reward function [D. L. Margules and L. Stein, *Am. J. Physiol.* **217**, 475 (1969); E. F. Domino and M. E. Olds, *J. Pharmacol. Exp. Ther.* **164**, 202 (1968); P. Stark and E. S. Boyd, *J. Pharmacol.* **205**, 745 (1963); L. M. Newman, *J. Comp. Physiol. Psychol.* **79**, 394 (1972)]. Our data do not contradict this conclusion; measures of refractory period distributions are based on relative comparisons between single-pulse and paired-pulse effectiveness, whereas evidence of overall suppressive effects are based on analysis of absolute measures of stimulation effectiveness. It is possible that other muscarinic systems, independent of and antagonistic to the one identified here, usually mask the positive contribution revealed here.

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Multiple Organ Carcinogenicity of 1,3-Butadiene in B6C3F$_1$ Mice After 60 Weeks of Inhalation Exposure

Abstract. *Groups of 50 male and 50 female B6C3F$_1$ mice were exposed 6 hours per day, 5 days per week, for 60 to 61 weeks to air containing 0, 625, or 1250 parts per million 1,3-butadiene. These concentrations are somewhat below and slightly above the Occupational Safety and Health Administration standard of 1000 parts per million for butadiene. The study was designed for 104-week exposures but had to be ended early due to cancer-related mortality in both sexes at both exposure concentrations. There were early induction and significantly increased incidences of hemangiosarcomas of the heart, malignant lymphomas, alveolar-bronchiolar neoplasms, 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. Current workplace standards for exposure to butadiene should be reexamined in view of these findings.*

1,3-Butadiene, in production volume one of the top 20 organic chemicals manufactured in the United States (*1*), is a colorless gas used mainly to make synthetic rubber (styrene-butadiene rubber and polybutadiene rubber) and thermoplastic resins (acrylonitrile-butadiene-styrene) (*2*). In 1983, 2.3 billion pounds of butadiene was produced in the United States (*1*). The maximum 8-hour time-weighted average workroom exposure concentration promulgated by the Occupational Safety and Health Administration (OSHA) is 1000 ppm (*3*).

Butadiene is mutagenic to *Salmonella typhimurium* strains (TA1530 and TA1535), which are sensitive to base-pair substitution mutagens (*4*). Mutagenicity of butadiene apparently requires metabolic activation (*4*) and may be due to the epoxide intermediates butadiene monoxide (1,2-epoxybutene-3) and diepoxybutane. Butadiene monoxide is the primary metabolite of butadiene biotransformation by rat liver microsomal monooxygenase (*5*) and may be conjugated with glutathione or further metabolized to diepoxybutane or 3,4-epoxy-1,2-butanediol (*6*).

Butadiene monoxide and diepoxybutane have been found to induce local neoplasms when applied to the skin of mice or when administered to mice or rats by subcutaneous injection (*7*). In the study reported here malignant neoplasms were observed at multiple sites in B6C3F$_1$ mice exposed to butadiene vapors for only 60 to 61 weeks.

Groups of 50 male and 50 female B6C3F$_1$ mice (Charles River) were exposed 6 hours per day, 5 days per week to air containing butadiene at target concentrations of 0 (chamber control), 625, or 1250 ppm (*8*). The animals, which were housed individually, were 8 to 9 weeks of age when first exposed to the butadiene vapors. The study was intended to last 103 weeks, but was ended after 60 weeks (for males) or 61 weeks (for females) because of reduced survival due to fatal tumors. Tap water and food (NIH-07 diet) were freely available except during exposure periods, when only water was available.

All animals that died during the study or that were killed at the end of the exposure period were subjected to a gross necropsy and a complete histopathologic examination (*9*). Differences in survival were analyzed by life table methods (*10*). Incidences of neoplastic lesions were analyzed by life table methods and by the Fisher exact test for pairwise comparisons of high-dose or low-dose groups with controls and the Cochran-Armitage test for dose-response trends (*10*).

Mean body weights of male or female mice did not appear to be affected by exposure to butadiene. However, survival was significantly ($P < 0.01$) reduced in all exposure groups. Survival rates were as follows: for males at 60 weeks, 49 of 50 (controls), 11 of 50 (625 ppm), and 7 of 50 (1250 ppm); for females at 61 weeks, 46 of 50 (controls), 15 of 50 (625 ppm), and 30 of 50 (1250 ppm).

Early deaths were due primarily to malignant neoplasms involving multiple organs. Because the study was stopped while background tumor incidences were low (*11*), it was possible to examine the effect of butadiene on total tumor rates. At the end of the study there were tumors in 20 percent of the control males and 12 percent of the control females, compared to 80 to 94 percent of the exposed mice. The total number of primary malignant and benign neoplasms per animal was also much greater ($P < 0.01$) in the butadiene-exposed groups.

Primary tumors caused by exposure to butadiene are listed in Table 1. Malig-

Table 1. Incidence of primary tumors in B6C3F$_1$ mice exposed to butadiene by inhalation for 60 to 61 weeks. Values are numbers of animals and incidence (percent).

