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Inhalation Toxicology and Carcinogenicity of 1,3-Butadiene in B6C3F$_1$ Mice Following 65 Weeks of Exposure

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1,3-Butadiene, a large-production volume chemical used mainly in the manufacture of synthetic rubber, was found to induce multiple-organ carcinogenicity in male and female B6C3F$_1$ mice at exposure concentrations (625 and 1250 ppm) equivalent to and below the OSHA standard of 1000 ppm. Since this study was terminated after 60 weeks of exposure because of reduced survival due to fatal tumors, and because dose-response relationships for 1,3-butadiene-induced neoplastic and nonneoplastic lesions were not clearly established, a second long-term inhalation study of 1,3-butadiene in B6C3F$_1$ mice was conducted at lower exposure concentrations, ranging from 6.25 to 625 ppm. Both the histopathological findings from animals dying through week 65 and the results of evaluations of animals exposed for 40 and 65 weeks are presented in this report. Exposure to 1,3-butadiene caused a regenerative anemia at concentrations of 62.5 ppm and higher. Testicular atrophy was induced at 625 ppm, and ovarian atrophy was observed at 20 ppm and higher. During the first 50 weeks of the study, lymphocytic lymphoma was the major cause of death of mice exposed to 625 ppm 1,3-butadiene. Neoplasms of the heart, forestomach, lung, Harderian gland, mammary gland, ovary, and liver were frequently observed in 1,3-butadiene-exposed mice that died between week 40 and week 65 of the study. Studies in which exposure to 1,3-butadiene was stopped after limited periods were also included to assess the relationship between exposure levels and duration of exposures on the outcome of 1,3-butadiene-induced carcinogenicity. In these studies, lymphocytic lymphomas were induced in male mice exposed to 625 ppm 1,3-butadiene for only 13 weeks. The incidence of lymphocytic lymphoma in male mice exposed to 625 ppm 1,3-butadiene for 26 weeks was two times that in mice exposed to 625 ppm for 13 weeks. However, when the exposure concentration was reduced by half to 312 ppm and the exposure duration extended to 52 weeks, the incidence of lymphocytic lymphoma was reduced by 90%. Thus, the multiple of the exposure concentration times the exposure duration did not predict the incidence of lymphocytic lymphoma in mice. The early mortalities resulting from lymphocytic lymphomas in male mice exposed to 625 ppm 1,3-butadiene limited the expression of tumors at other sites. A clearer dose-response for 1,3-butadiene-induced neoplasia should be apparent from experiments in mice exposed to lower concentrations of this chemical for 2 years.

Introduction

1,3-Butadiene is a colorless, flammable, noncorrosive gas (bp: −4.4 °C, Vp: 1900 mmHg at 20 °C) used mainly in the production of synthetic rubber and thermoplastic resins (*1*). In production volume, 1,3-butadiene is one of the top 25 organic chemicals manufactured in the United States (*2*), with an annual production level of rubber-grade 1,3-butadiene in the U.S. of approximately 2.5 billion pounds (*3*). This gas has also been identified in automobile exhaust, cigarette smoke, and in incineration products of fossil fuels (*4*). 1,3-Butadiene was selected for evaluation of its toxicologic and carcinogenic potential because of its large production volume, its potential for human exposure in facilities that produce or process this chemical, and the lack of long-term toxicology and carcinogenicity data for this compound (*5*). The National Institute for Occupational Safety and Health estimates that about 65,000 workers are potentially exposed to 1,3-butadiene (*6*), and the U.S. Environmental Protection Agency estimates that between 5300 and 8200 workers are exposed to 1,3-butadiene in facilities that either produce the monomer or process the monomer into polymers (*7*). Recent surveys conducted by NIOSH at monomer and polymer manufacturing plants indicate that most occupational exposures to 1,3-butadiene are less than 20 ppm; however, excursions in certain jobs were as high as 374 ppm (*8*).

1,3-Butadiene has long been considered to have a low,

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noncumulative toxicity in animals and humans. The LC_{50} for 1,3-butadiene was reported to be 270,000 mg/m^3 (122,000 ppm) in mice exposed for 2 hr and 285,000 mg/m^3 (129,000 ppm) in rats exposed for 4 hr (*9*). Carpenter et al. (*10*) exposed rats, guinea pigs, rabbits, and dogs to 0, 600, 2300, or 6700 ppm 1,3-butadiene 7.5 hr/day, 6 days/week, for 8 months. Slight growth retardation and cloudy swelling in hepatocytes were observed in the highest dose group; there were no apparent treatment-related pathologic changes in the adrenal gland, heart, kidneys, lungs, skeletal muscle, pancreas, spleen, testes, or ovaries. Exposure of humans to 2000 ppm 1,3-butadiene or higher for 6 to 8 hr caused slight irritation of the eyes (*9*).

The National Toxicology Program (NTP) studied the long-term effects of inhalation exposure to 1,3-butadiene in mice (*5,11*). Groups of 50 male and 50 female B6C3F$_1$ mice were exposed 6 hr/day, 5 days/week to air containing 0 (chamber control), 625, or 1250 ppm 1,3-butadiene. This study, intended to last for 103 weeks, was ended after 60 to 61 weeks because of cancer-related mortality in both sexes at both exposure concentrations. There were early induction and increased incidences of malignant lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, and squamous cell neoplasms of the forestomach in males and females; and acinar cell carcinomas of the mammary gland, granulosa cell neoplasms of the ovary, and hepatocellular neoplasms in females. The observation of high incidences of hemangiosarcomas of the heart was a particularly unusual finding, since the historical rate for this uncommon endothelial cell tumor is about 0.04% in untreated B6C3F$_1$ mice and has rarely been induced in B6C3F$_1$ mice in long-term studies (*12,13*). In male mice, dose-responses for neoplasms of the heart, lung, and forestomach were not clearly demonstrated since both doses produced similar responses, whereas in female mice, the rate of malignant lymphomas was lower than that in males, and the dose-responses for other neoplastic lesions were more clearly defined.

