluated using the Tox-Risk program of Clement Associates (Ruston, LA). This program has the ability to fit 10 different models to quantal animal tumor incidence vs. dose data. In addition to the multistage model, the Weibull, Mantel-Bryan and Log-Normal models were fit to the BD experimental data. In general, the multistage model (where k = number of dose groups -1) or the Global 86 (above) gave better fits to the data than the other models did.

4.2.7 Interspecies Extrapolation

For purposes of cancer risk assessment, it was assumed that mg/unit surface area/day is an equivalent dose between species. Since surface area closely correlates with the 2/3 power of body weight (W), the dose units of $mg/W^{2/3}/d$ are also considered equivalent. BD is slightly soluble in water and can be considered a partially soluble gas. The dose in mg/d = m is proportional to O_2 consumption which in turn is proportional to $W^{2/3}$ and to the solubility of BD in body fluids indicated by an absorption fraction r. Expressing O_2 consumption as $kW^{2/3}$, where k is a constant independent of species, it follows that:

$$m = kW^{2/3}vr,$$

where v is the BD concentration in air (mg/m^3), or

$$\frac{m}{W^{2/3}} = kvr.$$

It is assumed that r, the absorbed fraction, is the same for all species. Therefore, for one method of extrapolation, OEHHA staff followed EPA practice (EPA, 1985) and assumed that a certain exposure concentration in ppm or mg/m^3 in experimental animals was equivalent to the same exposure concentration in humans (Anderson et al., 1983).

To convert doses between mg/kg-d internal and ppm external, the following factors were used: (1) a BD conversion factor of 2.21 mg/m^3/ppm at 25°C, 760 mm Hg; (2) the absorption fraction at low concentrations from Bond et al. (1986) of 0.16 for mice and 0.17 for rats; (3) the ventilation rate in cm^3/min. estimated from the relation of Guyton (1947), i.e., rats, 2.10 (body weight in grams)$^{3/4}$; mice, 2.54 (body weight in grams)$^{3/4}$. The resulting values were then converted to m^3/d. For a 700 g rat this conversion would appear as follows (EPA, 1985):

$$2.21\ mg/m^3/ppm \times 0.17 \times 0.41\ m^3/d \times 1/0.7\ kg = 0.22\ mg/kg\text{-}d/ppm.$$

The use of air concentration in ppm or mg/m^3 as an interspecies equivalent dose unit results in potency values about 2.5 fold lower than those based on mg/m^2 surface area (see 4.2.9 below).

4.2.8 Quantitative Estimation

4.2.8.1 NTP (1984) Mouse Inhalation Study (Mouse I)

Presented in Table 4-12 is a sample calculation of a cancer potency estimate for BD based on the mouse total significant tumor incidence data and estimated continuous internal doses.

The quantal data and doses were entered into Global 86 and the q_1 and q_1* values obtained. Intercurrent mortality corrections and the external low dose conversions are then performed to give potency estimates in units of $(ppm)^{-1}$.

Cancer potency estimates for mice using the three measures of dose and individual target tissue lesions as well as total tumor incidence are given in Table 4-13. Individual target tissue potency values ranged from 6.9 x 10^{-4} $(ppm)^{-1}$ for liver neoplasms in females using applied dose to 2.0 x 10^{-1} $(ppm)^{-1}$ for malignant lymphoma in males using continuous internal dose. Usually metabolized dose potencies were midrange between potencies estimated with applied and those estimated with internal doses. When total significant tumor incidence was input, the cancer potency values ranged from 0.016 to 0.32 $(ppm)^{-1}$ for males and from 0.01 to 0.17 $(ppm)^{-1}$ for female mice. These figures compare well with the sums of the individual potencies over tissues of 0.015 to 0.245 $(ppm)^{-1}$ and 0.009 to 0.19 $(ppm)^{-1}$ respectively. Cancer potency estimates from the Weibull 82 time to tumor analysis (Table 4-14) were quite similar to the Global 86 analysis. With total tumors and continuous internal doses, the potency values were 0.292 $(ppm)^{-1}$ for males and 0.162 $(ppm)^{-1}$ for females. For metabolized doses the values were 0.138 and 0.075 $(ppm)^{-1}$ respectively. For these analyses k = 2 and t_0 = 0. In order to compare the ppm^{-1} potency values in Table 4-13 with surface area scaled values in Table 4-15 the values should be multiplied by 2.5 (e.g., 0.32 ppm^{-1} unscaled x 2.5 = 0.80 ppm^{-1} scaled).

Table 4-12 Quantitative Estimation of Butadiene Potency With Mouse Internal Doses.

Nominal Exposure (ppm)	Males: Internal Dose (mg/kg-d)[a]	Males: Tumor Incidence[b]	Females: Internal Dose (mg/kg-d)	Females: Tumor Incidence
0	0	2/50 (4%)	0	4/48 (8%)
625	18.4	43/49 (88%)	18.4	31/48 (65%)
1250	29.0	40/45 (89%)	29.0	45/49 (92%)

[a] Continuous internal dose = daily internal dose x 5/7.
[b] Number of animals with at least one statistically significant tumor/total examined, eliminating animals that died prior to 20 weeks.

Initial Maximum Likelihood Estimates:

Males	Females
$q_0 = 0.042$	$q_0 = 0.087$
$q_1 = 0.091$ (mg/kg-d)$^{-1}$	$q_1 = 0$
	$q_2 = 2.8 \times 10^{-3}$ (mg/kg-d)$^{-2}$

Initial Estimates of 95% Upper Limit:

$q_1^* = 0.114$ (mg/kg-d)$^{-1}$	$q_1^* = 0.066$ (mg/kg-d)$^{-1}$

Intercurrent Mortality Correction:

$(104/60)^3 = 5.21$	$(104/61)^3 = 4.96$

Final Estimate of q_1^* (animal):

0.59 (mg/kg-d)$^{-1}$	0.32 (mg/kg-d)$^{-1}$

Final Estimate of q_1^* in (ppm)$^{-1}$ = q_1^* (human):

2.21 mg/m^3/ppm x 0.16 x 0.053 m^3/d x 1/0.035 kg = 0.54 mg/kg-d/ppm

$q_1^* = 0.32$ (ppm)$^{-1}$	$q_1^* = 0.17$ (ppm)$^{-1}$

Since human low external/internal dose conversion is 9.09 ppm/mg/kg-d,

$q_1^* = 2.91$ (mg/kg-d)$^{-1}$	$q_1^* = 1.58$ (mg/kg-d)$^{-1}$

Table 4-13 Human Cancer Potency Estimates $(ppm)^{-1}$ for Butadiene From Mouse Bioassay Data

Tissue/Lesion	Sex	Experimental Dose q_1^*		Internal Dose q_1^{*b}		Metabolized Dose q_1^{*b}	
		$(ppm)^{-1}$ [a]	$(ppm)^{-1}$ [b]	$(ppm)^{-1}$ [c]	$(mg/kg\text{-}d)^{-1}$ [d]	$(ppm)^{-1}$ [c]	$(mg/kg\text{-}d)^{-1}$ [d]
Heart/hemangiosarcoma	M	9.3×10^{-4}	4.8×10^{-3}	0.073	0.167	0.042	0.078
	F	5.2×10^{-4}	2.5×10^{-3}	0.044	0.099	0.025	0.047
Hematopoietic system malignant lymphoma	M	1.0×10^{-3}	5.2×10^{-3}	0.089	0.203	0.051	0.095
	F	3.2×10^{-4}	1.6×10^{-3}	0.029	0.065	0.016	0.030
Lung/alveolar and bronchiolar adenomma and carcinoma	M	4.6×10^{-4}	2.4×10^{-3}	0.041	0.094	0.024	0.044
	F	5.8×10^{-4}	2.9×10^{-3}	0.046	0.104	0.207	0.050
Mammary/acinar cell carcinoma	F	1.5×10^{-4}	7.4×10^{-4}	0.012	0.028	7.0×10^{-3}	0.013
Ovary/granulosa cell neoplasm	F	3.5×10^{-4}	1.7×10^{-3}	0.028	0.064	0.017	0.031
Forestomach/papilloma and carcinoma	M	5.4×10^{-4}	2.8×10^{-3}	0.041	0.094	0.025	0.046
	F	2.8×10^{-4}	1.4×10^{-3}	0.024	0.055	0.014	0.026
Liver/adenoma and carcinoma	F	1.4×10^{-4}	6.9×10^{-4}	0.012	0.027	7.1×10^{-3}	0.013
All significant tumors (program input)	M	3.0×10^{-3}	0.0156	0.32	0.59	0.150	0.278
	F	2.1×10^{-3}	0.0104	0.18	0.33	0.087	0.161
Sum of individual sites	M	2.9×10^{-3}	0.0152	0.245	0.557	0.142	0.263
	F	1.9×10^{-3}	9.3×10^{-3}	0.194	0.441	0.113	0.210

NOTE: [a]Initial estimate of 95% upper bound on low exposure potency q_1^*.

[b]Final Estimates of q_1^* corrected for length of study Le in weeks by a factor $(104/Le)^3$, i.e. 5.21 for male mice, 4.96 for female mice.

[c]Animal/Human equivalent potencies using external low dose conversion factor of 0.54 mg/kg-d/ppm for the mouse.

[d]Mouse potency estimates derived from low dose extrapolations.

Table 4-14 Weibull Time to Tumor Analysis of NTP 1984 Mouse Data for All Significant Tumors Related to Butadiene Exposure

Sex	Dose	Dose for 10^{-6} Risk (lower bound)	Risk for Unit Dose (upper bound)	$(L/Le)^3$ [a]	q_1^* $(ppm)^{-1}$ [b]
M [c,d]	Continuous[e] Internal	1.1×10^{-5}	0.102	0.53	0.292[c]
M	Metabolized[f] 0, 38.8, 63.2 mg/kg-d	2.3×10^{-5}	4.9×10^{-2}	0.256	0.138
F [c,d]	Continuous[e] Internal	1.9×10^{-5}	0.06	0.30	0.160
F	Metabolized[f]	4.1×10^{-1}	0.028	0.14	0.075

NOTES:

[a] Intercurrent mortality correction: Males $(104/60)^3 = 5.21$; Females $(104/61)^3 = 4.96$.

[b] Internal dose to low dose external conversion = 0.54 mg/kg/d/ppm.

[c] Females include 1 missexed male and males exclude 1 missexed animal.

[d] Males-number of animals evaluated at control, low, high doses: 48,47,50; females: 50,46,45.

[e] 0, 18.4 and 29.3 mg/kg-d continuous internal doses.

[f] 0, 38.8, 63.2 mg/kg-d metabolized doses.

