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Carcinogenicity of 1,3-Butadiene

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1,3-Butadiene, a high-production volume chemical used largely in the manufacture of synthetic rubber, is a multiple organ carcinogen in rats and mice. In inhalation studies conducted in mice by the National Toxicology Program, high rates of early lethal lymphomas occurring at exposure levels of 625 ppm or higher reduced the development and expression of later developing tumors at other sites. Use of survival-adjusted tumor rates to account for competing risk factors provided a clearer indication of the dose responses for 1,3-butadiene-induced neoplasms. An increase in lung tumors in female mice was observed at exposure concentrations as low as 6.25 ppm, the lowest concentration ever used in a long-term carcinogenicity study of this gas. Human exposures to 1,3-butadiene by workers employed at facilities that produce this chemical and at facilities that produce styrene-butadiene rubber have been measured at levels higher than those that cause cancer in animals. Furthermore, epidemiology studies have consistently revealed associations between occupational exposure to 1,3-butadiene and excess mortality due to lymphatic and hematopoietic cancers. In response to the carcinogenicity findings for 1,3-butadiene in animals and in humans, the Occupational Safety and Health Administration has proposed lowering the occupational exposure standard for this chemical from 1000 ppm to 2 ppm. Future work is needed to understand the mechanisms of tumor induction by 1,3-butadiene; however, the pursuit of this research should not delay the reduction of human exposure to this chemical.

Introduction

1,3-Butadiene (CH_2=CH=CH=CH_2; CAS no. 106-99-0), a colorless, noncorrosive, flammable gas (boiling point: -4.4 °C), is produced mainly as a co-product in the steam cracking of petroleum fractions for the manufacture of ethylene (*1*). 1,3-Butadiene is a reactive chemical that can dimerize to 4-vinylcyclohexene or, upon exposure to air, form explosive peroxides (*1*). The major uses of 1,3-butadiene are in the manufacture of synthetic rubber (such as styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resins. Butadiene elastomers are used in the manufacture of rubber tires, footwear, sponges, hoses, pipes, luggage, packaging, and a variety of other molded products. 1,3-Butadiene is also used as an intermediate in the production of other industrial chemicals. The annual production volume of 1,3-butadiene is approximately 12 billion pounds worldwide and 3 billion pounds in the United States (*2,3*).

Based on its high volatility and low water solubility, environmentally released 1,3-butadiene partitions almost entirely into the atmosphere. According to a 1984 survey by the U.S. Environmental Protection Agency, atmospheric emissions of 1,3-butadiene from facilities that produce or process this chemical totaled approximately 10 million pounds per year, with 70% of these emissions being attributed to equipment leaks and 30% to process venting (*4*). 1,3-Butadiene has also been identified in automobile exhaust, cigarette smoke, and gasoline formulations, and small amounts are released by the burning of plastics or rubber (*5*). Low levels of 1,3-butadiene (0.5–10 ppb) have been detected in ambient air in urban locations in the United States; however, in community air at the perimeter of the industrial complex in Port Neches, Texas, where 1,3-butadiene and styrene-butadiene rubber are produced, levels as high as 2 ppm have been detected (*6*).

Approximately 52,00 workers are potentially exposed to 1,3-butadiene in the United States, as estimated from data compiled from the National Occupational Exposure Survey (*7*). In recent industrial hygiene surveys at four monomer and five polymer manufacturing plants, occupational exposures to 1,3-butadiene were generally found to be less than 10 ppm; however, several maximum 8-hr time-weighted average exposures and short-term exposures (15–120 min) were between 10 and 150 ppm, and in one case as high as 374 ppm, in operations involving decontamination and maintenance of process equipment, sampling and analyzing quality control samples, and loading or unloading tank trucks or rail cars (*8*). It is likely that exposures, especially short-term exposures, during the intensive wartime start-up of the synthetic rubber industry were greater than those in following years because of the rapid growth of the synthetic rubber industry during World War II and because during the 1940s, 1,3-butadiene was not considered to be hazardous to human health. In addition a high temperature, batch process was used in the early manufacture of styrene-butadiene rubber.

1,3-Butadiene has long been considered to have a low and noncumulative toxicity in animals and humans. Early toxicology studies on 1,3-butadiene indicated that this chemical only caused irritation to mucous membranes, skin, and eyes, or narcosis at high concentrations (*9*). Human volunteers exposed to 2000,

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4000, or 8000 ppm 1,3-butadiene for 6–8 hr experienced minor irritation to the eyes and difficulty in visual focusing. The studies by Carpenter et al. (*9*) in the 1940s formed the basis for the 8-hr time-weighted average workroom exposure concentration standard of 1000 ppm subsequently promulgated by the U.S. Occupational Safety and Health Administration (OSHA). Results of recent carcinogenicity studies of 1,3-butadiene in rats and mice (*10–13*) prompted the American Conference of Governmental Industrial Hygienists to lower their recommended threshold limit value (TLV) for 1,3-butadiene in the work environment from 1000 ppm to 10 ppm (*14*). Meanwhile, OSHA has proposed lowering the occupational exposure standard for 1,3-butadiene to a permissible exposure limit (PEL) of 2 ppm with a 15-min short-term exposure limit (STEL) of 10 ppm (*15*). Hearings have been held, and a final decision on the proposed change is expected this year. Proceedings of an international symposium on the Toxicology, Carcinogenesis, and Human Health Aspects of 1,3-Butadiene were published in 1990 in *Environmental Health Perspectives* (*16*).