Nonneoplastic lesions associated with exposure of B6C3F$_1$ mice to 1,3-butadiene included epithelial hyperplasia of the forestomach, endothelial hyperplasia of the heart, alveolar epithelial hyperplasia, hepatocellular necrosis, testicular atrophy, ovarian atrophy, and nasal cavity lesions (chronic inflammation, fibrosis, and osseous and cartilagenous metaplasia in the olfactory region) in high-dose males (*5,11*).

Stimulated by the unusual effects of 1,3-butadiene in mice, the fact that the studies were terminated early, the sometimes unclear dose-response relationships for various lesions, and the importance of 1,3-butadiene as a high-production volume chemical with potential human exposure, additional toxicology/carcinogenicity studies of various durations, including a 2-year exposure, were initiated to better characterize dose-response relationships for 1,3-butadiene-induced nonneoplastic and neoplastic lesions in B6C3F$_1$ mice. Data from the first 65 weeks (15 months) of that study (early death animals and interim evaluations at 40 and 65 weeks) are presented. The data from the 2-year exposures are not yet available and will be reported separately.

Materials and Methods

Chemical and Exposure System

Liquified 1,3-butadiene of 99$^+$% purity, containing trace amounts of *t*-butyl catechol, a peroxide inhibitor, was obtained from Phillips Chemical Co. (Borger, 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. 1,3-Butadiene gas was metered to the exposure chambers 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 with a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector (oven temperature: 120°C; GC column: 12″ × 1/8″ nickel column packed with 1% SP-1000 on 60/80 Carbopack B; 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.

Animal Maintenance

Five-week-old male and female B6C3F$_1$ mice, obtained from 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 (2.3 m^3) 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 75 ± 3°F and 55 ± 15% relative humidity, with a chamber air flow of 15 air changes/hr. 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-week necropsies.

Treatment Groups

Groups of 70 male and 70 female B6C3F$_1$ mice were exposed 6 hr/day, 5 days/week for periods up to 65 weeks to 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene; groups of 90 male and 90 female mice were similarly exposed to 625 ppm 1,3-butadiene. The additional animals in the 625-ppm exposure group were included out of concern that high mortality rates observed previously (*5,11*) at this exposure concentration might interfere with the interim evaluations at weeks 40 and 65. Fifty animals per group were included for evaluation of 1,3-butadiene-induced carcinogenicity after exposure for up to 103 weeks. The 625-ppm concentration corresponds to the low exposure level in the previous inhalation study (*5,11*), while 6.25 ppm and 20 ppm more closely represent occupational exposure levels to 1,3-butadiene (*8*).

Animals were observed twice daily for moribundity and mortality, and body weights were measured weekly for the first 13 weeks of the study and at 4-week intervals thereafter.

Studies in which the exposure to 1,3-butadiene was stopped after limited exposure periods (stop-exposure studies, SE) were included to assess the relationship between exposure level and duration of exposure on the outcome of 1,3-butadiene-induced carcinogenicity. Groups of 50 male mice were exposed to one of the following regimens: *a*) 625 ppm for 13 weeks (SE 13 WK), *b*) 200 ppm for 40 weeks (SE 40 WK), *c*) 625 ppm for 26 weeks (SE 26 WK), or *d*) 312 ppm for 52 weeks (SE 52 WK). After the exposures were terminated, these groups of animals were placed in control chambers for the remainder of the 104-week study. The total exposure to 1,3-butadiene (concentration multiplied by duration of exposure, CT) was approximately equivalent for the first and second treatments (8000 and 8125 ppm-weeks), and it provided about half the total exposure given in the third and fourth treatments (16,224 and 16,250 ppm weeks).

Hematology

Blood samples were obtained at weeks 40 and 65 from the supraorbital sinus of CO_2-anesthetized mice and collected into tubes containing EDTA. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (HGB), volume of packed red cells (VPRC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet counts were determined with an Ortho ELT-8/ds hematology analyzer.

Histopathology

All moribund animals and those sacrificed at 40 and 65 weeks were killed by CO_2 asphyxiation and then immediately necropsied. 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.

Statistics

Mean body weights, organ weights, organ weight/body weight ratios, and hematology results of treated groups were compared to those of control groups using Dunnett's *t*-test. The minimum level of probability accepted for significance was $p<0.05$.

Results

Survival, Body Weight, and Organ Weights

Exposure of $B6C3F_1$ mice to 625 ppm 1,3-butadiene caused a sharp decrease in survival of males and females (Fig. 1). Increased mortality was apparent in exposed males at about week 25 and in exposed females at about week 30. Survival was reduced 50% by week 36 for males and by week 46 for females. All mice exposed to 625 ppm 1,3-butadiene were dead by week 65. Exposure of mice to 200 ppm 1,3-butadiene also caused a reduction in survival; however, the rate of mortality at this exposure level was much less than that at 625 ppm. At week 65 the survival of mice exposed to 200 ppm 1,3-butadiene was 43 of 50 for males and 31 of 50 for females. In the control and lower exposure concentration groups, survival was generally greater than 90% at week 65.