Table 4-15 Human Cancer Potency Estimates in $(ppm)^{-1}$ and $(mg/kg\text{-}d)^{-1}$ For Butadiene From Mouse II Bioassay Data (Melnick et al., 1990)

Tissue/ Lesion	Sex	Experimental Dose q_1^*				Internal Dose q_1^*				Metabolized Dose q_1^*			
		$(mg/kg\text{-}d)^{-1a}$	$(mg/kg\text{-}d)^{-1b}$	$(ppm)^{-1c}$	$(ppm)^{-1d}$	$(mg/kg\text{-}d)^{-1a}$	$(mg/kg\text{-}d)^{-1b}$	$(ppm)^{-1c}$	$(ppm)^{-1d}$	$(mg/kg\text{-}d)^{-1a}$	$(mg/kg\text{-}d)^{-1b}$	$(ppm)^{-1c}$	$(ppm)^{-1d}$
Heart/ hemangio-sarcoma	M	5.1×10^{-3}	0.064	2.8×10^{-3}	7.0×10^{-3}	0.067	0.84	0.04	0.092	0.037	0.47	0.020	0.052
	F	1.2×10^{-3} f	0.015	6.5×10^{-4}	1.6×10^{-3}	0.013	0.16	7.0×10^{-3}	1.4×10^{-3}	6.4×10^{-3} e	0.08	3.5×10^{-3}	$8.8 \times 10^{-}$
Hematopoietic system/ All malignant lymphomas	M	4.1×10^{-3}	0.052	2.2×10^{-3}	5.7×10^{-3}	0.034	0.43	8.1×10^{-3}	0.047	0.025	0.32	0.014	0.035
	F	5.8×10^{-3}	0.073	3.1×10^{-3}	8.0×10^{-3}	0.093	1.2	0.05	0.13	0.044	0.55	0.024	0.060
Lymphocytic lymphomas	M	1.5×10^{-3}	0.019	8.1×10^{-4}	2.1×10^{-3}	0.014	0.88	7.6×10^{-3}	0.097	7.0×10^{-3}	0.09	3.8×10^{-3}	9.9×10^{-3}
	F	4.5×10^{-3}	0.057	2.4×10^{-3}	6.3×10^{-3}	0.047	0.59	0.025	0.065	0.032	0.40	0.017	0.044
Lung/alveolar and bronchioler adenoma and carcinoma	M	0.017	0.21	9.2×10^{-3}	0.023	0.16	2.0	0.086	0.22	0.125 e	1.6	0.068	0.18
	F	0.033	0.42	0.018	0.046	0.27	3.4	0.15	0.37	0.136 e	1.7	0.073	0.19
Ovary/ granulosa cell neoplasm	F	5.2×10^{-3} f	0.066	2.8×10^{-3}	7.3×10^{-3}	0.018 e	0.23	9.7×10^{-3}	0.025	0.034	0.43	0.018	0.047
Forestomach/ papilloma and carcinoma	M	4.0×10^{-3}	0.050	2.2×10^{-3}	5.5×10^{-3}	0.026	0.33	0.014	0.036	0.029	0.36	0.016	.040
	F	4.2×10^{-3}	0.053	2.3×10^{-3}	5.8×10^{-3}	0.040	0.50	0.022	0.055	0.030	0.38	0.016	.042
Sum of individual sites	M	0.030	0.38	0.016	0.042	0.29	3.6	0.16	0.40	0.216	2.7	0.12	0.30
	F	0.049	0.62	0.026	0.068	0.43	5.4	0.23	0.59	0.250	3.2	0.14	0.35

NOTE: a q_1^* program output mouse - Experimental Dose - TOX RISK v.1; Internal Dose and Metabolized Dose - GLOBAL 86
b q_1^* human, via mouse/human surface area scaling $(70/0.035)^{1/3} = 12.6$
c q_1^* (ppm)-1 via mouse low dose external conversion factor 0.54 mg/kg-d/ppm (from a)
d q_1^* (ppm)-1 via human low dose external conversion factor 0.11 mg/kg-d/ppm (from b)
e high dose group(s) deleted by GLOBAL 86 to achieve p-value of $\geq$ 0.01 for chi-square goodness of fit statistic
f high dose group(s) deleted to obtain p-value of $\geq$ 0.01 for chi-square goodness of fit statistic

4.2.8.2 Melnick et al. (1990) Mouse Inhalation Study (Mouse II)
Cancer potency estimates for the mouse II study using three measures of dose (experimental, continuous internal, and metabolized) and two mouse/human equivalent dose units (ppm in air, $mg/m^2/day$) are presented in Table 4-15.

The tumor incidence data are located in Table 3-2 and Tables 1, 2, 4, and 5 of Melnick et al. (1990) (see Appendix). The continuous internal doses for the study doses, calculated as described above were: 0; 0.8; 1.75; 3.84; 8.40; and 18.4 mg/kg-d. The metabolized doses, obtained using the PBPK model of Hattis and Wasson (Best Value: Blood/Air = 0.35, alveolar ventilation = 0.023 L min^{-1}, V_{max} = 1.87 x 10^{-7} mols min^{-1}, Km = 2 x 10^{-5}M), were: 0; 0.62; 1.97; 6.12; 17.8; and 54.3 mg/kg-d. In Table 4-15 there are four potency estimates (a through d) under each dose measure heading. The first value (a) represents the unscaled potency value in $(mg/kg\text{-}d)^{-1}$. In the case of the applied or experimental dose this value was obtained by inputting quantal tumor incidence data as a function of experimental dose in ppm into the Tox Risk multistage program (Tox-Risk v3 KS Crump Division Clement International Corp, Ruston, LA). The animal to human conversion method was mg/kg body weight/day. In the cases of continuous internal and metabolized dose, the Global 86 program was used to obtain the respective q_1* values in column (a). The values given in column (b) represent human potency values for each dose measure assuming that the intra-species equivalent dose is mg/m^2 body surface area/day (ie., dose conversion factor = (body weight human/body weight animal)$^{1/3}$). The value given in column (c) assumes that the interspecies equivalent dose is ppm in air and the animal potency in $(mg/kg\text{-}d)^{-1}$ is converted into $(ppm)^{-1}$ units using the mouse conversion factor of 0.54 mg/kg-d/ppm. This is the same approach taken previously in Mouse I (Table 4-13) and by EPA (1985). The fourth column (d) is a separate estimate of human potency in ppm^{-1} obtained from the surface area scaled value in (b) using the human conversion factor of 0.11 mg/kg-d/ppm. Individual target tissue potencies ranged from 6.0 x 10^{-4} ppm^{-1} for heart hemangiosarcomas (experimental dose unscaled) in female mice to 0.37 ppm^{-1} (3.7 x 10^{-4} ppb^{-1}) for alveolar and bronchiolar neoplasms in female lung (continuous internal dose, scaled). Surface area scaled potency values in $(ppm)^{-1}$ were approximately 2.5 fold greater than unscaled values. Potency values obtained using the metabolized dose measure were approximately one-half to three-quarters those obtained with continuous internal dose, whereas those obtained with the experimental dose were about 1/10 those with internal dose.

4.2.8.3 Hazleton (1981, 1987) Rat Inhalation Study
Cancer potency estimates for rats using the three measures of dose and individual target tissue lesions as well as for total tumor incidence are given in Table 4-16. In nearly all cases the tumor incidence figures of EPA (1985) were used with a few minor revisions (Owen et al., 1987). Individual cancer potency values ranged from 5.3 x $10^{-6}(ppm)^{-1}$ for male Zymbal gland neoplasms using applied doses to 1.7 x $10^{-3}(ppm)^{-1}$ for mammary carcinomas using internal doses. Potencies based on metabolized doses were usually intermediate in value compared with experimental or internal doses. When total significant tumor incidence was input, the cancer potency values ranged from 3.6 x $10^{-5}(ppm)^{-1}$ to 1.5 x $10^{-3}(ppm)^{-1}$ for males, and from 9.0 x $10^{-5}(ppm)^{-1}$ to $0.021(ppm)^{-1}$ for females. These figures compare well with the sums of the individual potencies over tissues of 8.9 x $10^{-5}(ppm)^{-1}$

to $1.8 \times 10^{-3}(ppm)^{-1}$ and $1.9 \times 10^{-4}(ppm)^{-1}$ to $4.4 \times 10^{-3}(ppm)^{-1}$ respectively. Cancer potency estimates from the WEIBULL 82 time to tumor analysis (Table 4-16) were in general similar to the GLOBAL 86 analysis. The analysis of mammary adenomas and carcinomas gave the highest potencies: $3.6 \times 10^{-2}(ppm)^{-1}$ by GLOBAL 86 and $1.4 \times 10^{-2}(ppm)^{-1}$ by WEIBULL 82, but these tumors also showed a high incidence in unexposed control rats.

For the purpose of this risk assessment, following the lead of EPA (1985), cancer potency estimates for the rat data will be based on total significant tumor incidence less mammary fibroadenomas and uterine tumors rather than on a single target tissue or organ. The total incidence is the number of animals bearing one or more of the primary lesions in a given dose group divided by the number of animals examined that could have had the tumors in question. For a multi-target carcinogen such as butadiene it is believed that this approach gives a better estimate of potential cancer risk.

From Table 4-16 the best potency value from the rat data would be 3.5×10^{-3} $(ppm)^{-1}$ (3.5×10^{-6} ppb^{-1})based on the continuous internal dose. A comparable value based on an interspecies equivalent dose of $\mu g/m^2$ surface area/d would be 9.8×10^{-3} $(ppm)^{-1}$ (9.8×10^{-6} ppb^{-1}).

4.2.9 Conclusions

Of the various estimates of human cancer potency for 1,3 butadiene given in Tables 4-13 to 4-17, those based on the incidence of multiple significant cancers in female rats or individual sites in mice range from 6.5×10^{-4} $(ppm)^{-1}$ (6.5×10^{-7} ppb^{-1}) to 0.80 $(ppm)^{-1}$ (8.0×10^{-4} ppb^{-1}) with different estimates of the dose of BD. In view of our limited understanding of the toxicokinetics of BD and its metabolites and the lack of any direct human metabolic data on butadiene, it is not possible at present to rely on the current pharmacokinetic estimates of metabolized dose for estimating human health risks. The pharmacokinetic dose approach is preliminary and does not explain tumor frequencies in target tissues or species differences. For key data sets, the multistage model gave relatively poor fits to the pharmacokinetic doses. Unadjusted applied dose estimates may underestimate risk, as the animal exposure regimen resembled occupational exposures more closely than continuous ambient environmental exposures. For comparison purposes, cancer potency values obtained using a variety of dose measures have been presented here.

Since there are large differences in potency values between rats and mice that are not explained by differences in pharmacokinetics and tissue distributions of metabolites, it is still an open question as to which experimental animal is a better indicator of human risk. However, the most detailed evaluations of the carcinogenicity of BD have been conducted in the mouse. For the purpose of risk assessment, the quality of the mouse II bioassay data is superior to that of the rat data. The primary reasons for this conclusion are: 1) the use of the most relevant dose levels in the mouse II study; 2) the use of five dose levels, compared to two in the rat study; 3) the presence of two mouse studies with similar findings; 4) the consistency in sites of carcinogenicity between the two mouse studies; 5) the availability of the mouse data in greater detail, allowing in-depth analysis; 6) the fact that the rat study has not been replicated; and 7)

Table 4-16 Human Cancer Potency Estimates (ppm^{-1}) for Butadiene from Rat Inhalation Study (Hazleton 1981, 1987)

Tissue/Lesion	Sex	Experimental Dose q_1*	nternal Dose q_1*		Metabolized Dose q_1*[b]	
		$(ppm)^{-1}$	$(ppm)^{-1}$[c]	$(mg/kg\text{-}d)^{-1}$[d]	$(ppm)^{-1}$[c]	$(mg/kg\text{-}d)^{-1}$[d]
Mammary/carcinoma	F	1.9×10^{-4}	1.7×10^{-3}	7.9×10^{-3}	4.8×10^{-4}	2.2×10^{-3}
Thyroid follicular cell/ adenoma and carcinoma	F	2.6×10^{-5}	1.5×10^{-3}	7.0×10^{-3}	5.1×10^{-4}	2.3×10^{-3}
Uterus/cervical stromal sarcoma	F	1.1×10^{-5}	8.4×10^{-4}	3.8×10^{-3}	3.2×10^{-4}	1.5×10^{-3}
Testis/Leydig cell adenoma and carcinoma	M	2.0×10^{-5}	1.2×10^{-3}	5.4×10^{-3}	4.0×10^{-4}	1.8×10^{-3}
Pancreas/exocrine adenoma	M	1.6×10^{-5}	2.6×10^{-4}	1.2×10^{-3}	1.4×10^{-4}	6.2×10^{-4}
Zymbal Gland/ adenoma and carcinoma	M	5.3×10^{-6}	3.5×10^{-4}	1.6×10^{-3}	1.3×10^{-4}	5.9×10^{-4}
	F	8.4×10^{-6}	4.0×10^{-4}	1.8×10^{-3}	9.2×10^{-5}	4.2×10^{-4}
TOTAL SIGNIFICANT TUMORS	M[a]	8.4×10^{-5}	1.8×10^{-3}	8.4×10^{-3}	3.5×10^{-4}	1.6×10^{-3}
	F[b]	9.9×10^{-5}	3.5×10^{-3}	1.6×10^{-2}	6.6×10^{-4}	3.0×10^{-3}
SUM	M[a]	4.1×10^{-5}	1.8×10^{-3}	8.2×10^{-3}	6.6×10^{-4}	3.0×10^{-3}
	F[b]	2.2×10^{-4}	3.6×10^{-3}	1.6×10^{-2}	1.1×10^{-3}	4.9×10^{-3}

NOTE: [a]includes pancreatic, Zymbal gland and Leydig cell tumors.
[b]includes mammary carcinoma thyroid and Zymbal gland tumors.
[c]animal/human equivalent potencies using external low dose conversion factor of 0.22 mg/kg-d/ppm for the rat.
[d]rat potency estimates derived from low dose extrapolations.