This paper examines the carcinogenicity data of 1,3-butadiene in laboratory animals, with special emphasis on the influence of competing factors on tumor dose–response relationships, assesses the impact of recent mechanistic data on our understanding of species sensitivity to 1,3-butadiene-induced carcinogenicity, and compares results of exposure to 1,3-butadiene in humans to results obtained in animals.

Dose–Response for 1,3-Butadiene-Induced Carcinogenicity in Mice and Rats

In the absence of reliable epidemiological data, long-term studies in laboratory animals are the most reliable means of identifying potential health hazards, especially cancer, in humans. The carcinogenicity of inhaled 1,3-butadiene was studied in B6C3F$_1$ mice by the National Toxicology Program (NTP) (*10,11*) and in Sprague-Dawley rats by the International Institute of Synthetic Rubber Producers (IISRP) (*12,13*) to determine if there is a potential health hazard to humans. The NTP usually conducts experiments in Fischer 344 rats and B6C3F$_1$ mice, and the duration of exposure is generally 2 years. Because the IISRP study was in progress at the time of chemical selection, the NTP study was performed only in mice.

Inhalation Exposure of Mice to 625 or 1250 ppm 1,3-Butadiene

Two long-term inhalation exposure studies of 1,3-butadiene in B6C3F$_1$ mice have been completed by the NTP. In the first study (*10,11*), groups of 50 male and 50 female mice were exposed 6 hr/day, 5 days/week to air containing 0 (chamber control), 625, or 1250 ppm 1,3-butadiene. These concentrations bracketed the OSHA standard of 1000 ppm. This study, designed to last for 103 weeks, was terminated after 60–61 weeks for humane reasons because of reduced survival at both exposure concentrations due to malignant neoplasms involving multiple organs in both sexes. Malignant lymphomas, which appeared to originate in the thymus and were observed as early as week 20, were considered to be the major cause of early deaths.

Incidences of malignant lymphomas, hemangiosarcomas of the heart, and lung neoplasms were increased in both exposure groups of male and female mice compared with controls. Irons and co-workers confirmed by cytofluorometric analysis of cell surface markers that the type of lymphoma caused by 1,3-butadiene in B6C3F$_1$ mice is a T-cell lymphoma (*17,18*). The high incidences of hemangiosarcomas of the heart was a particularly unusual finding because these endothelial cell neoplasms are uncommon in B6C3F$_1$ mice, occurring only in one untreated male and one female mouse in the history of the NTP studies, and they have rarely been induced in long-term studies. Early induction and increased incidences of forestomach tumors were also observed in both sexes of mice, and increased incidences of tumors of the mammary gland, ovary, and liver were observed in females.

These results demonstrate that 1,3-butadiene is a potent multiple organ carcinogen in mice. However, dose–response relationships were not always clear; for example, the incidences of hemangiosarcomas of the heart in male mice were 0/50 for controls, 16/49 at 625 ppm, and 7/49 at 1250 ppm.

Inhalation Exposure of Mice to 0–625 ppm 1,3-Butadiene

A second long-term inhalation study of 1,3-butadiene in B6C3F$_1$ mice, over an expanded range of exposure concentrations, was performed to better characterize dose-response relationships for neoplastic lesions induced by this chemical (*19,20*). Male and female mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm of 1,3-butadiene, 6 hr/day, 5 days/week for up to 2 years. The top exposure level was included to coincide with the low exposure level in the first study.

Survival was reduced for males and females exposed to 20 ppm or higher concentrations (Fig. 1), largely due to the development of compound-related fatal tumors. Again, thymic lymphoma, occurring as early as week 23, was the major cause of death for male and female mice exposed to 625 ppm. The incidence of lymphoma was also increased in females exposed to 200 ppm.

The incidences of hemangiosarcomas of the heart were increased in male mice exposed to 62.5, 200, and 625 ppm, and in female mice exposed to 200 and 625 ppm. In addition, one animal from the 20-ppm exposure group of males and one from the 62.5 ppm group of females were observed with this uncommon endothelial cell tumor; these rare sarcomas are also most likely due to exposure to 1,3-butadiene.

Interestingly, the incidence of hemangiosarcomas of the heart was greater in male mice exposed to 200 ppm than in those exposed to 625 ppm. This unusual dose-response was attributed to the early and extensive induction of lymphomas at 625 ppm that resulted in a significantly reduced number of mice at risk for the later-developing hemangiosarcomas. The effect of "competing risks" of early occurring lethal thymic lymphomas on the development of hemangiosarcomas of the heart is evident from the plots of the cumulative incidences of these neoplastic lesions against the number of weeks on study for male mice exposed to 200 or 625 ppm of 1,3-butadiene (Fig. 2). In the 625-ppm exposure group, the incidence of early lymphoma was high (70%), and the incidence of hemangiosarcoma of the heart was low (5%), but in the 200-ppm exposure group, the incidence of early lymphoma was low (4%), and the incidence of hemangiosarcoma of the heart (42%) was much higher than that in the 625-ppm exposure group.