Survival data from the stop-exposure groups (Fig. 2) indicate that although mortality due to exposure to 1,3-butadiene was concentration related, the multiple of exposure concentration times duration of exposure (CT) did not predict the probability of survival. Mortalities occurred at a greater rate for male mice exposed to 625 ppm 1,3-butadiene for 26 weeks, compared to 312 ppm for 52 weeks (equivalent total exposure), and for male mice exposed to 625 ppm for 13 weeks, compared to 200 ppm for 40 weeks.

Exposure of male or female mice to 6.25 to 625 ppm 1,3-butadiene for 65 weeks did not produce any significant adverse effects on mean body weight gain. The initial mean body weight of male mice was 22.8 ± 0.3 g and of female mice was 18.5 ± 0.2 g. At week 65, the mean body weight of control male mice was 49.3 g, and the mean body weights of the groups of male mice exposed to 1,3-butadiene ranged from 46.0 to 47.4 g; the mean body weight of control female mice was 43.5 g, and the mean body weights of the groups of female mice exposed to 1,3-butadiene ranged from 42.9 to 49.7 g.

At the interim evaluations at weeks 40 and 65, liver, brain, kidney, spleen, lung, heart, thymus, and testis weights were measured. The only significant concentration-related effect was a decrease in testis weight for males exposed to 62.5 ppm and higher (Table 1). The testis weight/body weight ratio was decreased in the 200 and 625 ppm exposure groups, compared to controls. A similar dose-related decrease in testis weight was observed at the 65-week evaluation.

Hematology

Hematologic indices were measured in blood samples taken from male and female mice at the 40- and 65-week interim evaluations. For male mice at week 40, significant decreases were observed in red blood cell counts, hemoglobin concentration, and packed red cell volume at exposure concentrations of 62.5, 200, or 625 ppm 1,3-butadiene (Table 2). Similar decreases (data not shown) were observed in female mice exposed to 625 ppm 1,3-butadiene and further demonstrate the dose-related anemia caused by 1,3-butadiene in mice. These changes

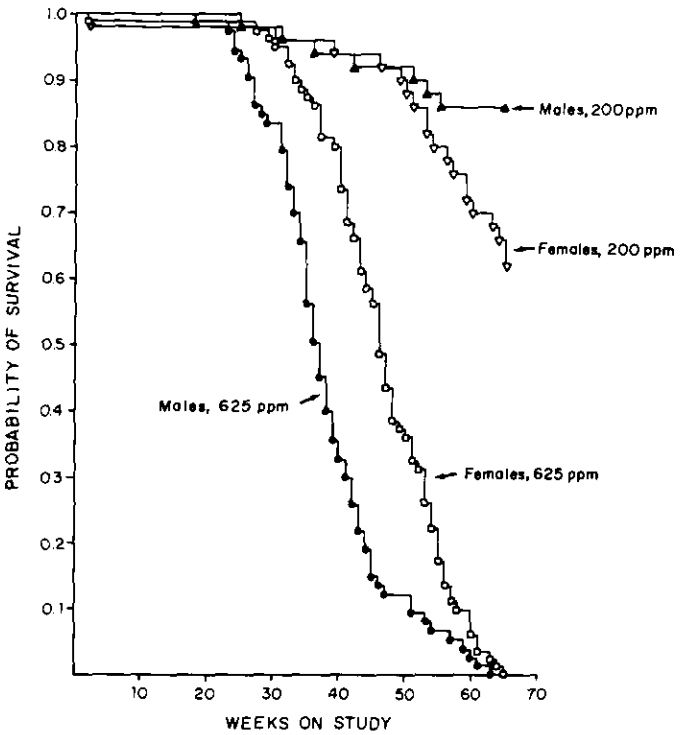

FIGURE 1. Survival curves for male and female B6C3F$_1$ mice exposed to 200 or 625 ppm 1,3-butadiene for 65 weeks.

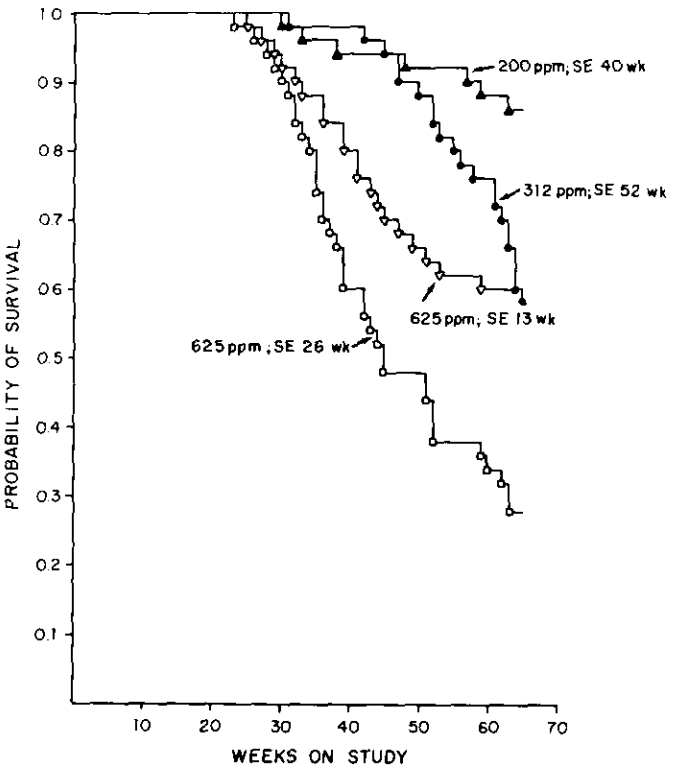

FIGURE 2. Survival curves for male B6C3F$_1$ mice exposed to 1,3-butadiene: stop-exposure groups. Exposure to 1,3-butadiene was stopped after 13 (SE 13 wk), 26 (SE 26 wk), 40 (SE 40 wk), or 52 weeks (SE 52 wk). After these limited exposure periods, animals were held in control chambers.