Tumor	Males			Females		
	Control	625 ppm	1250 ppm	Control	625 ppm	1250 ppm
Malignant lymphoma	0 of 50 (0)*	23 of 50 (46)†	29 of 50 (58)†	1 of 50 (2)*	10 of 49 (20)†	10 of 49 (20)†
Hemangiosarcoma of heart	0 of 50 (0)‡	16 of 49 (33)†	7 of 49 (14)†	0 of 50 (0)*	11 of 48 (23)†	18 of 49 (37)†
Alveolar-bronchiolar neoplasms	2 of 50 (4)*	14 of 49 (29)†	15 of 49 (31)†	3 of 49 (6)*	12 of 48 (25)†	23 of 49 (47)†
Squamous cell neoplasm of forestomach	0 of 49 (0)	7 of 40 (18)†	1 of 44 (2)	0 of 49 (0)*	5 of 42 (12)§	10 of 49 (20)†
Acinar cell carcinoma of mammary gland	0 of 50 (0)	0 of 50 (0)	0 of 50 (0)	0 of 50 (0)*	2 of 49 (4)	6 of 49 (12)§
Granulosa cell neoplasm of ovary				0 of 49 (0)*	6 of 45 (13)†	12 of 48 (25)†
Hepatocellular neoplasms	8 of 50 (16)	6 of 49 (12)	2 of 49 (4)	0 of 50 (0)‡	2 of 47 (4)	5 of 49 (10)§

*Increasing trend ($P < 0.01$). †Increased compared to control ($P < 0.01$). ‡Increasing trend ($P < 0.05$). §Increased compared to control ($P < 0.05$).

nant lymphomas, hemangiosarcomas of the heart, and alveolar-bronchiolar neoplasms occurred with significant positive trends in male and female mice, and the incidences of these neoplasms in both exposure groups were significantly higher than those in the controls. Malignant lymphomas, observed as early as week 20, were considered to be the major cause of early deaths. The lymphomas appeared to originate in the thymus, but involvement of the spleen, lymph nodes, liver, lung, kidney, heart, pancreas, and stomach was also common. Hemangiosarcomas of the heart were also a major cause of death. These are uncommon endothelial cell tumors (*11, 12*); their incidence in untreated male or female $B6C3F_1$ mice is about 0.04 percent. Hemangiosarcomas were also observed in the liver, lung, and kidney. The lesions in these organs were probably metastatic foci because early lesions were observed only in the heart, the incidence was highest in the heart, and, with one exception, a cardiac hemangiosarcoma was found in each animal having a liver, lung, or kidney hemangiosarcoma.

Forestomach papillomas or carcinomas were induced in males exposed to 625-ppm butadiene and in females exposed to either dose (Table 1). Acinar cell carcinomas of the mammary gland and granulosa cell tumors of the ovary occurred in female mice with a dose-related trend; the incidences of acinar cell carcinomas were increased at the 1250-ppm exposure level and the incidence of granulosa cell tumors were increased at both exposure levels. Liver tumors were increased in females exposed to 1250-ppm butadiene.

Brain gliomas were found in two males in the 625-ppm group and one male in the 1250-ppm group and Zymbal gland carcinomas were observed in two males in the 1250-ppm group and one female in the 1250-ppm group. Because these tumors are rare in untreated $B6C3F_1$ mice (*11*), their occurrence after 60 to 61 weeks of exposure may have been due to exposure to butadiene.

Nonneoplastic effects associated with exposure to butadiene included gonadal atrophy in 19 of 47 males at 625 ppm, 11 of 48 males at 1250 ppm, 40 of 45 females at 625 ppm, and 40 of 48 females at 1250 ppm (control rates were 0 of 50 males and 2 of 49 females). Nasal cavity lesions (chronic inflammation, fibrosis, osseous and cartilagenous metaplasia, and atrophy of the olfactory epithelium) were increased in the male mice exposed at 1250 ppm, yet no neoplastic lesions of the nasal cavity were observed in males or females.

The finding of increased incidences of neoplasms at multiple organ sites but a lack of neoplasms of the nasal cavity may reflect a requirement for biotransformation of butadiene to a reactive epoxide intermediate (such as butadiene monoxide or diepoxybutane). The carcinogenicity of butadiene, therefore, may be related to the rate at which these active epoxide intermediates are formed and further metabolized or inactivated.

The International Institute of Synthetic Rubber Producers sponsored a 2-year inhalation study of butadiene in male and female Sprague-Dawley rats exposed to 0, 1000, or 8000 ppm, males for 111 weeks and females for 105 weeks (*13*). A carcinogenic response was also observed (*13*), but the sites of neoplastic development were different from those observed in our study.

As a consequence of the unusual findings in mice and the differences between these long-term studies, pharmacokinetic experiments to determine whether absorption, metabolism, or dispositional factors might differently influence butadiene-induced toxicity in rats and mice have been initiated, as has an inhalation study of butadiene (6.25 to 625 ppm) to characterize the dose-response relation for butadiene-induced neoplastic and nonneoplastic lesions in male and female $B6C3F_1$ mice. The teratogenic potential of butadiene in $B6C3F_1$ mice and Fischer 344 rats and the effect of inhalation exposure to butadiene on the immune system of male $B6C3F_1$ mice should be assessed.

Since similar neoplastic responses were induced at both 625- and 1250-ppm butadiene, it is possible that this gas also elicits a carcinogenic response at lower concentrations. Another important public health concern raised here relates to the occurrence of gonadal atrophy in exposed male and female mice. Thus there is a public health need to reevaluate the current OSHA standard for exposure of workers to butadiene (*14*).

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9. Tissues were preserved in 10 percent neutral-buffered Formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically were gross lesions, mandibular lymph node, mammary gland, sternebra (including marrow), thymus, trachea, lung and bronchus, heart, thyroid gland, parathyroid, esophagus, stomach, colon, small intestine, liver, gallbladder, pancreas, spleen, kidney, adrenal gland, urinary bladder, prostate, testis, ovary, uterus, nasal cavity and turbinate, brain, pituitary, pharynx, and (if abnormal) eye.
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