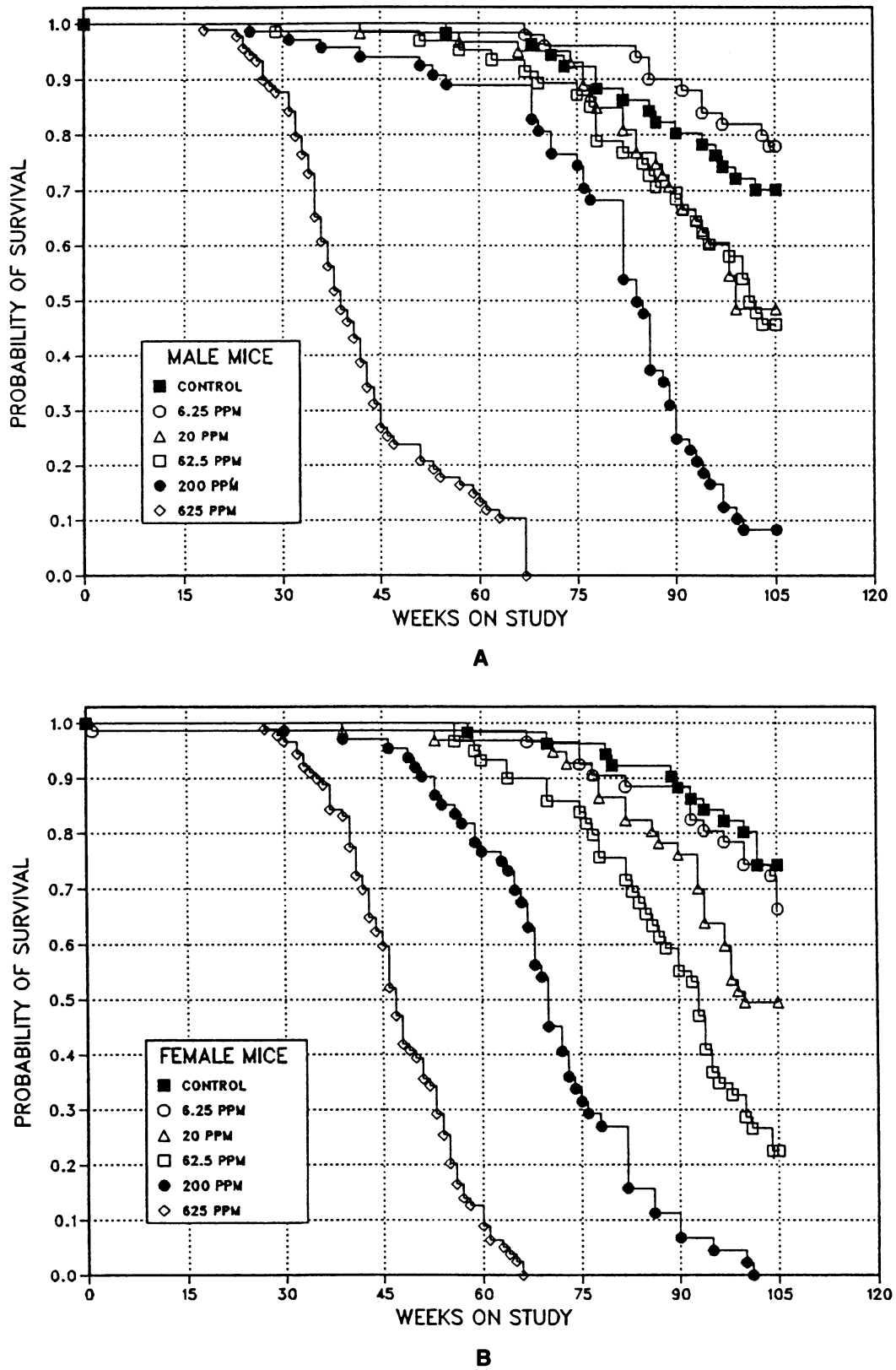

FIGURE 1. Kaplan-Meier survival curves for B6C3F$_1$ mice exposed to 1,3-butadiene for 2 years. (*A*) Male mice; (*B*) female mice.

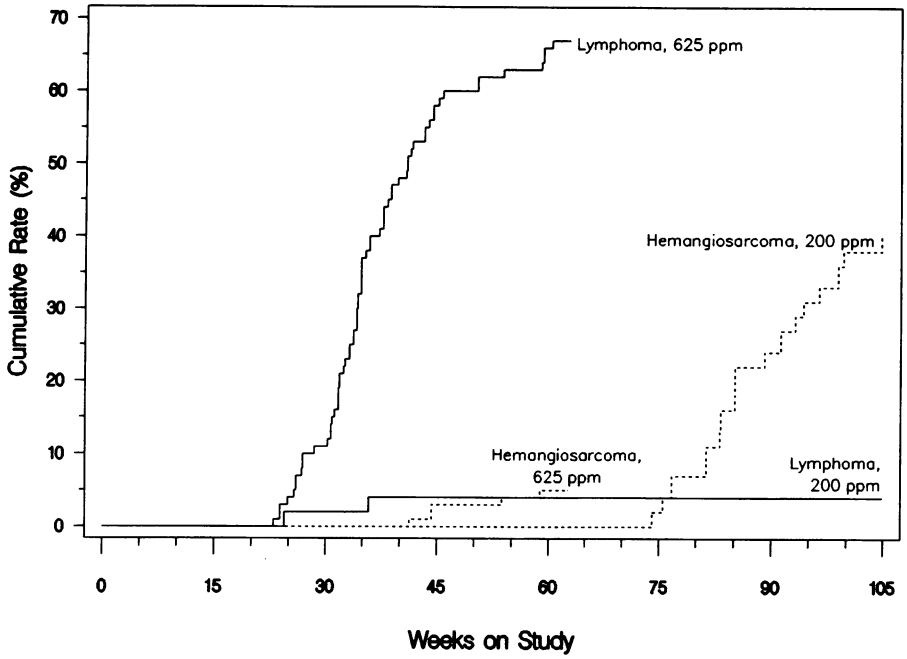

FIGURE 2. Cumulative death-with-tumor rates for lymphomas or hemangiosarcomas of the heart versus weeks on study in male B6C3F$_1$ mice exposed to 200 or 625 ppm 1,3-butadiene.