in male and female mice were not accompanied by increases in reticulocyte counts or in the frequency of polychromatophilic cells in peripheral blood, and they may indicate a partial or poorly regenerative response in the bone marrow of 1,3-butadiene-exposed mice to reduced levels of circulating erythrocytes. Another change caused by exposure of male and female mice to 1,3-butadiene was an increase in mean corpuscular volume and in mean corpuscular hemoglobin. A similar profile of hematologic changes was observed in male and female mice exposed to 625 ppm 1,3-butadiene for 65 weeks.

Table 1. Testis weights and testis weight/body weight ratios (×1000) for male mice exposed to 1,3-butadiene for 40 weeks.

Exposure concentration, ppm	Testis weight, mg[a]	Testis weight/body weight, ratio[a]
0	116 ± 6	2.89 ± 0.19
6.25	117 ± 9	2.93 ± 0.28
20	114 ± 9	2.78 ± 0.25
62.5	103 ± 12*	2.87 ± 0.36
200	102 ± 6*	2.54 ± 0.18*
625	60 ± 10*	1.60 ± 0.07*

[a]Results are given as means ± SD; $n = 10$.
*Different compared to control, $p < 0.05$.

40-Week Evaluation

Gross and microscopic evaluations of early-death mice at week 40 (i.e., those which died between the start of the study and week 40), indicated that the predominant cause of death in the 625-ppm exposure group was lymphocytic lymphoma (Table 3). The cumulative number of deaths by week 40 at the lower exposure levels was too

Table 2. Hematologic changes in peripheral blood of male mice exposed to 1,3-butadiene by inhalation for 40 weeks.[a]

Exposure concentration, ppm	RBC × 10^6/μL	HGB, g/dL	VPRC, mL/dL	MCV, fL	MCH, pg
0	10.4 ± 0.3	16.5 ± 0.4	48.1 ± 1.5	46.3 ± 0.8	15.9 ± 0.2
6.25	10.3 ± 0.3	16.4 ± 0.5	47.8 ± 1.7	46.4 ± 1.0	16.0 ± 0.3
20	10.4 ± 0.4	16.7 ± 0.7	48.2 ± 2.2	46.3 ± 0.8	16.1 ± 0.4
62.5	9.9 ± 0.4*	15.9 ± 0.6*	45.9 ± 2.1*	46.7 ± 1.2	16.1 ± 0.3
200	9.6 ± 0.5*	15.6 ± 0.9*	45.4 ± 2.7*	47.2 ± 1.0	16.2 ± 0.2*
625	7.6 ± 1.2*	13.5 ± 1.8*	39.9 ± 5.3*	53.2 ± 2.9*	17.9 ± 0.8*

[a] $n = 10$.
*Different from control (0 ppm), $p < 0.05$.

Table 3. Incidence of microscopic lesions in early death mice (through week-40) exposed to 625 ppm 1,3-butadiene.

Target: lesion	Males[a] n = 49	Females[a] n = 21	SE week 13[b] n = 10	SE week 26 n = 20
Lymphocytic lymphoma	43	16	9	19
Heart:				
Endothelial hyperplasia	2	0	0	0
Forestomach:				
Epithelial hyperplasia	22	11	3	9
Squamous cell neoplasm	1	2	0	1
Lung:				
Alveolar epithelial hyperplasia	3	1	0	0
Alveolar-bronchiolar neoplasm	1	2	0	1
Gonad:				
Atrophy	35	15	2	4

[a]Number of early death animals: males, n = 49; females, n = 21. There were no deaths in either the male or female control groups.
[b]SE, stop-exposure groups (males).

few (3/group or less) to demonstrate any potential trend. However, these deaths were generally due to lymphocytic lymphoma and included three mice at 200 ppm and one mouse at 62.5 ppm. Male mice were more susceptible to 1,3-butadiene-induced lymphoma than females; by week 40, lymphocytic lymphoma was observed in 43 of 49 early-death males and 16 of 21 early-death females exposed to 625 ppm 1,3-butadiene.

Lymphocytic lymphomas occurred in most early-death mice in the stop-exposure (SE) groups (Table 3). Ten of 50 male mice exposed to 625 ppm 1,3-butadiene for 13 weeks and 20 of 50 male mice exposed to 625 ppm 1,3-butadiene for 26 weeks died by week 40. Twenty-eight of these deaths were due to lymphocytic lymphoma. The mortality rate and the incidence of lymphocytic lymphoma was markedly reduced in those groups exposed to lower concentrations of 1,3-butadiene for a longer duration (comparable total exposures); lymphocytic lymphoma was present in only two male mice exposed to 200 ppm 1,3-butadiene for 40 weeks and one male mouse exposed to 312 ppm.

The incidence of other tumor types, typically later appearing than lymphocytic lymphoma, were too few to clearly demonstrate an exposure-related effect. The low incidences of squamous cell neoplasms of the forestomach and alveolar-bronchiolar neoplasms observed by week 40 are noteworthy since they are uncommon, especially in 46 to 47-week-old $B63CF_1$, and because they had been observed at increased incidences in the previous inhalation study in mice (*5,11*). Epithelial hyperplasia of the forestomach was observed in over 50% of the mice dying by week 40 that were exposed to 625 ppm 1,3-butadiene. Testicular and ovarian atrophy, lesions observed at high rates in the previous study (*5,11*), were prominent in this study as well.