TABLE 4-17 WEIBULL TIME TO TUMOR ANALYSES OF HAZLETON 1981 RAT DATA

SEX,N	DOSE	SITE	95% LCL DOSE FOR 10-6 Risk	95% UCL Potency $(mg/kg\text{-}d)^{-1}$	$(ppm)^{-1}$
M 106,102,107[a]	Continuous Internal 0,7.5,25.8 mg/kg-d	All[b] SIGNIF	$1.1x10^{-4}$	$9.5x10^{-3}$	$2.1x10^{-3}$
F 110,99,109	Continuous Internal 0,7.5,25.8 mg/kg-d	All[b] SIGNIF	$4.5x10^{-5}$	$2.2x10^{-2}$	$5.0x10^{-3}$
M 96,102,97 93,99,89[c]	Continuous Internal 0,7.5.25.8 mg/kg-d	All[b] SIGNIF	$1.1x10^{-4}$ $1.1x10^{-4}$	$9.5x10^{-3}$ $9.5x10^{-3}$	$2.1x10^{-3}$ $2.1x10^{-3}$
F 100,99,99 99,97,98[c]	Continuous Internal 0,7.5,25.8 mg/kg-d	ALL[b] SIGNIF	$4.7x10^{-5}$ $4.7x10^{-5}$	$2.2x10^{-2}$ $2.2x10^{-2}$	$5.0x10^{-3}$ $4.8x10^{-3}$
F 110,99,109[a]	Continuous Internal 0,7.5,25.8 mg/kg-d	ALL[c] Incl. Uterine Sarcoma	$3.8x10^{-5}$	$2.7x10^{-2}$	$5.9x10^{-3}$
F 100,99,99 99,97,98[c]	Continuous Internal 0,7.5,25.8 mg/kg-d	Mammary Benign and Malignant	$1.9x10^{-5}$ $1.9x10^{-5}$	$6.4x10^{-2}$ $6.4x10^{-2}$	$1.4x10^{-2}$ $1.4x10^{-2}$
M 96,102,97	Metabolized 0,34.7,58.7 mg/kg-d	ALL[c] SIGNIF	$4.2x10^{-4}$	$2.4x10^{-3}$	$5.3x10^{-4}$
F 100,99,99	Metabolized 0,34.7,58.7 mg/kg-d	ALL[c] SIGNIF	$1.4x10^{-4}$	$6.6x10^{-3}$	$1.4x10^{-3}$
F 100,99,99	Metabolized 0,34.7,58.7 mg/kg-d	Mammary Benign and Malignant	$4.4x10^{-4}$	$2.8x10^{-2}$	$6.0x10^{-3}$

a Analysis includes animals from 52 week interim sacrifice.

b All significant tumors; male - pancreatic exocrine adenoma, Zymbal gland adenoma and carcinoma, and Leydig cell tumors; female - mammary carcinoma, thyroid follicular adenoma and carcinoma, and Zymbal gland adenoma and carcinoma.

c Includes animals surviving: 54 weeks or longer (first mammary tumor); 66 weeks or longer (first significant male tumor).

suggestions from limited epidemiological observations that BD exposure may be associated in humans with lymphatic and hematopoetic cancers, effects that were seen in mice. Thus, the staff of OEHHA conclude that the best data for use in risk assessment are the mouse II bioassay data of Melnick et al. (1990).

Among the mouse continuous dose estimates, those based on an interspecies equivalent dose unit of mg/m^2 surface area/day are considered to be the most accurate. The data of Bond et al. (1986) indicate that the basis of the continuous dose measure, namely the ^{14}C BD equivalents absorbed and retained at 6 hours, appear to scale with body surface area (Rat/Mouse = 0.60 ± 0.26 (N = 5)) over 3 orders of magnitude. The corresponding values for body weight and ppm in air, using conversion factors of 0.54/mg/kg-d/ppm for mouse and 0.22 mg/kg-d/ppm for rat, were 0.25 ± 0.11 and 0.54 ± 0.23 respectively.

Assuming that the continuous internal, surface area scaled dose is the best measure currently available, the range of human potencies or slope factors would be 9.8×10^{-6} $(ppb)^{-1}$ to 8.0×10^{-4} $(ppb)^{-1}$ [9.8×10^{-3} $(ppm)^{-1}$ to 0.80 $(ppm)^{-1}$]. The single best estimate is 3.7×10^{-4} $(ppb)^{-1}$ [0.37 $(ppm)^{-1}$] based on lung tumors in female mice. These values can be compared with the current EPA mouse-based geometric mean value of 6.4×10^{-4} $(ppb)^{-1}$. This latter figure is based on multiple significant cancers in mice, a 54% absorption fraction from early unpublished data of the study of Bond et al. (1986), and continuous internal doses of the Mouse I bioassay (NTP, 1984). The unit risk values from the current analyses range from 4.4×10^{-6} to 1.7×10^{-4} $(\mu g/m^3)^{-1}$ and the lifetime BD exposure level associated with an extra risk of cancer of 1 in 10^6 ranges from 6.0×10^{-3} to 0.23 $\mu g/m^3$. These key values are summarized in Table 4-18.

Table 4-18 Human Cancer Potency And Risk Estimates Based on Rodent Data

Measure	Units	Mouse (Melnick et al., 1990)	Rat (Hazleton, 1981)	EPA[a] (NTP, 1984)
Cancer Potency	$(ppm)^{-1}$	0.37	9.8×10^{-3}	0.64
Cancer Potency	$(mg/kg\text{-}d)^{-1}$	3.4	0.42	1.8
Unit Risk	$(\mu g/m^3)^{-1}$	1.7×10^{-4}	4.4×10^{-6}	2.9×10^{-4}
Dose For a 10^{-6} Risk	$\mu g/m^3$	6.0×10^{-3}	0.23	3.5×10^{-3}

[a]EPA (1985) figures based on geometric mean of male and female mouse total significant tumor incidence and a continuous internal dose calculation using absorption fraction of 54%.

5.0 RISK CHARACTERIZATION

5.1 Population Burden

As reported in Part A of this document, the California Air Resources Board has found an overall statewide population-weighted average ambient butadiene concentration of 0.37 ppb. Air basin-specific population-weighted mean concentrations ranged from 0.22 to 0.44 ppb.

Assuming a range of potency values from a rat-based low value of 9.8×10^{-6} $(ppb)^{-1}$ to a mouse-based best value of 3.7×10^{-4} $(ppb)^{-1}$, community exposure to 1 ppb ambient BD could be associated with up to 10 (9.8 rounded) to 370 additional cancers per million individuals exposed for their lifetimes. Acutal exposures in California are generally lower than 1 ppb, however. Using the air basin-specific average BD concentrations observed would yield lower figures, namely up to 2 to 160 additional cancers per million exposed.

5.2 Hot Spots

Since an important source of BD emissions appears to be equipment leaks in industrial plants using or producing BD, the potential for hot spots of community exposure in the vicinity of these facilities exists. The EPA has estimated lifetime extra cancer risks for individuals living near two butadiene production plants in California. A facility in southern California, which emits 48,500 kg BD/yr, would have a lifetime cancer risk at the border of the facility of roughly 10^{-3}. A facility in northern California, which emits 12,600 kg BD/yr, would have a lifetime cancer risk at its fenceline of roughly 10^{-4}. The estimates are based on EPA dispersion modeling and other typical assumptions. The actual risks could be lower (sources: California Air Resources Board, Stationary Source Division and EPA Office of Air Quality Planning and Standards).

5.3 Comparison With Epidemiology

EPA (1985) compared the risk estimates based on the mouse study data with the findings in the epidemiology studies of Meinhardt et al. (1982), Matanoski et al. (1982), and McMichael et al. (1976). They used the estimated exposure of 13.5 ppm from Plant B in Meinhardt et al. to derive a continuous lifetime dose of 0.64 ppm from an average of 10.74 working years. They concluded that there was no evidence that unit risk extrapolation from animal to human was unreasonable or that it seriously over-predicted cancer risk.

A similar comparison can be made between the risk estimates derived in the preceding section and the more recent epidemiological reports of Downs et al. (1987) and Matanoski and Schwartz (1987). Although occupational exposure estimates are not given by these authors, values of 1 and 10 ppm based on the earlier studies seem reasonable (Fajen et al., 1986). These represent 10 and 100% of the current 8 hour weighted average TLV for butadiene (ACGIH, 1987). For the Downs et al. study the average years of employment or exposure were 11.3 and age at entry 30.6. The continuous lifetime equivalent exposure based on 11.3 working years out of about 40 remaining years is:

$$10 \text{ ppm} \times \frac{11.3}{40} \text{ years} \times \frac{240}{365} \text{ days} \times \frac{8}{24} \text{ hours} = 0.62 \text{ ppm}$$

The initial estimate of q_1, the maximum likelihood estimate or MLE, from the incidence of malignant lymphoma in male mice was 3.12×10^{-2} kg-d/mg or 1.68×10^{-2} $(ppm)^{-1}$ while the cancer potency estimate (q_1*) for this site was 2.03×10^{-1} kg-d/mg or 8.9×10^{-2} $(ppm)^{-1}$. The initial estimate is used for comparison with the EPA analysis of the data of Meinhardt et al. (1982) (Table 5.1). Based on the initial MLE of 1.68×10^{-2} $(ppm)^{-1}$ the individual extra lifetime risk would be:

$$R = 1.68 \times 10^{-2} (ppm)^{-1} \times 0.62 \text{ ppm} = 1.04 \times 10^{-2}.$$

Of 2586 workers at risk for 25 years out of their 40 remaining years this risk represents the following extra number of lymphatic cancers:

$$1.04 \times 10^{-2} \times 2586 \times \frac{25}{40} = 16.8$$

Adding 16.8 to the 4.4 cancer deaths expected (Table 5.1) we could expect to observe 21 cancers of the lymphatic system for occupational exposure to 10 ppm BD. For 1 ppm exposure we could expect to observe 1.7 additional cases or 6 total cases. These estimates are remarkably close to 8 cases actually observed in the Downs et al. study. These data are also consistent with predictions obtained using the potency or upper 95% confidence level; namely 13 at 1 ppm and 94 at 10 ppm. If similar estimates are employed with the Matanoski and Schwartz study (i.e. 10 working years average exposure, 36 years observed/50 years lifetime remaining), the mouse data would predict 21.7 to 115 cases at 10 ppm and 2.2 at 1 ppm where the observed excess was only 0.5. This may indicate a lower average continuous exposure for this larger cohort of workers. Alternatively, it may indicate that the potency estimate is too high.