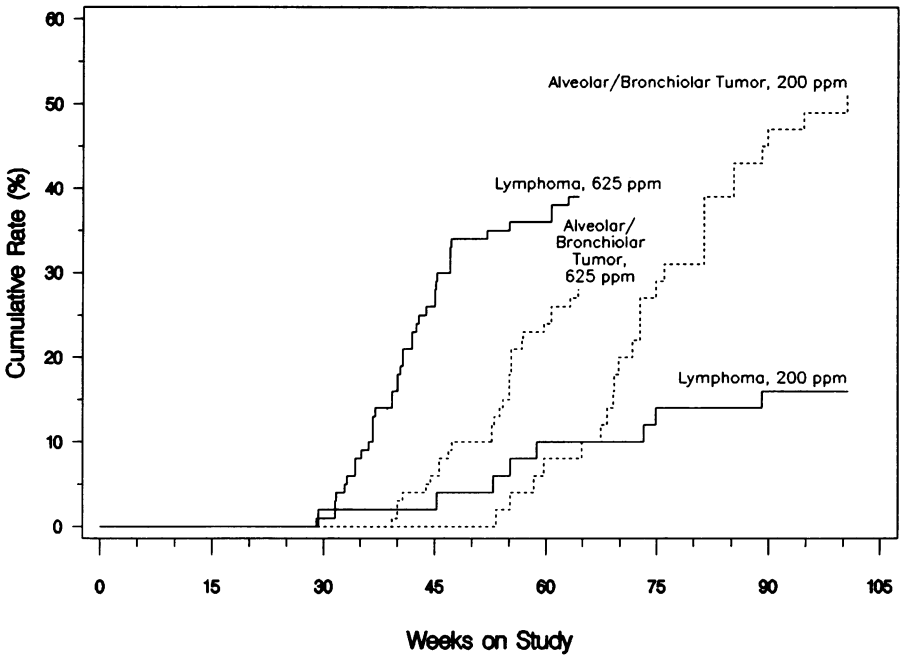

FIGURE 3. Cumulative death-with-tumor rates for lymphomas or alveolar-bronchiolar neoplasms versus weeks on study in female B6C3F$_1$ mice exposed to 200 or 625 ppm 1,3-butadiene.

The incidences of lung neoplasms in female mice were increased in all exposure groups. Thus, even at 6.25 ppm, 1,3-butadiene is carcinogenic to B6C3F$_1$ mice. Because there were no exposure levels at which a carcinogenic response was not induced, it is likely that exposure concentrations below 6.25 ppm would also cause cancers in laboratory animals. Extrapolation of the dose–response curves to 2 ppm, the proposed OSHA standard for 1,3-butadiene, results in an estimated 2-fold increase in lung tumors in female mice. The reduced incidence of lung neoplasms at 625 ppm compared with the incidence at 200 ppm

Table 1. Sites of neoplasia and lowest significant effect level in male and female B6C3F$_1$ mice in the 2-year inhalation study of 1,3-butadiene.[a]

Site	Male mice	Female mice
Lymphoma	+, 200 ppm	+, 20 ppm
Heart	+, 62.5 ppm	+, 200 ppm
Lung	+, 62.5 ppm	+, 6.25 ppm
Forestomach	+, 200 ppm	+, 625 ppm
Liver	+, 200 ppm	+, 62.5 ppm
Harderian gland	+, 62.5 ppm	+, 62.5 ppm
Preputial gland	+, 200 ppm	NA
Ovary	NA	+, 62.5 ppm
Mammary gland	−	+, 62.5 ppm
Brain	+(U)	−
Kidney	+(U)	±
Small intestine	±	−
Zymbal gland	±	±
Skin	−	±

[a](+) Increase relative to controls; (±) marginal increase relative to controls; (−) no difference from controls; NA, not applicable; U, uncommon tumor with nonsignificant increased incidence.

was attributed to the high rate of early deaths due to competing risks of 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 than with exposure to 200 ppm; however, because all the female mice exposed to this concentration of 1,3-butadiene died by week 65, the final incidence of this later-developing and rarely lethal (lung) tumor was less than that for female mice exposed to 200 ppm.

Neoplastic lesions of the forestomach, mammary gland, ovary, and liver, the other organ sites identified in the first study, were again increased in mice exposed to 1,3-butadiene. In addition, the Harderian gland, preputial gland, brain, and kidney were identified as sites of 1,3-butadiene-induced neoplasia (Table 1).

In unexposed chamber-control female mice, only benign neoplastic lesions were observed in the lung, Harderian gland, forestomach, and ovary; however, in female mice exposed to 1,3-butadiene, malignant neoplasms were observed in each of these organs (*19,20*). In particular, high incidences of alveolar-bronchiolar carcinomas were observed in exposed female mice. Of the lung tumors observed in female mice exposed to 6.25 ppm 1,3-butadiene, 33% were alveolar-bronchiolar carcinomas. The greater tendency toward malignancy in mice exposed to 1,3-butadiene further demonstrates the strong carcinogenic potency of this chemical.