The incidence of selected histopathologic lesions in groups of 10 male or 10 female mice killed at week 40 are shown in Table 4. Lymphocytic lymphomas were observed in two males and one female exposed to 625 ppm 1,3-butadiene. The low incidence of lymphocytic lymphoma in these animals, compared to the early-death mice (Table 3), reflects the rapid onset of a moribund condition in lymphoma-bearing mice. Proliferative lesions of the forestomach (epithelial hyperplasia and squamous cell papilloma) were present in both males and females exposed to 625 ppm 1,3-butadiene. Epithelial hyperplasia of the forestomach was observed in one female mouse exposed to 200 ppm 1,3-butadiene. The incidences of alveolar epithelial hyperplasia and alveolar-bronchiolar neoplasms were increased in male and female mice exposed to 625 and 200 ppm 1,3-butadiene, but not to 62.5 ppm 1,3-butadiene. Testicular atrophy, of minimal to mild severity, was present in 6 of 10 males exposed to 625 ppm 1,3-butadiene. Ovarian atrophy was marked in female mice exposed to 200 or 625 ppm 1,3-butadiene, while ovaries of mice exposed to 62.5 ppm appeared normal. The atrophic ovaries had no identifiable oocytes, follicles, or corpora lutea. A granulosa cell neoplasm of the ovary was observed in one female mouse in the 200-ppm dose group. Hemangiosarcomas of the heart were not observed in animals sacrificed at the 40-week interim evaluation; however, endothelial hyperplasia, a proliferative vascular lesion of the heart, was observed in one male and one female exposed to 625 ppm 1,3-butadiene for 40 weeks and two early death male mice exposed to 625 ppm 1,3-butadiene.

65-Week Evaluation

Data from the 65-week interim sacrifice of male mice (Table 5) show that exposure to 1,3-butadiene resulted in dose-related increases in the incidences of proliferative lesions of the heart, forestomach, lung, and Harderian gland (a site not identified in the previous study). Only 7 of the 90 male mice exposed to 625 ppm butadiene survived until week 65. Proliferative responses were induced in male mice at concentrations as low as 62.5 or 20 ppm after 65 weeks of exposure. Testicular atrophy was observed only in the 625-ppm exposure group.

The interim sacrifice of female mice at week 65 (Table 6) also revealed 1,3-butadiene-induced proliferative

Table 4. Incidence of microscopic lesions in mice exposed to 1,3-butadiene for 40 weeks.[a]

	Exposure concentration, ppm					
	Males			Females		
Target: lesion	0	200	625	0	200	625
Lymphocytic lymphoma	0	0	2	0	0	1
Forestomach:						
Epithelial hyperplasia	0	0	6	0	1	6
Squamous cell papilloma	0	0	3	0	0	2
Lung:						
Alveolar epithelial hyperplasia	0	3	3	0	0	1
Alveolar-bronchiolar neoplasm	1	2	4	0	3	1
Gonad:						
Atrophy	0	0	6	0	9	8

[a]n = 10, except for female mice exposed to 625 ppm 1,3-butadiene where n = 8.

Table 5. Incidence of microscopic lesions in male mice exposed to 1,3-butadiene for 65 weeks.[a]

Target: lesion	Exposure concentration, ppm					
	0	6.25	20	62.5	200	625
Lymphocytic lymphoma	0	0	0	0	0	2
Heart:						
Endothelial hyperplasia	0	NE[b]	0	1	1	1
Hemangiosarcoma	0	NE	0	0	1	3
Forestomach:						
Epithelial hyperplasia	0	0	0	1	0	2
Squamous cell neoplasm	0	0	0	1	3	4
Lung:						
Alveolar epithelial hyperplasia	0	0	1	1	3	2
Alveolar-bronchiolar neoplasm	0	0	0	2	4	5
Harderian gland:						
Adenoma	0	0	2	4	3	3
Liver:						
Hepatocellular neoplasm	3	1	5	3	4	6
Testis:						
Atrophy	0	NE	NE	NE	0	4

[a]n = 10, except for mice exposed to 625 ppm 1,3-butadiene where n = 7.
[b]NE, not examined microscopically.

Table 6. Incidence of microscopic lesions in female mice exposed to 1,3-butadiene for 65 weeks.[a]

Target: lesion	Exposure concentration, ppm					
	0	6.25	20	62.5	200	625
Lymphocytic lymphoma	1	0	0	0	3	0
Heart:						
Endothelial hyperplasia	0	NE[b]	NE	0	4	0
Hemangiosarcoma	0	NE	NE	0	1	2
Forestomach:						
Epithelial hyperplasia	0	0	0	0	2	1
Squamous cell neoplasm	0	0	0	1	2	1
Lung:						
Alveolar epithelial hyperplasia	0	0	2	1	4	0
Alveolar-bronchiolar neoplasm	0	0	0	3	4	2
Liver:						
Hepatocellular neoplasm	1	1	1	1	3	1
Ovary:						
Atrophy	0	0	2	9	8	2

[a]n = 10, except for mice exposed to 625 ppm 1,3-butadiene where n = 2.
[b]NE, not examined microscopically.

changes in the heart, forestomach, and lung, especially at 200 and 62.5 ppm 1,3-butadiene. Only two female mice exposed to 625 ppm 1,3-butadiene survived until week 65. Ovarian atrophy was observed at exposure levels of 20 ppm and higher.