However, the greatest source of uncertainty in this comparison is the assumption of site-specific concordance among species. Since BD is clearly a multi-site carcinogen, BD exposure may result in tumors at different sites in different species. In conclusion, the cancer potency estimates (q_1*), and derived from initial MLE values of the male mouse assay for malignant lymphoma, appear to give a reasonable range of predicted incidence of human lymphatic cancer possibly induced by occupational exposure to BD as described in recent epidemiological studies. This supports EPA's earlier conclusion with regard to their potency estimate (geometric mean of all significant sites) and the earlier epidemiological data. While the recent studies are limited by lack of BD exposure data, the overall conclusion, considering the various uncertainties involved, is that the mouse bioassay data does not greatly over predict the extra cancer risk, at least in those cases where exposures are reasonably close to the bioassay doses (i.e., 2-3 orders of magnitude).

An additional comparison can be made using the mouse-based "best value" unit risk presented in Section 4.2.9 and epidemiologic data from the more recent

Table 5-1 Comparison of Recent (BD) Occupational Epidemology with Incremental Risks Based on Initial MLE and Potency Values of Mouse Data

Study	Sample Size	All Cancers			Lymphatic System Neoplasms			Mouse Data Prediction		Exposure Estimate ppm
		OBS	EXP	SMR	OBS	EXP	SMR	a	b	
Downs et al. (1987)	2586	122	161	76	8	4.4	182	17 1.7	90 9	10 1
Matanoski and Schwartz (1987)[c]	3269	94	106	89	11	10.5	104	21.7[c] 2.2	115 12	10 1
Meinhardt[d] et al. (1982) Plant B	1094	11	20.89	58	2	2.55	78	6.3	-	13.5

a) Male mouse MLE(q_1) for malignant lymphoma = 3.12×10^{-2} kg-d/mg or 1.68×10^{-2} $(ppm)^{-1}$

b) Male mouse potency (q_1*) for malignant lymphoma = 2.03×10^{-1} kg-d/mg or 8.9×10^{-2} $(ppm)^{-1}$

c) Assumed 10 human years average exposure, 36 years observed/50 years lifetime remaining

d) EPA (1985) initial MLE 2.5×10^{-2} $(ppm)^{-1}$ geometric mean of sexes, all significant sites

reports of Matanoski et al. (1990) and Divine (1990). An example is presented here, using subcohorts and tumor sites in which a significantly elevated SMR was observed. This comparison is presented in Table 5-1b.

For consistency with the use of a 95% upper-bound mouse-based potency estimate, an upper-bound 95% confidence limit (95% U.C.L.) on the observed number of cancer cases is calculated for each cohort and site selected. To calculate a U.C.L. for each observed number of cases, a table is used which specifies the U.C.L.s for given expected values of a Poisson distribution. For example, 5 cases of lymphosarcoma or reticulosarcoma were observed in the routinely exposed workers of the Divine (1990) cohort. The 95% U.C.L. on this number, assuming a Poisson distribution, is 11.7; rounded to the nearest integer, this U.C.L. is 12 (Table 5-1b). Since the number of cases observed for a given site is partially dependent on the number of person-years in a study, and the confidence interval around an observed number of cases narrows as the number increases, the bigger the cohort or the greater the number of person-years in a study, the smaller the difference between the observed value and the 95% U.C.L.

In Table 5-1b, 95% U.C.L.s on observed numbers of cancer cases are listed beside cancer incidence counts that would be predicted from the 95% upper-bound mouse-based unit risk estimate. As in the comparisons described above, workplace BD exposures of 1 and 10 ppm are assumed.

Since the epidemiologic studies are based on workers exposed for and followed for only parts of their lifetimes, and the mouse-based upper-bound unit cancer risk value is for lifetime exposure and follow-up, adjustments are applied to the unit risk to generate predictions of cancer cases among the workers. A description of these adjustments follows.

For the Matanoski et al. (1990) study, it is assumed that a time-weighted average exposure would adequately account for the less than lifetime exposure, and that workers were exposed for 10 years. For example, a lifetime time-weighted average (TWA) BD concentration may be estimated for black production workers assumed to be exposed to 1 ppm BD during working hours:

$$1 \text{ ppm} \times \frac{10 \text{ yrs}}{70 \text{ yrs}} \times \frac{50 \text{ wks}}{52 \text{ wks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{8 \text{ hrs}}{24 \text{ hrs}} = 0.032 \text{ ppm (TWA)}$$

At this exposure concentration, the lifetime excess individual cancer risk would be estimated as:

$$0.032 \text{ ppm BD} \times 0.37 \text{ per ppm BD} = 0.012$$

From this, it may be estimated that approximately 4.5 cancer cases (0.012 x 371) associated with BD exposure would occur among the 371 black production workers over their lifetimes.

Here it is assumed that the proportion of butadiene-associated cancer cases that would have appeared by the end of a study would be the same as the proportion of the study group that had died by the conclusion of the study. Thus, based on the overall mortality of Matanoski et al.'s study group (25%), it may be "predicted" that approximately 1.1 BD-linked cases (4.5 cases x 25%)

Table 5-1b. Comparison of Cancer Incidence in Worker Cohorts with Predictions from Mouse-Based Butadiene Risk Assessment.

			Number of Cancer Cases In Workers			Number of Cases Predicted from 95% U.C.L. of Mouse-Based Potency Estimate[a]	
Study	Cohort	Cancer Type	Exp.	Obs.	95% U.C.L. of Obs.	At 10 ppm BD	At 1 ppm BD
Matanoski et al., 1990	Black Production Workers (N=371)	All	16.5	19	30	28	18
		All Lymphopoietic	1.2	6	13	12	2
Divine, 1990	Routinely Exposed Workers (N=705)	All	46.3	42	57	50 - 80[b]	47 - 50[b]
		Lymphosarcoma & reticulosarcoma	0.9	5	12	5 - 40	1 - 5

Abbreviations: Exp., expected. Obs., observed. U.C.L., upper confidence limit. BD, butadiene. ppm, parts per million.

[a]Based on occupational exposure to the indicated concentrations of BD. These concentrations represent a range of plausible estimates (the actual workplace concentrations were not measured). See text for exposure assumptions and description of calculations.

[b]Ranges given here result from using different numbers of stages in multistage model of human cancer incidence. See text for details.

would appear during the study period. Overall, approximately 18 cancer deaths would be predicted to occur in the cohort (Table 5-1b), based on 1.1 BD-linked cases and 16.5 cases expected due to background incidence.

For the Divine (1990) study, there is sufficient information to apply a Doll-Armitage multistage correction (Brown and Chu, 1983) to account for the less than lifetime exposure and follow-up. The formula used here is:

$$\text{Correction Factor} = (1/L)^{k-1}\,[(T_e - T_a)^{k-1} - (T_e - T_b)^{k-1}]$$

where L = lifespan (70 years)
k = stages of the multistage model
T_e = average age of conclusion of study
T_a = average age at first exposure
T_b = average age at last exposure

Using data reported by Divine (1990), T_e = 57.9 years, T_a = 27.5 years, and T_b = 39.9 years. The k parameter, which represents the number of stages in the multistage model of carcinogenesis, is assumed to be between 4 and 7, in consideration of data reported by Brown and Chu (1983). Using these values, the correction factor was estimated as:

Stages (k)	Correction Factor
4	0.065
5	0.031
6	0.014
7	0.0064

These correction factors are used (in place of the partial lifetime exposure factor and mortality correction used for the Matanoski et al. cohort) to estimate equivalent lifetime exposure concentrations (for the Divine cohort). For example, using 4 stages in the multistage model, the equivalent lifetime exposure is:

$$1 \text{ ppm} \times 0.065 \times \frac{50 \text{ wks}}{52 \text{ wks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{8 \text{ hrs}}{24 \text{ hrs}} = 0.015 \text{ ppm (TWA)}$$

At this lifetime equivalent exposure concentration, the lifetime excess individual cancer risk is estimated to be:

0.015 ppm BD x 0.37 per ppm BD x 705 workers = 3.9 cases

Thus, approximately 50 cancer deaths are predicted to occur in the cohort at 1 ppm BD (Table 5-1b, far right column), based on 3.9 BD-linked cases plus 46.3 cases expected due to background incidence. This prediction decreases with increasing values of k.

The predictions that use this document's mouse-based "best value" cancer potency estimate for BD are remarkably consistent with epidemiologic observations (Table 5-1b). The concordance is most striking when the workplace concentration of BD is considered to be 10 ppm. Then, the 95% U.C.L.s on the epidemiologic observations are closest to the predictions from the 95% U.C.L. mouse-based potency estimate: 30 vs. 28, 13 vs. 12, 57 vs. 50

to 80, and 12 vs. 5 to 40 (the low end of the ranges corresponds to k=7 and the high end corresponds to k=4).

One cannot draw sweeping conclusions from this comparison, however. Both the mouse and the human studies have their limitations. The degree to which mice are like humans (or vice versa) is still largely unknown. Nevertheless, from the foregoing, the mouse-based "best value" upper-bound unit risk estimate derived in this document appears sufficiently valid for use in human health risk assessment.

5.4 Relative potency

In order to compare the potency of 1,3-butadiene with other toxic air contaminants evaluated by OEHHA, potency slope factors were also expressed in molar units (Table 5-2). The 95% upper confidence limit on the slope of the low dose response of female mice for lung tumors is 0.37 ppm^{-1}. This is converted to $(mg/kg\text{-}d)^{-1}$ for humans as follows, assuming 17% low dose absorption fraction:

$$2.21\ mg/m^3 \times 0.17 \times 20\ m^3/d \times 1/70\ kg =$$

$$0.11\ mg/kg\text{-}d/ppm$$

$$0.37\ (ppm)^{-1} \times \frac{1}{0.11} \quad \frac{ppm}{mg/kg\text{-}d} = 3.36\ (mg/kg\text{-}d)^{-1}$$

By further converting to molar potency using the molecular weight of butadiene (54.1) the following value is obtained:

$$3.36 \left[\frac{mg}{kg\text{-}d}\right]^{-1} \times \left[\frac{1\ \mu mol}{0.0541 mg}\right]^{-1} = 62.2 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1}$$

$$\log_{10} 62.2 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1} = 1.8 \left[\frac{\mu mol}{kg\text{-}d}\right]^{-1}$$

In Table 5-2 the molar potencies and their logarithims are listed for ease of comparison. Based on this comparison butadiene ranks fourth in relative potency among this group of toxic air contaminants. In a similar analysis EPA (1985) found butadiene to rank in the third quartile of some 55 carcinogens evaluated by EPA in various environmental media.

5.5 Interspecies Comparisons

Despite the apparent consistency between human cancer epidemiology and the cancer potency values derived from mouse bioassay data, the large differences seen between mouse and rats in BD/BMO metabolism and carcinogenic susceptibility raises some questions about the choice of animal model for extrapolation to humans. Studies of `monooxygenase' and other xenobiotic metabolizing enzymes in human biopsy and in surgical and postmortem tissue specimens, particularly lung, have been quite variable to date. Monooxgenase activities in lung ranged from less than 0.1 (Watanabe and Abe, 1981) to 135 pmol/mg protein/min with benzo[a]pyrene and naphthalene as substrates (Table 5-3).