Survival-adjusted Tumor Rates

The impact of early mortality on the expression of late developing tumors was largely accounted for by adjusting for differences in survival patterns. Survival-adjusted tumor rates for selected neoplasms are shown in Table 2 for male mice and in Table 3 for female mice. For the tumor incidences in these tables, numerators represent the number of animals bearing the specific neoplastic lesion, and denominators represent the adjusted number of animals at risk. The latter values were determined by combining the partial life experience for each of the animals in a particular exposure group. This combination was achieved by calculating the proportion of lifetime tumor risk observed for each animal. Animals that survived to the end of the study and animals that had the particular tumor in question (early death animals or animals that lived until the end of the study) were given a weight of one unit. The weight for an animal that died

Table 2. Survival-adjusted rates of selected neoplasms in male B6C3F$_1$ mice in the 2-year inhalation study of 1,3-butadiene.[a]

	Exposure concentration, ppm					
Site: neoplasm	0	6.25	20	62.5	200	625
Lymphoma	4/44.3 (9.0)	2/46.0 (4.3)	4/40.0 (10.0)	6/39.3 (15.3)	2/27.1 (7.4)	51/52.5 (97.2)*
Heart: hemangiosarcoma	0/43.2 (0.0)	0/45.0 (0.0)	1/38.8 (2.6)	5/37.3 (13.4)*	20/31.3 (63.8)*	4/7.6 (52.6)*
Lung:alveolar-bronchiolar neoplasm	21/44.3 (47.4)	23/47.0 (48.9)	19/42.4 (44.8)	31/41.9 (74.0)*	35/39.9 (87.7)*	3/6.7 (44.8)

[a]Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomic site divided by the adjusted number of animals at risk determined by the poly-3 survival-adjusted quantal response method of Portier and Bailer (*21*). Survival-adjusted tumor rates are given in parentheses, as percentages.
*Increased compared with chamber control (0 ppm), $p < 0.05$.

Table 3. Survival-adjusted rates of selected neoplasms in female B6C3F$_1$ mice in the 2-year inhalation study of 1,3-butadiene.[a]

	Exposure concentration, ppm					
Site: neoplasm	0	6.25	20	62.5	200	625
Lymphoma	6/46.0 (13.0)	12/44.3 (27.1)	11/40.1 (27.4)	7/35.0 (20.0)	9/22.6 (39.8)*	32/37.5 (85.3)*
Heart: hemangiosarcoma	0/45.4 (0.0)	0/44.1 (0.0)	0/38.8 (0.0)	1/32.7 (3.1)	21/29.3 (71.6)*	23/27.6 (83.2)*
Lung:alveolar-bronchiolar neoplasm	4/45.5 (8.8)	15/45.5 (33.0)*	19/41.0 (46.3)*	24/39.4 (60.9)*	25/30.8 (81.3)*	22/26.8 (82.2)*
Ovary:granulosa cell neoplasm	1/44.4 (2.3)	0/44.1 (0.0)	1/38.2 (2.6)	9/34.2 (26.3)*	8/19.5 (41.1)*	6/12.9 (46.5)*
Mammary gland: adenocarcinoma or adenoacanthoma	0/45.4 (0.0)	2/44.1 (4.5)	4/39.2 (10.2)*	12/36.8 (32.6)*	15/26.6 (56.4)*	16/24.0 (66.8)*

[a]Incidence is given as the number of animals bearing a neoplastic lesion at a specific anatomic site divided by the adjusted number of animals at risk determined by the poly-3 survival-adjusted quantal response method of Portier and Bailer (*21*). Survival-adjusted tumor rates are given in parentheses, as percentages.
*Increased compared with chamber control (0 ppm), $p < 0.05$.

before study termination but was free of the tumor being analyzed was estimated by calculating the fractional survival time on study for that animal raised to the third power. For example, an animal that did not have a hemangiosarcoma of the heart and died due to lymphoma after about 1/3 of the study (approximately 35 weeks) had $(1/3)^3$ or about 0.04 risk for the development of the heart tumor, as compared to an animal living for the full study; however, if that animal had survived for 2/3 of the study (approximately 70 weeks), it would have contributed a weight of $(2/3)^3$ or about 0.30. This survival-adjusted "poly-3 quantal response" method has been described by Portier and Bailer (*21*). The use of the third power was selected because cumulative tumor rates have been found to occur generally as a third- to fourth-order function of age in animals. Simulation experiments have shown that this method is valid (correct false-positive error rate) even when the actual values for the power are as low as 1 or as high as 5 (*22*).

The survival-adjusted tumor rates provide a clearer indication of the dose–responses for neoplasms caused by 1,3-butadiene. The apparent downturn in response for hemangiosarcomas of the heart and lung neoplasms in male mice exposed to 625 ppm 1,3-butadiene was substantially, but not totally, reversed by adjusting for reduced survival. Because of the large number of early deaths due to lymphoma at this exposure level, the adjusted

number of animals at risk for developing heart or lung tumors was less than 10. At lower exposure concentrations, where early death due to lymphoma was not a major competing factor, there was a much larger adjusted number of animals at risk for these later developing tumors. Thus, not unexpectedly, the 200 ppm exposure group had the largest number of animals with heart and lung tumors.

In female mice, the incidence of lymphoma at 625 ppm was less than that in male mice. Consequently, tumor responses at other sites were not as drastically affected in females as in males at this exposure level. Except for the ovary, the adjusted number of animals at risk for the later-developing tumors was approximately 25. As the concentration of 1,3-butadiene was reduced from 625 ppm to 6.25 ppm, survival was increased and the adjusted number of animals at risk increased steadily for each of these sites. The high adjusted number of male or female mice at risk for lymphoma at 625 ppm reflects the high incidence of this life-shortening neoplastic lesion.

For tumors showing exposure related effects, the shapes of the dose–response curves were estimated by fitting a modified Weibull model (*23*) to the poly-3 survival-adjusted tumor rates (*20*). For approximately half of the tumors evaluated, the dose response was consistent with a linear model (forestomach neoplasms in males and females, hemangiosarcomas of the heart and preputial gland tumors in males, and lymphoma, liver, and ovarian neoplasms in females). In most instances in which a departure from linearity was evident, the shape parameter indicated a dose–response curve that was supralinear (concave downward) in the low dose region (lung and Harderian gland neoplasms in males and females, liver neoplasms in males, and mammary gland tumors in females). Only for lymphoma in male mice and hemangiosarcoma of the heart in female mice was there evidence of a sublinear (concave upward) dose–response curve.