The cumulative incidence of microscopic lesions in early-death mice through week 65 shows that lymphocytic lymphoma was the major cause of death at the 625 ppm exposure level, especially for male mice (Table 7). Between week 40 and week 65, 24 male mice exposed to 625 ppm 1,3-butadiene died; 18 had lymphocytic lymphoma. Increased incidences of hemangiosarcomas of the heart, forestomach neoplasms, alveolar-bronchiolar neoplasms, Harderian gland adenomas, and hepatocellular neoplasms were also evident in early-death male mice exposed to 625 ppm 1,3-butadiene. The number of early deaths in male mice exposed to 62.5 or 200 ppm 1,3-

Table 7. Incidence of microscopic lesions in early-death mice (through week 65) exposed to 1,3-butadiene.

Target: lesion	Exposure concentration, ppm					
	Males			Females		
	62.5 (4)[a]	200 (7)	625 (73)	62.5 (6)	200 (19)	625 (80)
Lymphocytic lymphoma	1	2	61	1	6	36
Heart:						
Endothelial hyperplasia	0	0	6	1	4	9
Hemangiosarcoma	0	0	3	0	3	24
Forestomach:						
Epithelial hyperplasia	0	1	39	3	5	41
Squamous cell neoplasm	0	0	6	0	0	26
Lung:						
Alveolar epithelial hyperplasia	0	0	9	1	4	10
Avelolar-bronchiolar neoplasm	1	3	3	1	5	22
Harderian gland:						
Adenoma	0	1	4	0	5	6
Mammary gland:						
Adenocarcinoma				1	3	11
Ovary:						
Granulosa cell neoplasm				0	2	5
Liver:						
Hepatocellular neoplasm	1	1	5	0	4	2
Gonad:						
Atrophy	0	1	53	4	15	68

[a]Numbers in parentheses equal the number of early death animals in each group. Only one control male mouse and one control female mouse died during the first 65 weeks of the study; hepatocellular adenoma was observed in the male mouse.

butadiene was too low to ascertain any potential carcinogenic effects. Fifty-nine female mice that were exposed to 625 ppm died between week 40 and week 65; 20 had lymphocytic lymphoma. The incidence of other primary tumors was high in these 59 mice: 24 hemangiosarcomas of the heart, 24 squamous cell neoplasms of the forestomach, 20 alveolar-bronchiolar neoplasms, 6 Harderian gland adenomas, 11 mammary gland adenocarcinomas, and 5 granulosa cell neoplasms of the ovary. Elevated incidences of neoplasms at these sites were also apparent in early-death female mice exposed to 200 ppm 1,3-butadiene. The development of neoplasms in the heart, forestomach, and lung were supported by high incidences of hyperplasias in these organs in both males and females. Testicular atrophy was observed at a high incidence in male mice exposed to 625 ppm, while ovarian atrophy was observed in female mice exposed to 62.5, 200, or 625 ppm 1,3-butadiene.

The tumor incidence profiles in the stop-exposure groups (Table 8) indicate that at comparable total exposures, the occurrence of lymphocytic lymphoma is more likely with exposure to a higher concentration of 1,3-butadiene for a short time, compared to exposure to a lower concentration for an extended duration. This is evident by comparing the incidence of lymphocytic lymphoma in the 625 ppm SE 13 WK group (15 lymphocytic lymphomas) with that in the 200 ppm SE 40 WK group (3 lymphocytic lymphomas), and comparing the incidence in the 625 ppm SE 26 WK group (30 lymphocytic lymphomas) with that in the 312 ppm SE 52 WK group (3 lymphocytic lymphomas). An obscuring effect of a high

incidence of lymphocytic lymphoma on 1,3-butadiene-induced neoplasia at other primary sites is evident by comparing the tumor profile in the 312 ppm SE 52 WK group with that in the 625 ppm SE 26 WK group. The incidences of hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, and hepatocellular neoplasms were higher in the group with the lower incidence of lymphocytic lymphoma (312 ppm SE 52 WK), indicating that longer survival allows these other chemically induced tumors to manifest. Graphic presentation of the incidences of lymphocytic lymphomas and hemangiosarcomas of the heart versus weeks on study for these two stop-exposure groups (Fig. 3) shows that hemangiosarcomas of the heart are more likely to be observed when the incidence of early lymphocytic lymphomas is low; conversely, when the rate of early lymphocytic lymphoma is high, the incidence of 1,3-butadiene-induced hemangiosarcoma of the heart is low.

Discussion

1,3-Butadiene has been shown to induce multiple organ carcinogenicity in rats and mice (*5,11,14*). In $B6C3F_1$ mice, exposure to 625 or 1250 ppm 1,3-butadiene caused early induction and significantly increased incidences of malignant lymphomas, hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, squamous cell neoplasms of the forestomach, acinar cell carcinomas of the mammary gland, granulosa cell neoplasms of the ovary, and hepatocellular neoplasms (*5,11*); Sprague-Dawley rats exposed to 1000 or 8000 ppm 1,3-butadiene had increased incidences at multiple sites: pancreatic exocrine adenomas, uterine sarcomas, Zymbal gland carcinomas, mammary gland neoplasms, thyroid follicular cell neoplasms, and Leydig cell neoplasms of the testis (*14*). Morphological descriptions and illustrations of the neoplastic lesions induced by 1,3-butadiene in $B6C3F_1$ mice are presented in the following paper (*15*). The large number of primary organ sites of tumor induction by 1,3-butadiene was unusual for chemicals studied by the NTP; only 1.9% of approximately 360 chemicals evaluated showed evidence of carcinogenicity at six or more primary sites.