Table 5-2 Relative Potencies of BD and Selected Toxic Air Contaimnants

Toxic Air Contaminant	Unit Risk $(ug/m^3)^{-1}$	Potency $(mg/kg\text{-}d)^{-1}$	MW	Molar Potency $(umol/kg\text{-}d)^{-1}$	log_{10} Molar Potency
1. TCDD	24 to 38	1.33×10^5	322	4.1×10^5	5.6
2. Chromium (VI) (Cr_O4^{-2})	(1.2 to 14.6) x 10-2		100	5.1×10^3	3.7
3. Cadmium (II)	$(0.2 \text{ to } 18) \times 10^{-2}$	40	112.4	356	2.6
4. Butadiene	$(0.04 \text{ to } 1.6) \times 10^{-4}$	3.36	54.1	62.2	1.8
5. Ethylene Oxide	$(0.6 \text{ to } 8.8) \times 10^{-5}$	0.3	44.1	6.8	0.83
6. Benzene	(0.75 to 5.3) x 10-5	0.17	78	2.2	0.34
7. Ethylene Dibromide	(1.3 to 7.1) x 10-5	2.4×10^{-1}	187.9	1.3	0.11
8. Carbon Tetrachloride	$(1 \text{ to } 15) \times 10^{-5}$	0.15	153.8	0.98	-0.011
9. Ethylene Dichloride	(1.3 to 2.2) x 10-5	7.2×10^{-2}	96.9	.74	-0.13
10 Methylene Chloride	$(2.6 \text{ to } 3.4) \times 10^{-6}$	1.4×10^{-2}	84.9	0.16	-0.78

Table 5-3 Human Lung Xenobiotic Metobolizing Enzyme Activities

Study	Specimen(N)	Cytochrome P450[a]	BaP monoxygenase[b]	NADPH[c] cytochrome[c] reductase	NADH[d] cytochrome[b5] reductase
Watanabe & Abe (1981)	surgical (16)	<0.01	<0.1 to 6.0	0.04	3.6
Jakobson etal.	post mortem (N)	trace (14)	11.5 ± 4.8(19)	20.9(9)	--
McManus etal. (1980)	surgical (10)	ND	0.23	0.033	0.68
Leboeuf etal.	post mortem (1)	--	0.32	--	--
Devereux etal. (1986)	surgical (5) Alveolar Type II cells	ND	--	0.018-0.029	--
Buckpitt & Bahnson (1986)	surgical (1)	--	42-104	-- --	
	(1)naphthalene	--	11-135	--	--
Mori etal. (1986)	surgical (3)	<.012	0.37	--	--

NOTE: [a] nmol cytochrome P450/mg protein
[b] pmol/min/mg protein
[c] nmol cytochrome c reduced/min/mg protein
[d] pmol ferricyanide reduced/min/mg protein
[e] ND - not determined

According to Lorenz et al. (1984), with the exception of monooxygenase activity in human lung which was two orders of magnitude lower, rodent and human subcellular fractions from liver or lung had specific activity ratios (liver/lung) within one order of magnitude (monooxgenase, epoxide hydrolase, glutathione-S-transferase).

Similar results were obtained by Mori et al. (1986) and are summarized in Table 5-4. Even highly sensitive immunocytochemical techniques using monoclonal antibody to a major form of human hepatic cytochrome P450, P450hA7, have shown only weak immunoreactivity in proximal tubules of the kidney, pancreatic acini, gall bladder epithelium, squamous epithelium and sebaceous glands of the skin, interstitial cells of the testis and luteal cells of the ovary. No activity was detected in adrenal gland, placenta, colonic epithelium and alveolar type II cells and Clara cells of the lung (Murray et al., 1988). Clearly more work is needed to determine whether the mouse is a good model for prediction of human susceptibility to BD induced carcinogenesis or if it is simply a particularly sensitive species due to its enzymatic profile.

Table 5-4 Cytochrome P450 Contents and Aniline Hydroxylase Activities in Animal or Human Lung S-9 (Mori et al., 1986)

Species	N	Sex	P450 Content nmol/mg Protein	AH Activity pmol/mg S9 protein/min
Rat	5	M	0.036 ± 0.009	14 ± 3
	5	F	0.042 ± 0.002	10 ± 3
Hamster	3	M	0.034 ± 0.010	40 ± 8
Mouse	10	M	0.060 ± 0.013	30 ± 2
Rabbit	2	M	0.041 ± 0.004	19 ± 2
Monkey	1	M	0.081	6
	1	F	0.086	10
Human	A	M	<0.012	0.3
	B	M	<0.012	0.5
	C	M	<0.012	0.3

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APPENDIX A

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Carcinogenicity of 1,3-Butadiene in C57BL/6 × C3H F_1 Mice at Low Exposure Concentrations

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ABSTRACT

The carcinogenicity of inhaled 1,3-butadiene was evaluated in C57BL/6 × C3H F_1 mice exposed to concentrations of this gas ranging from 6.25 to 625 ppm. Butadiene is a high production volume chemical, used mainly in the manufacture of synthetic rubber. In these 2-yr inhalation studies, a potent multisite carcinogenic response was observed, including neoplasms of the lung at concentrations as low as 6.25 ppm. Early occurrence and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm of butadiene reduced the number of animals at risk for the expression of later developing neoplasms at other sites; at lower exposure concentrations, dose responses were demonstrated for hemangiosarcomas of the heart and neoplasms of the lung, forestomach, Harderian gland, preputial gland, liver, mammary gland, and ovary. So far, no long-term studies on butadiene have been conducted at exposure concentrations that have not shown a carcinogenic response. In separate experiments with reduced exposure durations, butadiene induced neoplastic responses at multiple organ sites even after only 13 wk of exposure. Because of the correspondence between these animal data and recent epidemiology findings, there is a worldwide public health need to reevaluate current workplace exposure standards for 1,3-butadiene.

INTRODUCTION

1,3-Butadiene, a colorless gas (bp, −4.4°C) generally produced as a coproduct of ethylene production, is used mainly in the manufacture of synthetic rubber (styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resins (1). The annual production volume of butadiene is approximately 12 billion pounds worldwide and 3 billion pounds in the United States (2). The United States Environmental Protection Agency estimates that between 5300 and 8200 workers are exposed to butadiene in facilities which produce the monomer or which process the monomer into polymers (3). Occupational exposure to butadiene is generally less than 20 ppm; however, in a recent industrial hygiene survey, it was found that exposures in certain jobs were as high as 375 ppm (4). Butadiene has also been identified in automobile exhaust and cigarette smoke (5).

Butadiene is mutagenic to *Salmonella typhimurium* (strains TA1530 and TA1535) in the presence of S9 metabolic activation systems (6). The mutagenicity of butadiene may be due to its oxidative intermediates, butadiene monoxide and diepoxybutane (7), which are direct acting mutagens in *Salmonella* (8, 9). Butadiene was also genotoxic to bone marrow cells of exposed mice, causing an increase in the frequency of chromosomal aberrations, sister chromatid exchanges, and micronucleated erythrocytes (10).

At exposure concentrations nearly equal to and one-half the Occupational Safety and Health Administration 8-h time-weighted average workroom exposure standard of 1000 ppm, butadiene was carcinogenic in mice (11) and rats (12). A species difference with respect to sites of tumor induction and magnitude of response was demonstrated in these carcinogenicity studies of butadiene (11, 12). The results of those studies prompted the American Conference of Governmental Industrial Hygienists to lower their recommended threshold limit value for butadiene in the work environment from 1000 ppm to 10 ppm (13). Meanwhile, the Occupational Safety and Health Administration has determined that there are significant predicted risks associated with occupational exposure to 100 ppm of butadiene over a working lifetime (14). Most importantly, recent epidemiological findings from the rubber industry indicate an association between occupational exposure to 1,3-butadiene and the development of lymphatic and hematopoietic cancers (15, 16).

This paper reports our 2-yr inhalation studies of butadiene in mice exposed to concentrations ranging from 6.25 to 625 ppm and separate studies using variable exposures and durations. Dose-response relationships are reported for the various neoplastic lesions induced by butadiene, and the relationship between exposure concentrations and durations of exposure on the outcome of butadiene-induced carcinogenicity has been assessed.

MATERIALS AND METHODS

Chemicals. Liquified 1,3-butadiene of 99+% purity, containing trace amounts of *t*-butyl catechol, a peroxide inhibitor, was obtained from Phillips Chemical Co. (Borner, TX). Concentrations of the dimer, 4-vinyl-1-cyclohexene, in the cylinder headspace were determined by gas chromatographic analysis. Cylinders were not retained for use if the dimer content was greater than 150 ppm. Butadiene gas was metered to Hazelton 2000 chambers (Harford Division of Lab Products, Inc., Aberdeen, MD) via a distribution system and diluted in the fresh air chamber inlets. Chamber concentrations of 1,3-butadiene were monitored continuously during the exposure periods utilizing a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector [oven temperature, 120°C; gas chromatography column, 12- x ⅛-in nickel column packed with 1% SP-1000 on 60/80 Carbopack B (Supelco, Inc., Bellefonte, PA); carrier gas, nitrogen (20 ml/min)]. The daily mean concentrations of 1,3-butadiene, distributed uniformly in the chambers, were within 2% of the target concentrations.

Animals. Five-wk-old male and female C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mice, obtained from the Frederick Cancer Research Facility (Frederick, MD), were quarantined and acclimatized for 10 days prior to the start of the study. Stainless steel Hazelton 2000 chambers were used, and animals were exposed in individual wire mesh cage units, each having a floor area of 106 cm^2. The chamber environment was maintained at 24 ± 2°C and 55 ± 15% relative humidity with a chamber air flow of 15 air changes/h. Fresh softened tap water and NIH-07 diet were available *ad libitum*, except when the feed was removed during the exposure periods and overnight prior to the 40- and 65-wk necropsies.

Exposure Regimen. Groups of 70 to 90 male and female B6C3F_1 mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm of butadiene, 6 h/day, 5 days/wk for up to 2 yr. As many as 10 animals/group were killed and evaluated after 40 and 65 wk of exposure. Additional studies, in which exposure to butadiene was stopped after limited exposure periods, were included to assess the relationship between exposure level

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and duration of exposure on the outcome of butadiene-induced carcinogenicity. In the stop-exposure studies, groups of 50 male mice were exposed to one of the following regimens: (*a*) 625 ppm for 13 wk; (*b*) 200 ppm for 40 wk; (*c*) 625 ppm for 26 wk; or (*d*) 312 ppm for 52 wk. After the exposures were terminated, these groups of animals were placed in control chambers for the remainder of the 104-wk studies. The total exposure to butadiene (concentration × duration of exposure) was approximately equivalent for Groups *a* and *b* and provided about half the total exposure given to Groups *c* and *d*.

Histopathology. All animals that died during the study or that were killed at the end of the exposure period received a complete gross necropsy and histopathological examination. All moribund animals and those remaining at the end of the study were killed by carbon dioxide asphyxiation and necropsied immediately. Tissue samples, preserved in 10% neutral buffered formalin, were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: gross lesions and tissue masses; bronchial, mediastinal, mandibular, and mesenteric lymph nodes; salivary gland; sternebrae (including marrow); thyroid; parathyroid; small intestine; large intestine; liver; gall bladder; prostate; testes, epididymis, and seminal vesicles; ovaries; lungs and mainstem bronchi; nasal cavity and turbinates; heart; esophagus; stomach; uterus; brain; thymus; larynx; trachea; pancreas; spleen; kidneys; adrenals; urinary bladder; pituitary; and mammary gland.