The inhalation studies conducted at 6.25–625 ppm 1,3-butadiene provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced cancers than did the studies at 625 and 1250 ppm. The observation of carcinogenicity at all exposure levels studied demonstrates that carcinogenic responses occur over a 200-fold concentration range in mice.

Stop-Exposure Studies

Stop-exposure studies were designed to assess the relationship between exposure level and duration of exposure on the outcome of 1,3-butadiene-induced carcinogenicity and to consider the effect of variable worker exposure patterns. These studies were conducted by exposing groups of 50 male mice to one of the following regimens: *a*) 200 ppm for 40 weeks; *b*) 625 ppm for 13 weeks; *c*) 312 ppm for 52 weeks; or *d*) 625 ppm for 26 weeks (*19,20*). After the particular exposures were terminated, these groups of animals were placed in control chambers for the remainder of the 104 week studies. The total exposure to 1,3-butadiene (concentration times duration of exposure) was approximately equivalent for the first two groups (8000 ppm-weeks for groups *a* and *b*) and provided about half the total exposure given to the latter two groups (16,000 ppm-weeks for groups *c* and *d*).

The tumor incidence profiles in the stop-exposure groups showed that lymphomas, hemangiosarcomas of the heart, and tumors of the lung, forestomach, Harderian gland, and preputial gland were increased even after only 13 weeks of exposure to 625 ppm of 1,3-butadiene (*19,20*). It is likely that even shorter exposure durations would also produce a positive carcinogenic response. Furthermore, at comparable total exposures, the incidence of lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for an extended duration. This is evident by comparing the adjusted incidence of lymphoma in the 625 ppm 13-week stop-exposure group (56%) with that in the 200-ppm 40-week stop-exposure group (24%), or by comparing the incidence in the 625 ppm 26-week stop-exposure group (87%) with that in the 312 ppm 52-week stop-exposure group (35%) (Table 4). Thus, for the development of thymic lymphomas, the concentration of 1,3-butadiene is a greater contributing factor than is the duration of exposure. For other tumor types, e.g., hemangiosarcomas of the heart, the rates appeared to be dependent on the multiple of exposure concentration times the duration of exposure.

Table 4. Survival-adjusted rates for lymphoma and hemangiosarcoma of the heart in the stop-exposure (SE) groups of male B6C3F$_1$ mice exposed to 1,3-butadiene.[a]

	Exposure concentration, ppm				
Site: neoplasm	0 (control)	200 ppm SE 40 weeks (8,000)[b]	625 ppm SE 13 weeks (8,125)	312 ppm SE 52 weeks (16,224)	625 ppm SE 26 weeks (16,250)
Lymphoma	9.0	24.1	56.1*	35.0*	87.2*
Heart: hemangiosarcoma	0.0	47.1*	30.9*	85.2*	74.5*

[a]Survival-adjusted tumor rates, determined by the poly-3 survival-adjusted quantal response method of Portier and Bailer (*21*), are given as percentages.
[b]Total exposure expressed as ppm-weeks.
*Increased compared with chamber control (0 ppm), $p < 0.05$.

Carcinogenicity of 1,3-Butadiene in Rats

In the carcinogenicity study conducted by the IISRP in rats, groups of 100 animals of each sex were exposed to 0, 1000, or 8000 ppm 1,3-butadiene for 6 hr/day, 5 days/week for 2 years (*12,13*). Under the conditions of this study, 1,3-butadiene was carcinogenic at multiple organ sites, as evidenced by increased incidences and/or dose–response trends for several organ-specific cancers: pancreatic exocrine neoplasms and Leydig cell tumors of the testis in males and uterine stromal sarcomas, Zymbal gland carcinomas, mammary gland fibroadenomas and carcinomas, and thyroid follicular cell neoplasms in females. The increase in mammary gland fibroadenomas was further related to exposure to 1,3-butadiene because the average number of mammary gland fibroadenomas per rat bearing a mammary gland tumor was increased in both exposure groups compared to controls. The occurrence of uncommon glial cell tumors of the brain in exposed male rats may also have been related to exposure to 1,3-butadiene. The incidences of brain, uterine, and mammary gland tumors were not much higher in the 8000-ppm exposure group compared to the 1000 ppm exposure group. The nearly level dose response for these tumors was probably due to saturation of 1,3-butadiene metabolism in Sprague-Dawley rats, which occurs at exposure concentrations between 1000 and 2000 ppm. Unlike the response in mice, there were no increases in the incidences of lymphoma/leukemia, or neoplasms of the lung, heart, forestomach, ovary, liver, Harderian gland, or preputial

gland in Sprague-Dawley rats of either sex exposed to 1000 or 8000 ppm 1,3-butadiene.

Mechanistic Studies of 1,3-Butadiene Carcinogenicity and Relevance to Human Risk

Influence of Murine Retrovirus

Recent experimental research has been aimed at understanding the mechanisms of tumor induction by 1,3-butadiene. Irons and co-workers compared the induction of thymic lymphomas and the expression of murine leukemia retrovirus in B6C3F$_1$ mice and in NIH Swiss mice exposed to 1,3-butadiene for 52 weeks (*17,18,24*). The Swiss strain of mouse was used because it does not express the ecotropic murine leukemia viruses expressed in B6C3F$_1$ mice, and it has a background rate of nearly zero for thymic lymphoma. The finding that 1 year of exposure to 1250 ppm 1,3-butadiene caused a 14% incidence of thymic lymphomas in NIH Swiss mice shows clearly that 1,3-butadiene induces this neoplasm independently of these activated retroviruses.