Although the previous mouse study was ended after only 60 weeks of exposure because of high rates of early mortality (*5,11*), the doses used in that study are not considered excessive for evaluation of carcinogenicity, since survival was not reduced from toxic effects other than from the induction of fetal neoplasms. Furthermore, those concentrations (625 and 1250 ppm) are equivalent to and below the OSHA standard of 1000 ppm for occupational exposure to 1,3-butadiene (*16*). Announcements have been made to lower this level considerably (*17*).

The results of the studies of 1,3-butadiene in mice are unusual in that uncommon tumors, such as hemangiosarcoma of the heart and squamous cell neoplasms of the forestomach, were induced after inhalation exposure. In untreated $B6C3F_1$ mice 110 to 112 weeks of age, the historical rate for hemangiosarcomas of the heart is about 0.04% (1 in 2500), while that of squamous cell neoplasms of the forestomach is about 0.5% (1 in 200) (*12*). In the present studies, Harderian gland adenomas were observed in early-death mice (through week 65) exposed to 200 or 625 ppm 1,3-butadiene and at the 65-week interim evaluation. These observations indicate an additional site of tumor induction by 1,3-butadiene;

Table 8. Incidence of microscopic lesions in early-death male mice: stop-exposure (SE) groups through week 65.

	Exposure concentration, ppm[a]			
Target: lesion	200 SE 40 (8000)[a]	625 SE 13 (8125)	312 SE 52 (16224)	625 SE 26 (16250)
Lymphocytic lymphoma	3	15	3	30
Heart:				
Endothelial hyperplasia	0	1	1	5
Hemangiosarcoma	1	0	12	4
Forestomach:				
Epithelial hyperplasia	2	5	12	13
Squamous cell neoplasm	0	3	3	9
Lung:				
Alveolar eipthelial hyperplasia	1	0	3	4
Alveolar-bronchiolar neoplasm	1	3	9	5
Harderian gland:				
Adenoma	1	1	3	2
Liver:				
Hepatocellular neoplasm	2	0	8	4
Testis:				
Atrophy	0	2	1	5

[a]Number of early-death animals in each group (n): 200 ppm, SE 40, n = 7; 625 ppm, SE 13, n = 20; 312 ppm, SE 52, n = 21; 625 ppm, SE 26, n = 36.
[a]Total exposure expressed as ppm-weeks.

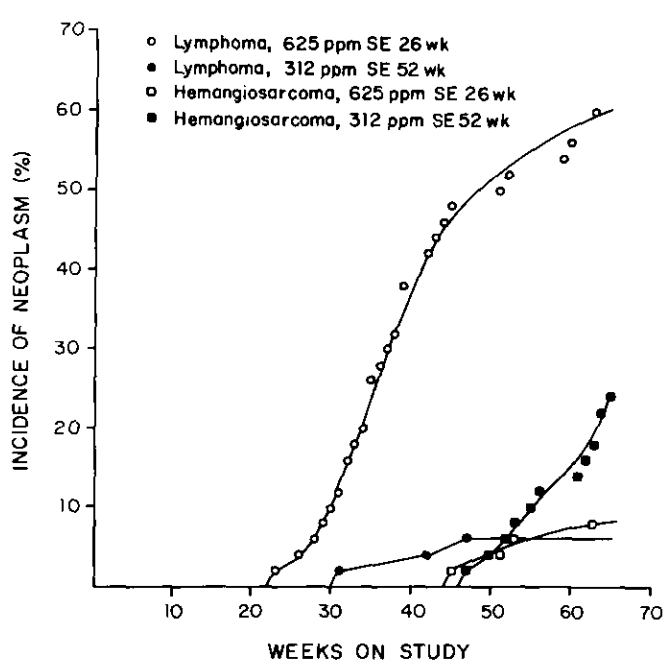

FIGURE 3. Cumulative incidence of lymphocytic lymphomas or hemangiosarcomas of the heart versus weeks on study in two stop-exposure groups for which the total exposures to 1,3-butadiene were comparable (625 ppm SE 26 wk and 312 ppm SE 52 wk).

the historical rate of Harderian gland neoplasms in untreated 110- to 112-week-old $B6C3F_1$ mice is approximately 2% (*12*).

The present inhalation studies of 1,3-butadiene were performed to better characterize dose-response relationships for butadiene-induced neoplastic and nonneoplastic lesions in $B6C3F_1$ mice at various intervals and after 2 years of exposure. The following conclusions are based on the results of the 40-week and 65-week evaluations. 1,3-Butadiene is a gonadal toxicant in mice, inducing testicular atrophy in males at 625 ppm, and ovarian atrophy in females at 20 ppm and higher. This gas was also toxic to the bone marrow of mice, causing a partially or poorly regenerative dose-dependent anemia at exposure concentrations of 62.5 ppm and higher. The increase in MCV and MCH at 625 ppm 1,3-butadiene indicates that although 1,3-butadiene caused suppression of hematopoiesis in the bone marrow, young larger cells may have been released into the blood from extramedullary sites. It has been reported that exposure of $B6C3F_1$ mice to 1250 ppm 1,3-butadiene produces a macrocytic-megaloblastic anemia (*18*) and promotes extramedullary hematopoiesis in the spleen (*19*).