Statistical Analyses. Differences in survival were analyzed by life table methods (17), and incidences of neoplastic lesions (the ratio of the number of animals bearing such lesions at a specific anatomical site to the number of animals in which that site was examined) including information on time of death were analyzed by life table tests, logistic regression analyses, Fisher exact analyses, and Cochran-Armitage trend analyses (18, 19). Tumor rates were adjusted for intercurrent mortality using the survival-adjusted quantal response test described by Portier and Bailer (20), with a power value equal to 3 (21).

RESULTS

Survival was reduced for male and female mice exposed to 20 ppm of butadiene or higher concentrations, largely due to the development of compound-related malignant tumors (Tables 1 and 2). All of the mice exposed to 625 ppm died by Wk 65. The percentage of animals bearing malignant tumors increased from about 30% in the control groups to nearly 90% in the 625-ppm exposure groups. However, the best approach for detecting potential carcinogenic effects of chemicals is to compare specific tumor incidences between dosed and control groups rather than comparing the overall proportion of animals with malignant tumors.

Lymphocytic lymphomas, of thymic origin, appeared as early as Wk 23 and were the major cause of death for male or female mice exposed to 625 ppm; the incidence of these neoplasms was greater in males than in females (Tables 1 and 2). Malignant lymphomas were classified according to the criteria of Frith and Wiley (22). Most butadiene-induced lymphomas were well differentiated and lymphocytic, and they appeared to originate in the thymus. Furthermore, the thymus was the most frequently and prominently affected organ. The incidence of lymphocytic lymphomas was also increased in females exposed to 200 ppm, but not at lower concentrations. After Wk 65, malignant lymphomas had less thymic involvement, and the other histological types (histocytic and mixed-cell lymphomas) more commonly associated with the spontaneous lymphoma of $B6C3F_1$ mice were observed in all remaining groups.

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 animal from both the 20-ppm male group and the 62.5-ppm female group was observed with this endothelial cell tumor. These neoplasms are particularly important because hemangiosarcomas of the heart are uncommon in untreated male or female $B6C3F_1$ mice, occurring at a rate of about 0.04%, or one of every 2500 mice. Interestingly, the incidence of hemangiosarcomas of the heart was greater in male mice exposed to 200 ppm than in those exposed to 625 ppm. Yet, it is evident from Fig. 1 (where the cumulative incidence of lymphocytic lymphoma or hemangiosarcoma of the heart is plotted against the number of weeks on study) that, for male mice exposed to 200 or 625 ppm of butadiene, the early and extensive development of lethal lymphocytic lymphomas at 625 ppm resulted in a reduced number of mice at risk in that exposure group for the later developing hemangiosarcomas. The impact of early mortality on the expression of late developing tumors is largely accounted for in the mortality-adjusted tumor rates shown in Tables 1 and 2. Furthermore, when the rates of hemangiosarcoma of the heart in early death animals were compared over 25-wk intervals for male mice exposed to 200 or 625 ppm of butadiene, it was found that the rates of this neoplastic lesion during these time intervals were nearly equivalent at these two exposure concentrations for the first 75 wk of the study. After that time, a high rate of hemangiosarcoma of the heart (50%) was observed in the 200-ppm exposure group; there were no surviving animals in the 625-ppm exposure group. Thus, for male mice exposed to concentrations of butadiene below 625 ppm, the dose response for butadiene-induced hemangiosarcomas of the heart is more clearly demonstrated. Mortality-adjusted dose-response curves for lymphocytic lymphoma, hemangiosarcoma of the heart, and neoplasms of the lung, forestomach, and Harderian gland in male mice are shown in Fig. 2.

The impact of an early occurring lethal tumor on the detection of later developing neoplasms at other sites is further demonstrated by the finding that, in 15 male mice in the 625-ppm exposure group which did not die early with lymphocytic lymphoma (17% of the animals), the occurrences of hemangiosarcomas of the heart (five), alveolar-bronchiolar neoplasms (six), forestomach neoplasms (eight), and Harderian gland neoplasms (six) accounted for 66% of the total occurrences of these neoplasms in that exposure group.

The incidence of alveolar-bronchiolar neoplasms of the lung in male mice was increased at 62.5 ppm and higher concentrations compared with controls. Statistical significance at 625 ppm was obtained when tumor rates were adjusted for early mortality. In female mice, the incidence of alveolar-bronchiolar neoplasms was increased at all exposure concentrations compared with controls. Thus, even at 6.25 ppm, butadiene is carcinogenic to $B6C3F_1$ mice. The reduced incidence of lung neoplasms at 625 ppm compared with the incidence at 200 ppm is attributed to the high rate of early deaths due to lymphocytic lymphoma in female mice exposed to 625 ppm (Fig. 3). For alveolar-bronchiolar neoplasms, the time to tumor detection was slightly shorter with exposure to 625 ppm; however, because all of the female mice exposed to this concentration of butadiene died by Wk 65, the final incidence of this neoplastic lesion was less than that for female mice exposed to 200 ppm. Furthermore, the rates of alveolar-bronchiolar neoplasms in early death female mice exposed to 200 or 625 ppm of butadiene were nearly equivalent during 25-wk intervals for the first 75 wk of the study. After that time, a high rate of alveolar-bronchiolar neoplasms (78%) was observed in the 200-ppm exposure group. Mortality-adjusted dose-response curves

Table 1 *Survival and incidence of primary tumors in male B6C3F₁ mice exposed to 1,3-butadiene for up to 2 yr*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Initial no.[a]	70	70	70	70	70	90
No. of survivors	35	39	24[b]	22[b]	3[b]	0[b]
Target (neoplasm)						
Lymphocytic lymphoma	2 3% (4%)	1 1% (2%)	2 3% (5%)	4 6% (10%)	2 3% (7%)	62[c] 69% (96%)
All lymphomas	4 6% (8%)	3 4% (6%)	8 11% (19%)	11[c] 16% (25%)	9[c] 13% (27%)	69[c] 77% (97%)
Heart (hemangiosarcoma)	0 0% (0%)	0 0% (0%)	1 1% (2%)	5[c] 7% (13%)	20[c] 29% (57%)	6[c] 7% (53%)
Lung (alveolar-bronchiolar neoplasm)	22 31% (46%)	23 38% (48%)	20 33% (45%)	33[c] 48% (72%)	42[c] 60% (87%)	12[c] 13% (73%)
Forestomach (squamous cell neoplasm)	1 1% (2%)	0 0% (0%)	1 1% (2%)	5 7% (13%)	12[c] 17% (36%)	13[c] 14% (75%)
Harderian gland (neoplasm)	6 9% (13%)	7 10% (15%)	11 16% (25%)	24[c] 34% (53%)	33[c] 47% (77%)	7[c] 8% (58%)
Preputial gland (adenoma or carcinoma)	0 0% (0%)	0 0% (0%)	0 0% (0%)	0 0% (0%)	5[c] 7% (17%)	0 0% (0%)
Liver (hepatocellular neoplasm)	31 44% (55%)	27 45% (54%)	35 59% (68%)	32 54% (69%)	40[c] 59% (87%)	12 13% (75%)

[a] Initial number includes animals removed from the study for interim sacrifices at 40 and 65 wk.
[b] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[c] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

for lymphocytic lymphoma, hemangiosarcoma of the heart, and neoplasms of the lung, forestomach, and Harderian gland in female mice are shown in Fig. 4. The sharp and rapid concentration-dependent increase in the incidence of alveolar-bronchiolar neoplasms in female mice is particularly noteworthy. The estimated rate of alveolar-bronchiolar neoplasms in female mice after adjustment for intercurrent mortality is 8% at 0 ppm (controls), 32% at 6.25 ppm, 44% at 20 ppm, 61% at 62.5 ppm, 81% at 200 ppm, and 83% at 625 ppm.

The incidences of neoplastic lesions of the forestomach (squamous cell neoplasms), mammary gland (adenocarcinomas), ovary (granulosa cell neoplasms), and liver (hepatocellular neoplasms), the other organ sites identified in the first study (11, 23), were again increased. In addition, the Harderian gland and preputial gland were identified as sites of butadiene-induced neoplasia. The concentrations of butadiene at which these lesions were not significantly increased compared with controls were 62.5 ppm for forestomach squamous cell neoplasms and preputial gland neoplasms, 20 ppm for Harderian gland neoplasms, mammary gland adenocarcinomas, and ovarian granulosa cell neoplasms, and 6.25 ppm for hepatocellular neoplasms.

In the stop-exposure groups, survival was also markedly reduced due to the development of compound-related malignant tumors (Table 3). The tumor incidence profiles in the stop-exposure groups show that lymphocytic lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, forestomach squamous cell neoplasms, Harderian gland neoplasms, and preputial gland neoplasms were increased compared with controls even after only 13 wk of exposure to 625 ppm of butadiene. Furthermore, at comparable total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of butadiene for a short time compared with exposure to a lower concentration for an extended duration. This is evident by comparing the incidence of lymphocytic lymphoma in the 625-ppm stop-exposure 13-wk group (34%) with that in the 200-ppm stop-exposure 40-wk group (12%), or more notably by comparing the incidence in the 625-ppm stop-exposure 26-wk group (60%) with that in the 312-ppm stop-exposure 52-wk group (6%). Renal tubular cell adenomas, which rarely occur in untreated B6C3F₁ mice (<0.1%), were also observed at increased rates in the stop-exposure groups at the lower butadiene concentrations.

Increased incidences of proliferative, nonneoplastic lesions in the heart (endothelial hyperplasia), lung (alveolar epithelial hyperplasia), forestomach (epithelial hyperplasia), Harderian gland (hyperplasia), and ovary (granulosa cell hyperplasia) probably represent preneoplastic changes caused by butadiene at these target sites, while the distinction between adenoma and carcinoma further reveals the biological progression of chemical-induced neoplastic lesions to malignant neoplasia. The spectrum of these proliferative lesions in mice exposed to butadiene is shown in Tables 4 (males) and 5 (females). At each of these sites, the neoplastic responses were generally supported by accompanying increases in the incidences of related nonneoplastic, proliferative lesions. In the lung of male mice, progression from alveolar-bronchiolar adenoma to carcinoma was evident in the 200-ppm exposure group and in all of the stop-exposure groups. In control female mice, only benign neoplastic lesions were observed in the lung, forestomach, Harderian gland, and ovary; however, in female mice exposed to butadiene, malignant neoplasms were observed in these organs. In partic-