DNA Adducts

Reaction products of guanine with 1,2-epoxy-3-butene or diepoxybutane were detected in liver DNA of B6C3F$_1$ mice, but not in liver DNA of Wistar rats, exposed to 500 ppm [^{14}C]1,3-butadiene (*25*). Future work is needed on DNA adduct formation in the major target sites of 1,3-butadiene carcinogenicity in B6C3F$_1$ mice and in Sprague-Dawley rats at the exposure concentrations used in the 2-year studies. Furthermore, the nature of the covalently bound [^{14}C]1,3-butadiene-derived radioactivity to rat liver DNA (*26*) is yet to be determined.

Metabolism and Pharmacokinetics

Studies on the metabolism and pharmacokinetics of 1,3-butadiene in rats and mice have been undertaken to investigate and explain species differences in organ site specificity and potencies of 1,3-butadiene-induced carcinogenicity. Biotransformation of 1,3-butadiene is probably an important factor in the carcinogenicity of this compound because the *in vitro* mutagenicity of 1,3-butadiene requires metabolic activation (*27*), and epoxide intermediates of 1,3-butadiene metabolism are mutagenic in *Salmonella typhimurium* (*28,29*) and are carcinogenic in rats and mice (*30,31*).

1,2-Epoxy-3-butene, the first intermediate of 1,3-butadiene metabolism (*32*), is formed by an inducible rat liver microsomal cytochrome P-450 monooxygenase (*33*) (Fig. 4). 1,2-Epoxy-3-butene has been detected in expired air of Sprague-Dawley rats (*33,34*) and of B6C3F$_1$ mice (*35*) exposed to 1,3-butadiene, indicating that this epoxide intermediate is systemically available in exposed animals. Further metabolic transformation of 1,2-epoxy-3-butene involves conjugation with glutathione by glutathione-*S*-transferase, oxidation to 1,2:3,4-diepoxybutane, or hydrolysis by epoxide hydrolase and further oxidation to 3,4-epoxy-1,2-butanediol (*36*).

Saturation of 1,3-butadiene metabolism in Sprague-Dawley rats and in B6C3F$_1$ mice was reported to occur at atmospheric concentrations between 1000 and 2000 ppm (*37*). The rate of metabolism of 1,3-butadiene in mice is about two times higher

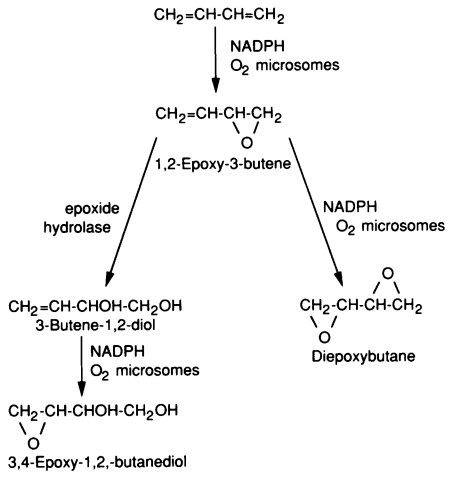

FIGURE 4. Metabolism of 1,3-butadiene.

than that in rats (*37,38*). This increase is probably due to the higher respiratory frequency of mice (*37*). Thus, risk assessment models that account for breathing rate differences between species adjust for the major factor that distinguishes the increased metabolic rate for 1,3-butadiene in mice compared to rats.

Steady-state concentrations of 1,2-epoxy-3-butene are approximately six times higher in mice than in rats exposed to the same atmospheric concentration of 1,3-butadiene; however, about half of this difference is attributed to the higher breathing rate of mice compared to rats (*39*). Pharmacokinetic studies of 1,3-butadiene in rats and mice have not revealed species differences of sufficient magnitude to account for the different carcinogenic responses seen in these two species (*39*). Evidently, other factors are involved in distinguishing site-specific differences in the carcinogenicity of 1,3-butadiene between species, including humans. More work is needed to understand species differences in 1,3-butadiene pharmacokinetics, metabolic differences and reactivity of metabolic intermediates with DNA at target sites, differences in detoxification or mechanisms of repair, and the impact these factors may have on 1,3-butadiene-induced carcinogenesis.

Epidemiology Studies

Associations between occupational exposure to 1,3-butadiene and increased risk of cancer have been evaluated in retrospective mortality studies of workers employed at facilities that produce 1,3-butadiene (*40,41*) and at facilities that produce styrene-butadiene rubber (*42–44*). Excess mortalities from lymphatic and hematopoietic cancers among subgroups of occupationally exposed workers in each of these studies provides strong evidence for the carcinogenicity of 1,3-butadiene to humans. Rates of mortality for lymphosarcoma and reticulum cell sarcoma were increased by as much as 5.6-fold among workers in a 1,3-butadiene manufacturing plant (*40*), while 5- to 6.6-fold increases in mortality from all lymphopoietic cancers and leukemia were reported among black workers in the production areas of styrene-butadiene-rubber plants (*44*). In a nested case–control study in which the cases of lymphopoietic cancers were compared to an internal population of workers who did not have cancer, Matanoski et al. (*45*) found that the excess leukemias in

the styrene-butadiene rubber industry were associated with exposure to 1,3-butadiene and not to styrene.