For male or female mice exposed to 625 ppm 1,3-butadiene, lymphocytic lymphoma was the major cause of death in the first 50 weeks of the study. Prior to week 65, the lowest exposure concentration at which lymphocytic lymphomas were observed was 62.5 ppm; lymphocytic lymphoma was observed in one male mouse exposed to this concentration of 1,3-butadiene for 29 weeks. Lymphocytic lymphomas were also induced in male mice exposed to 625 ppm 1,3-butadiene for only 13 weeks (i.e., stop-exposure study). The incidence of lymphocytic lymphoma was two times higher in male mice exposed to 625 ppm 1,3-butadiene for 26 weeks, compared to male mice exposed to 625 ppm for 13 weeks. When the exposure concentration was reduced to 312 ppm and the exposure duration was extended to 52 weeks (312 ppm SE 52 WK), the incidence of lymphocytic lymphoma was 10% of the incidence in the group of mice that received the same total exposure to 1,3-butadiene (625 ppm SE 26 WK). Thus, the multiple of exposure concentration times exposure duration does not predict the incidence of lymphocytic lymphoma in mice. At equivalent total exposures, the likelihood for induction of lymphocytic lymphoma is greater with exposure to a higher concentration of 1.3-butadiene for a shorter time than for exposure to a lower concentration of 1,3-butadiene over an extended duration.

The early mortalities due to lymphocytic lymphomas, especially in male mice exposed to 625 ppm 1,3-butadiene, appear to limit the expression of neoplasms at other sites. Increased incidences of hemangiosarcomas of the heart, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, Harderian gland adenomas, adenocarcinomas of the mammary gland, granulosa cell neoplasms of the ovary, and hepatocellular neoplasms were frequently observed in 1,3-butadiene-exposed mice that died between weeks 45 and 65 of the study. Since early lymphocytic lymphomas were not as prevalent at concentrations of 6.25 to 200 ppm, it is likely that a clearer dose response for 1,3-butadiene-induced neoplasia at these sites will be obtained over this concentration range. The lack of a clear dose response for some of the neoplastic and nonneoplastic lesions observed in the previous inhalation study of 1,3-butadiene in $B6C3F_1$ mice (*5,11*) was likely due to reduced survival caused by early development of malignant lymphomas. Proliferative, nonneoplastic lesions of the forestomach, heart, and lung which were observed in early-death mice and at the 40-week and 65-week evaluations may represent treatment related preneoplastic changes at these target sites.

The carcinogenicity studies of 1,3-butadiene in rats and in mice demonstrate a species difference with respect to sites of tumor induction and magnitude of response. Irons and coworkers (*20,21*) have suggested that activation of an endogenous ecotropic retrovirus may play a critical role in butadiene-induced thymic lymphoma/leukemia in $B6C3F_1$ mice. However, the finding that exposure to 1250 ppm 1,3-butadiene for up to 1 year also caused a 14% incidence of thymic lymphoma/leukemia in NIH Swiss mice, a strain that does not express the endogenous ecotropic retrovirus recovered from tissues of $B6C3F_1$ mice. Further, Swiss mice have a background rate of zero for this neoplasm, indicating that 1,3-butadiene can induce thymic lymphoma independently of this activated retrovirus.

Butadiene monoxide (1,2-epoxybutene-3) has been identified as the primary metabolite of 1,3-butadiene biotransformation by rat liver microsomal monooxygenase (*22*). This intermediate may be further metabolized by liver monooxygenase or epoxide hydrolase to diepoxybutane or 3,4-epoxy-1,2-butanediol, respectively (*23*). Butadiene monoxide and diepoxybutane have been shown to be mutagenic to *Salmonella typhimurium* strains, which are sensitive to base pair substitution mutagens (*24,25*), and to induce local neoplasms when applied to the skin of mice or when administered to rats or mice by subcutaneous injection (*26,27*). Since 1,3-butadiene is mutagenic to *Salmonella typhimurium* only when incubated with liver S-9 activation systems (*28*), epoxide intermediates may be the ultimate carcinogenic forms of this chemical. Butadiene monoxide has been identified in the expired air of Sprague-Dawley rats exposed to 1,3-butadiene (*29*). Although the rate of butadiene metabolism is greater in $B6C3F_1$ mice than Sprague-Dawley rats at equivalent exposure concentrations (*30–32*), the magnitude of this difference at carcinogenic exposure levels in these species does not seem to be sufficient to account for the species difference in carcinogenicity of 1,3-butadiene. The present studies indicate that 200 ppm butadiene is carcinogenic to the heart, lung, Harderian gland, mammary gland, and ovary of $B6C3F_1$ mice; however, other than for the mammary gland, neoplasms were not induced at these sites in Sprague-Dawley rats exposed to 1000 or 8000 ppm 1,3-butadiene. The metabolism of

1,3-butadiene is greater in Sprague-Dawley rats exposed to 1000 ppm 1,3-butadiene than in $B6C3F_1$ mice exposed to 200 ppm 1,3-butadiene (*30,31*). Perhaps steady-state tissue concentrations of these intermediates at target sites are sufficiently different to account for the species difference in carcinogenicity of 1,3-butadiene.

The inhalation carcinogenicity studies of 1,3-butadiene in rats and mice have raised 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 neoplasms (*33–36*). These findings are consistent with the results of the inhalation studies of 1,3-butadiene in mice.

NOTE ADDED IN PROOF: Data from the 2-year exposure studies clearly showed that the incidence of alveolar-bronchiolar neoplasms in female $B6C3F_1$, mice was increased at all exposure concentrations of of 1,3-butadiene, including 6.25 ppm, compared to controls (R.L. Melnick et al., Cancer Res., in press).

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