Table 2 *Survival and incidence of primary tumors in female B6C3F₁ mice exposed to 1,3-butadiene for up to 2 yr*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Initial no.[a]	70	70	70	70	70	90
No. of survivors	37	33	24[b]	11[b]	0[b]	0[b]
Target (neoplasm)						
Lymphocytic lymphoma	2 3% (4%)	4 6% (9%)	6 9% (14%)	3 4% (8%)	11[c] 16% (41%)	36[c] 40% (86%)
All lymphomas	10 14% (20%)	14 20% (30%)	18[c] 26% (41%)	10 14% (26%)	19[c] 27% (58%)	43[c] 48% (89%)
Heart (hemangiosarcoma)	0 0% (0%)	0 0% (0%)	0 0% (0%)	1 1% (3%)	20[c] 29% (64%)	26[c] 29% (84%)
Lung (alveolar-bronchiolar neoplasm)	4 6% (8%)	15[c] 25% (32%)	19[c] 32% (44%)	27[c] 39% (61%)	32[c] 46% (81%)	25[c] 28% (83%)
Forestomach (squamous cell neoplasm)	2 3% (4%)	2 3% (4%)	3 4% (8%)	4 6% (12%)	7[c] 10% (31%)	28[c] 31% (85%)
Harderian gland (adenoma or adenocarcinoma)	9 13% (18%)	10 14% (21%)	7 10% (17%)	16[c] 23% (40%)	22[c] 31% (67%)	7 8% (48%)
Liver (hepatocellular neoplasm)	17 25% (35%)	20 33% (41%)	23[c] 38% (52%)	24[c] 40% (60%)	20[c] 33% (68%)	3 3% (28%)
Mammary gland (adenocarcinoma)	0 0% (0%)	2 3% (4%)	2 3% (5%)	6[c] 9% (16%)	13[c] 19% (47%)	13[c] 14% (66%)
Ovary (granulosa cell neoplasm)	1 1% (2%)	0 0% (0%)	0 0% (0%)	9[c] 13% (24%)	11[c] 16% (44%)	6 7% (44%)

[a] Initial number includes animals removed from the study for interim sacrifices at 40 and 65 wk.
[b] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[c] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

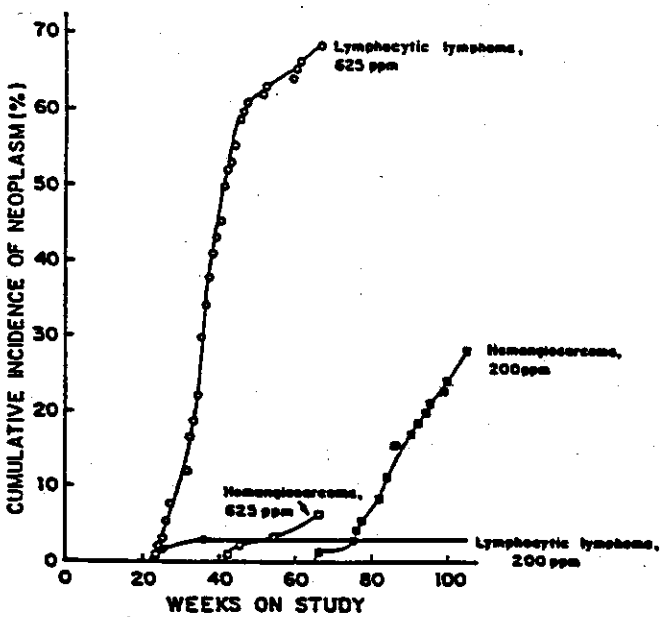

Fig. 1. Cumulative incidence of lymphocytic lymphomas or hemangiosarcomas of the heart versus weeks on study for male B6C3F₁ mice exposed to 200 or 625 ppm of 1,3-butadiene.

ular, high incidences of alveolar-bronchiolar carcinomas were observed in exposed female mice, even at 6.25 ppm of butadiene. Also, the increased incidence of hepatocellular neoplasms in female mice exposed to butadiene compared with controls appears to be largely due to the increase in hepatocellular carcinomas. The greater tendency to malignant neoplasia in mice exposed to butadiene further demonstrates the strong carcinogenic potency of this chemical.

Squamous cell papillomas and squamous cell carcinomas of the forestomach were detected as late as Wk 88 and Wk 105 in male mice that had been exposed to 625 ppm of butadiene for 13 wk and then held in control chambers to allow time for progression or regression of butadiene-induced lesions. The observation of both benign and malignant neoplasms of the forestomach near the end of the 2-yr studies in mice exposed to butadiene for only 13 wk shows that forestomach lesions induced by butadiene may persist and progress to malignant neoplasms in the absence of further exposure to this carcinogenic compound.

The high incidences of neoplastic lesions at multiple organ sites in mice exposed to butadiene also resulted in numerous occurrences of animals with tumors at more than one primary site. The frequencies of multiple organ site neoplasia in male mice and in female mice are shown in Tables 6 and 7. For these evaluations, lung tumors were not considered for male mice and liver tumors were not considered for female mice because of the high incidences of these neoplastic lesions in the respective control groups. Neoplasms were observed in two or more of the designated organ sites in 54% of the female mice and approximately 30% of the male mice exposed to 200 ppm of butadiene. Eight female mice developed primary tumors at 4

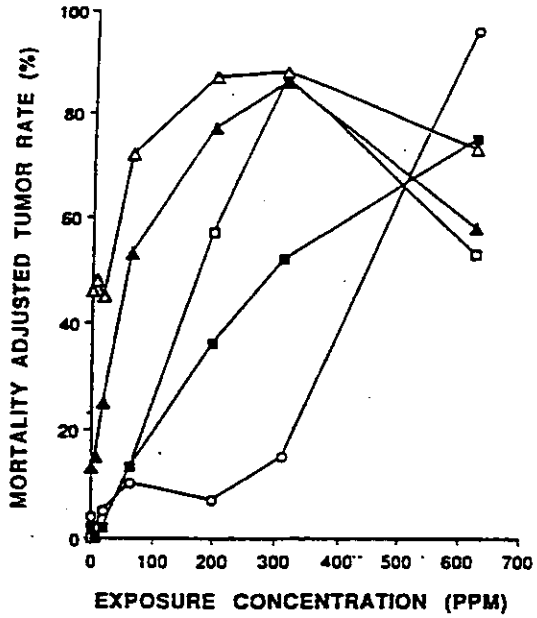

Fig. 2. Dose-response *curves* for lymphocytic lymphomas (O), hemangiosarcomas of the heart (□), alveolar-bronchiolar neoplasms (Δ), Harderian gland neoplasms (▲), and squamous cell neoplasms of the forestomach (■) in male B6C3F$_1$ mice exposed to 1,3-butadiene. Results from the 312-ppm, 52-wk stop-exposure group are also included, because that was the longest exposure duration of the stop-exposure groups and the incidence of lymphocytic lymphoma was not increased in that group compared with controls. Mortality-adjusted tumor rates were determined by the method of Portier and Bailer (20) using a power value equal to 3 (21).

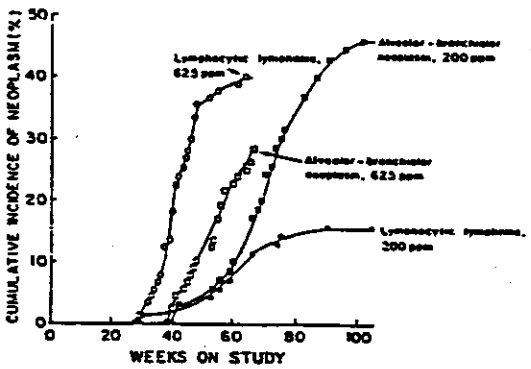

Fig. 3. Cumulative incidence of lymphocytic lymphomas or alveolar-bronchiolar neoplasms *versus* weeks on study for female B6C3F$_1$ mice exposed to 200 or 625 ppm of 1,3-butadiene.

different organ sites, while one female mouse had 5 different primary sites of tumor development.

DISCUSSION

The present studies demonstrate that 1,3-butadiene is a potent multiple organ carcinogen in mice and is carcinogenic at all concentrations evaluated, ranging from 6.25 to 625 ppm. Because there were no exposure concentrations in these studies that did not result in carcinogenicity, the possibility exists that exposure levels below 6.25 ppm will also cause cancers in laboratory animals. The concentrations of butadiene used in these studies are within the linear range of metabolic elimination of butadiene in B6C3F$_1$ mice (24). Miller and Boorman (25) have provided morphological descriptions and illustrations of neoplastic and nonneoplastic lesions induced by 1,3-butadiene in B6C3F$_1$ mice.

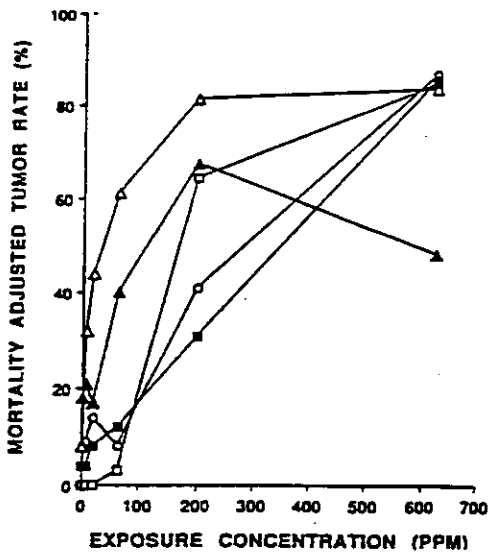

Fig. 4. Dose-response *curves* for lymphocytic lymphomas (O), hemangiosarcomas of the heart (□), alveolar-bronchiolar neoplasms (Δ), Harderian gland neoplasms (▲), and squamous cell neoplasms of the forestomach (■) in female B6C3F$_1$ mice exposed to 1,3-butadiene. Mortality-adjusted tumor rates were determined by the method of Portier and Bailer (20) using a power value equal to 3 (21).

In male or female mice exposed to 625 ppm of butadiene, or in female mice exposed to 200 ppm, competing risks of early occurring lethal thymic lymphomas limited the detection of later developing neoplasms at other organ sites. However, by adjusting for intercurrent mortality or by comparing tumor rates in early death animals at discrete time intervals of the study, it was possible to account for the influence of reduced survival resulting from induction of lymphocytic lymphoma on the dose responses of the later appearing tumors. At lower exposure concentrations where lymphocytic lymphoma was not a major cause of early death, the incidences of neoplastic lesions in the heart, lung, forestomach, Harderian gland, preputial gland, liver, mammary gland, and ovary were nearly linearly related to the concentration of butadiene. In the lungs of female mice, alveolar-bronchiolar carcinomas were induced at the lowest exposure concentration of 6.25 ppm of butadiene. Malignant lung tumors are uncommon in untreated female B6C3F$_1$ mice (26) and were not observed in control female mice in this study. Thus, butadiene is carcinogenic in mice at concentrations which are less than those to which humans are potentially exposed in occupational settings (4).

The stop-exposure studies show that multiple organ site neoplasia occurs in mice after only 13 wk of exposure to butadiene. It is likely that shorter exposure durations would also produce a positive carcinogenic response. These studies also demonstrate that butadiene-induced forestomach lesions can persist for long periods of time after exposure has ceased and may progress to malignant neoplasms. The development of thymic lymphomas in mice exposed to butadiene is not equivalent under different exposure regimens which result in the same total exposure; the stop-exposure studies show that the concentration of butadiene is a much greater contributing factor than is the duration of exposure. Likewise, time-weighted average exposures to butadiene in the workplace may not accurately reflect the potential human risk of cancer.

The mechanism of butadiene-induced carcinogenicity is not known; however, butadiene monoxide and/or diepoxybutane, oxidative intermediates of butadiene biotransformation (7), is

Table 3 *Survival and incidence of primary tumors in the stop-exposure groups of male B6C3F₁ mice exposed to 1,3-butadiene*

Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomical site. Overall rates, based on the number of animals in which that site was examined, are given below the incidence values. Mortality-adjusted tumor rates are given in parentheses.