Because the epidemiology studies lacked quantitative exposure data, some have considered duration of employment within broad work job classifications to be an indication of human exposure (*46,47*). This relationship is probably not valid for the 1,3-butadiene or styrene-butadiene rubber production industries because there have been large variabilities in exposures associated with specific tasks, especially during the rapid growth of the synthetic rubber industry during World War II, and because process changes in the production of 1,3-butadiene and in the production of styrene-butadiene rubber probably affected workplace exposures over the past 50 years. Thus, not surprisingly, increased mortality for lymphopoietic cancers was most striking among workers first hired in the 1,3-butadiene and styrene-butadiene-rubber plants during World War II (*40–42*). Relevant to this issue are the results of the stop-exposure studies in mice, where the exposure concentration of 1,3-butadiene was a greater contributing factor to the development of thymic lymphomas than was the duration of exposure (*19,20*).

Conclusions

1,3-Butadiene is a high-production-volume chemical used largely in the manufacture of synthetic rubber. The production and use of 1,3-butadiene increased dramatically during World War II with the development of the synthetic rubber industry. Before the 1980s, 1,3-butadiene was not considered to be particularly hazardous to human health; therefore, OSHA established a permissible limit of 1000 ppm for occupational exposure to this chemical. Results of recent inhalation carcinogenicity studies have demonstrated clearly that 1,3-butadiene is a multiple-organ carcinogen in Sprague-Dawley rats and in B6C3F$_1$ mice. Particularly noteworthy in mice were the early occurrences and extensive development of lymphomas, the induction of uncommon hemangiosarcomas of the heart, and the development of malignant lung tumors at exposure concentrations as low as 6.25 ppm. Because 6.25 ppm was the lowest concentration ever used in a long-term carcinogenicity study of this gas, it is likely that lower exposure levels would also cause cancers in laboratory animals. In addition, multiple organ site neoplasia was induced in mice after only 13 weeks of exposure.

The conclusion that the marginally increased incidences of hepatocellular neoplasms in male and female mice were chemically related is strengthened by the detection of activated K-*ras* oncogenes with a specific codon 13 mutation in liver neoplasms obtained from mice exposed to 1,3-butadiene (*48*) in the carcinogenicity studies (*19,20*). Activated K-*ras* oncogenes have never been detected in liver tumors from untreated B6C3F$_1$ mice (*49*). Activated K-*ras* genes by codon 13 mutations were also found in lung neoplasms and in some of the lymphomas induced by exposure to 1,3-butadiene. In addition, Wiseman and Barrett (*50*) showed that tumor-suppressor genes are inactivated during 1,3-butadiene carcinogenesis. The detection of activated K-*ras* oncogenes and inactivated tumor suppressor genes in tumors induced by 1,3-butadiene adds further relevance to the potential carcinogenicity of 1,3-butadiene in humans because K-*ras* is the most commonly detected oncogene in human cancers, and allele losses are common in human cancers.

The important issue of competing risks has received little attention in the evaluation of long-term chemical carcinogenesis experiments. The studies of 1,3-butadiene in mice illustrate the need to adjust tumor incidence data for survival differences among groups to provide reliable dose–response data when competing risk factors obscure the development and detection of later-developing neoplastic lesions. The poly-3 quantal response method was used to adjust for the number of animals at risk and thereby provide survival-adjusted tumor rates. For most of the tumors evaluated, the dose responses were consistent with a linear curve or supralinear curve in the low dose region. For only two tumor responses was there evidence of a sublinear dose–response curve.

Two reactive epoxides, 1,2-epoxy-3-butene and diepoxybutane, have been identified as intermediates in the biotransformation of 1,3-butadiene in rats and mice. Metabolism is probably an important factor in the carcinogenicity of 1,3-butadiene because *in vitro* mutagenicity of 1,3-butadiene requires metabolic activation, whereas these epoxide intermediates are direct-acting mutagens in bacteria and are carcinogens in rats and mice. The metabolism of 1,3-butadiene in rats and mice is linear up to concentrations of at least 1000 ppm. Pharmacokinetic studies on 1,3-butadiene and on 1,2-epoxy-3-butene have revealed certain quantitative differences in metabolic rates for these chemicals in Sprague-Dawley rats and B6C3F$_1$ mice; however, these differences were not of sufficient magnitude to account for the reported different target site carcinogenic responses in these two species. Thus, additional factors must be involved in distinguishing site specificity in the carcinogenicity of 1,3-butadiene between species.

Epidemiology studies of workers employed in the production of 1,3-butadiene or of styrene-butadiene rubber have consistently revealed associations between occupational exposure to 1,3-butadiene and excess mortality due to lymphatic and hematopoietic cancers. The excess leukemias in the styrene-butadiene rubber industry were associated with exposure to 1,3-butadiene and not to styrene. The results from studies in humans correspond to the increases in lymphoma observed in mice exposed to 1,3-butadiene.

Based on the evidence of carcinogenicity for 1,3-butadiene in humans and in experimental animals, the International Agency for Research on Cancer has concluded that 1,3-butadiene is probably carcinogenic to humans (*5*). In response to the findings that 1,3-butadiene is a potent carcinogen in animals and that human exposure to 1,3-butadiene is associated with excess mortalities due to lymphatic and hematopoietic cancers, OSHA has proposed to lower the occupational standard for this chemical from 1000 ppm to 2 ppm (*15*). The determination of this 500-fold decrease was based largely on data from the NTP carcinogenicity studies of 1,3-butadiene in mice.

Future research on 1,3-butadiene is needed to elucidate mechanisms of 1,3-butadiene-induced carcinogenicity and to more fully assess human mortality outcomes with better quantitative estimates of past and present exposures. The pursuit of these research objectives should not delay the reduction of human exposure to this chemical.

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