	Exposure concentration (ppm)				
	0 Control	200 Stop-exposure 40 wk (8,000)[a]	625 Stop-exposure 13 wk (8,125)	312 Stop-exposure 52 wk (16,224)	625 Stop-exposure 26 wk (16,250)
Initial no.[b]	70	50	50	50	50
No. of survivors	35	9[c]	5[c]	1[c]	0[c]
Target (neoplasm)					
Lymphocytic lymphoma	2 3% (4%)	6 12% (19%)	17[d] 34% (47%)	3 6% (15%)	30[d] 60% (84%)
All lymphomas	4 6% (8%)	12[d] 24% (35%)	24[d] 48% (61%)	15[d] 30% (55%)	37[d] 74% (90%)
Heart (hemangiosarcoma)	0 0% (0%)	15[d] 30% (47%)	7[d] 14% (31%)	33[d] 66% (87%)	13[d] 26% (76%)
Lung (alveolar-bronchiolar neoplasm)	22 31% (46%)	35[d] 70% (88%)	27[d] 54% (87%)	32[d] 64% (88%)	18[d] 36% (89%)
Forestomach (squamous cell neoplasm)	1 1% (2%)	6[d] 12% (20%)	8[d] 16% (33%)	13[d] 26% (52%)	11[d] 22% (63%)
Harderian gland (adenoma or adenocarcinoma)	6 9% (13%)	27[d] 54% (72%)	23[d] 46% (82%)	28[d] 56% (86%)	11[d] 22% (70%)
Preputial gland (carcinoma)	0 0% (0%)	1 2% (3%)	5[d] 10% (21%)	4[d] 8% (21%)	3[d] 6% (31%)
Kidney (renal tubular adenoma)	0 0% (0%)	5[d] 10% (16%)	1 2% (5%)	3[d] 6% (15%)	1% 2% (11%)

[a] Total exposure expressed as ppm-wk.
[b] Initial number of control mice includes 20 animals removed from the study for interim sacrifices at 40 and 65 wk.
[c] Decreased compared with chamber control (0 ppm), $P < 0.05$.
[d] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

Table 4 *Incidence of nonneoplastic lesions and neoplastic lesions in the heart, lung, forestomach, and Harderian gland of male mice exposed to 1,3-butadiene*

	Exposure concentration (ppm)									
Site and lesion	0	6.25	20	62.5	200	625	200 Stop-exposure 40 wk	625 Stop-exposure 13 wk	312 Stop-exposure 52 wk	625 Stop-exposure 26 wk
Heart	(70)[a]	(49)	(60)	(58)	(68)	(90)	(50)	(50)	(50)	(50)
Endothelial hyperplasia	0	1	0	3	8	8	6	7	3	6
Hemangiosarcoma	0	0	1	5[b]	20[b]	6[b]	15[b]	7[b]	33[b]	13[b]
Lung	(70)	(49)	(60)	(69)	(70)	(89)	(50)	(50)	(50)	(50)
Alveolar epithelial hyperplasia	1	5	4	12	19	18	13	11	13	10
Alveolar-bronchiolar adenoma	14	14	11	23[b]	25[b]	10[b]	19[b]	13[b]	24[b]	11[b]
Alveolar-bronchiolar carcinoma	9	13	11	15	24[b]	5	22[b]	20[b]	16[b]	11[b]
Forestomach	(70)	(50)	(60)	(60)	(68)	(89)	(48)	(50)	(48)	(50)
Epithelial hyperplasia	4	3	2	4	3	47	7	8	16	16
Squamous cell papilloma	1	0	1	5	9[b]	9[b]	6[b]	5[b]	8[b]	4[b]
Squamous cell carcinoma	0	0	0	0	3	4[b]	0	4[b]	5[b]	7[b]
Harderian gland	(63)	(59)	(60)	(57)	(60)	(53)	(48)	(42)	(48)	(36)
Hyperplasia	1	3	4	4	9	7	5	2	7	8
Adenoma	6	7	10	23[b]	32[b]	7[b]	26[b]	20[b]	26[b]	11[b]
Adenocarcinoma	0	1	1	2	2	0	2	4[b]	2	0

[a] Numbers in parentheses are the number of animals in which that site was examined microscopically.
[b] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

likely involved. These metabolites are direct acting mutagens in *S. typhimurium* (8, 9), whereas the detection of mutagenicity of butadiene requires metabolic activation (6). 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 s.c. injection (27, 28). Also, exposure of B6C3F₁ mice to 1250 ppm of butadiene did not result in any persistent defects in humoral or cell-mediated immunity (29).

In groups of 100 Sprague-Dawley rats exposed to 1000 or 8000 ppm of 1,3-butadiene for 2 yr, Owen *et al.* (12) reported that there were increased incidences and dose-response trends

Table 5 *Incidence of nonneoplastic lesions and neoplastic lesions in the heart, lung, forestomach, Harderian gland, liver, and ovary of female mice exposed to 1,3-butadiene*

Site and lesion	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Heart	(70)[a]	(50)	(50)	(59)	(70)	(90)
Endothelial hyperplasia	0	2	1	4	12	10
Hemangiosarcoma	0	0	0	1	20[b]	26[b]
Lung	(70)	(60)	(60)	(70)	(70)	(88)
Alveolar epithelial hyperplasia	6	4	6	11	13	11
Alveolar-bronchiolar adenoma	4	8	10[b]	18[b]	20[b]	14[b]
Alveolar-bronchiolar carcinoma	0	8[b]	13[b]	10[b]	20[b]	12[b]
Forestomach	(70)	(50)	(57)	(68)	(70)	(89)
Epithelial hyperplasia	2	3	4	6	16	48
Squamous cell papilloma	2	1	2	3	6[b]	22[b]
Squamous cell carcinoma	0	1	1	1	1	6[b]
Harderian gland	(63)	(49)	(48)	(59)	(59)	(73)
Hyperplasia	1	3	9	1	4	9
Adenoma	9	9	6	16[b]	22[b]	7
Adenocarcinoma	0	1	1	0	1	0
Liver	(69)	(51)	(51)	(60)	(60)	(90)
Hepatocellular adenoma	12	9	14	15[b]	13[b]	2
Hepatocellular carcinoma	7	13	11	11[b]	12[b]	1
Ovary	(69)	(59)	(59)	(70)	(70)	(89)
Granulosa cell hyperplasia	0	2	1	4	7	4
Granulosa cell tumor, benign	1	0	0	7[b]	8[b]	6
Granulosa cell tumor, malignant	0	0	0	2	3[b]	0

[a] Numbers in parentheses are the number of animals in which that site was examined microscopically.
[b] Increased compared with chamber control (0 ppm), $P < 0.05$, based on logistic regression analyses with adjustment for intercurrent mortality.

Table 6 *Frequency of multiple-site neoplasia in male B6C3F₁ mice exposed to 1,3-butadiene: lymphocytic lymphoma; hemangiosarcoma of the heart; squamous cell neoplasm of the forestomach; and Harderian gland neoplasm*

Exposure group	No. of organ sites/animal			
	0	1	2	3
	No. of animals			
Control	62 (89)[a]	7 (10)	1 (1)	0
6.25 ppm	52 (87)	8 (13)	0	0
20 ppm	47 (78)	12 (20)	1 (2)	0
62.5 ppm	36 (52)	28 (41)	5 (7)	0
200 ppm	27 (39)	23 (33)	16 (23)	4 (6)
625 ppm	15 (17)	65 (72)	7 (8)	3 (3)
200 ppm; stop-exposure, 40 wk	15 (30)	20 (40)	10 (20)	5 (10)
625 ppm; stop-exposure, 13 wk	8 (16)	32 (64)	8 (16)	2 (4)
312 ppm; stop-exposure, 52 wk	4 (8)	22 (44)	17 (34)	7 (14)
625 ppm; stop-exposure, 26 wk	5 (10)	27 (54)	16 (32)	2 (4)

[a] Numbers in parentheses, percentage.

Table 7 *Frequency of multiple-site neoplasia in female B6C3F₁ mice exposed to 1,3-butadiene: lymphocytic lymphoma; hemangiosarcoma of the heart; alveolar-bronchiolar neoplasm; squamous cell neoplasm of the forestomach; Harderian gland neoplasm; adenocarcinoma of the mammary gland; and granulosa cell neoplasm of the ovary*

Exposure group	No. of organ sites/animal					
	0	1	2	3	4	5
	No. of animals					
Control	55 (79)[a]	12 (17)	3 (4)	0	0	0
6.25 ppm	34 (57)	19 (32)	7 (12)	0	0	0
20 ppm	31 (52)	22 (37)	6 (10)	1 (2)	0	0
62.5 ppm	27 (39)	24 (34)	16 (23)	2 (3)	1 (1)	0
200 ppm	13 (19)	19 (27)	20 (29)	15 (21)	3 (4)	0
625 ppm	17 (19)	29 (32)	26 (29)	13 (14)	4 (4)	1 (1)

[a] Numbers in parentheses, percentage.

for pancreatic exocrine adenomas (control, 3; low dose, one; high dose, 10) and Leydig cell neoplasms of the testis (control, 0; low dose, 3; high dose, 8) in males and uterine sarcomas (control, one; low dose, 4; high dose, 5), Zymbal gland carcinomas (control, 0; low dose, 0; high dose, 4), mammary gland neoplasms (control, 50; low dose, 79; high dose, 81), and thyroid follicular cell neoplasms (control, 0; low dose, 4; high dose, 11) in females. The sites of tumor induction and the magnitude of response in rats were different from those reported here for B6C3F₁ mice. The reason for this species difference is not known; however, it is not readily accounted for by differences in rates of butadiene metabolism (23).

Irons and coworkers (30, 31) compared the induction of thymic lymphomas in NIH Swiss mice and B6C3F₁ mice exposed to butadiene and suggested that activation of an endogenous ecotropic retrovirus may play a role in butadiene-induced lymphoma in the B6C3F₁ strain of mice. The NIH Swiss mouse rarely expresses endogenous retrovirus and has a background rate of zero for thymic lymphoma. Thus, the finding that exposure of NIH Swiss mice to 1250 ppm of butadiene for 1 yr caused a 14% incidence of thymic lymphoma (31) indicates that butadiene can induce this neoplasm independently of an activated retrovirus.

The conclusion that the marginally increased incidences of hepatocellular neoplasms in male and female mice were chemically related is strengthened by the detection of an activated K-*ras* oncogene in liver neoplasms obtained from mice exposed to butadiene (32); activated K-*ras* has never been detected in liver tumors from untreated B6C3F₁ mice (33). Exposure to butadiene also caused activation of the K-*ras* oncogene in mouse lymphomas and lung neoplasms. These findings add further relevance to the potential carcinogenicity of butadiene in humans, since K-*ras* is the most commonly detected oncogene in human tumors.

Other toxic effects associated with exposure of mice to butadiene, and relevant to humans, include testicular atrophy in males exposed to 625 ppm, ovarian atrophy in females exposed to 6.25 ppm or higher concentrations, and bone marrow toxicity at 62.5 ppm and higher concentrations (34).

The carcinogenicity of butadiene in laboratory animals at concentrations as low as 6.25 ppm and the activation of the K-*ras* oncogene by butadiene raise further concerns of risk for humans exposed to this chemical. Results of epidemiology studies indicate an association between occupational exposure to 1,3-butadiene and the development of lymphatic and hematopoietic cancers (15, 16); for example, standardized mortality ratios were 500% for lymphopoietic cancers and 660% for leukemia in black production workers (15). In a follow-up nested case-control study of the styrene-butadiene rubber industry, Matanoski *et al.* (35) found that leukemia cases were associated with exposure specifically to butadiene (odds ratio, 9.4; 95% confidence interval, 2.1–22.9), whereas there was not a significant increased risk associated with exposure to styrene. Because of the correspondence between the animal data and the epidemiology findings, there exists an urgent worldwide public health need to reevaluate current workplace exposure standards for 1,3-butadiene.

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