hprt Mutant Lymphocyte Frequencies in Workers at a 1,3-Butadiene Production Plant

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1,3-Butadiene is a major industrial chemical that has been shown to be a carcinogen at multiple sites in mice and rats at concentrations as low as 6.25 ppm. Occupational exposures have been reduced in response to these findings, but it may not be possible to determine by using traditional epidemiological methods, whether current exposure levels are adequate for protection of worker health. However, it is possible to evaluate the biological significance of exposure to genotoxic chemicals at the time of exposure by measuring levels of genetic damage in exposed populations. We have conducted a pilot study to evaluate the effects of butadiene exposure on the frequencies of lymphocytes containing mutations at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus in workers in a butadiene production plant. At the same time, urine specimens from the same individuals were collected and evaluated for the presence of butadiene-specific metabolites. Eight workers from areas of the plant where the highest exposures to butadiene occur were compared to five workers from plant areas where butadiene exposures were low. In addition, six subjects with no occupational exposure to butadiene were also studied as outside controls. All of the subjects were nonsmokers. An air sampling survey conducted for 6 months, and ending about 3 months before the study, indicated that average butadiene levels in the air of the high-exposure areas were about 3.5 ± 7.5 ppm. They were 0.03 ± 0.03 ppm in the low-exposure areas. Peripheral blood lymphocytes from the subjects were assayed using an autoradiographic test for *hprt* mutations. The weighted mean variant (mutant) frequency (Vf) ($\pm$ SE) in the eight exposed subjects was $3.84(\pm0.70) \times 10^{-6}$ per evaluatable cell, as compared to $1.16(\pm0.27) \times 10^{-6}$ in the low-exposed and $1.03(\pm0.07) \times 10^{-6}$ in the outside controls. The Vf of the low-exposed controls and the outside controls were not significantly different, but the mean frequency of mutant lymphocytes in the seven exposed subjects was significantly higher when compared to the mean Vf of the nonexposed controls ($p<0.01$) and the low-exposed controls ($p<0.05$). A single metabolite of butadiene, 1,2-dihydroxy-4-(*N*-acetlylcysteinyl-*S*) butane, was detected in the urine of all subjects. The concentration in the urine of the workers in the high-exposed group was significantly greater than in the low-exposed or nonexposed groups. The correlation between the level of the metabolite in urine and the frequency of *hprt* mutants was $r = 0.85$. The observation of an elevated Vf in the exposed subjects and the strong correlation of Vf with the level of excreted metabolite suggests that butadiene exposures under these conditions were sufficient to induce somatic cell mutations. This degree of increase in Vf is similar to what we have observed in cigarette smokers. The results available at this time indicate that current levels of occupational exposure to butadiene may not be sufficiently low to protect workers from the adverse effects that may result from exposure to mutagens. — Environ Health Perspect 102(Suppl 9):79–85 (1994)

Key words: human, *hprt* mutation, 1,3-butadiene, occupation, metabolite, lymphocyte, urine, biomarker

Introduction

1,3-Butadiene (CAS no. 106-99-0) is used in the manufacture of styrene–butadiene

rubber, polybutadiene, and other polymers. It ranks 34 in production in the United States among commodity chemicals, with total production in 1987 of 2.7 billion pounds (*1*). Approximately 65,000 U.S. workers are engaged in jobs in which there is some level of exposure to butadiene (*2*). In chronic studies, 1,3-butadiene is carcinogenic to laboratory animals. Lifetime exposures of mice to as little as 6.25 parts per million (ppm) of butadiene induced significant increases in lung tumors, and at 625 ppm, lethal lymphomas occurred in most of the animals (*3*). In rats, tumors were observed in multiple organs of both sexes of animals treated at 8000 ppm and tumors were significantly increased in the mammary glands of females at 1000 ppm (*4*). At comparable exposure levels the incidence of tumors was higher in mice than in rats.

Butadiene is genotoxic in several *in vitro* test systems. It induced base substitution mutations in *Salmonella* strains TA1530 and 1535 after exogenous meta-

bolic activation (*5*). Sister chromatid exchanges were induced in human lymphocytes exposed to butadiene, with or without exogenous metabolic activation, when the agent was added directly to the culture medium (*6*) but not when cells were exposed to an atmosphere containing butadiene (*7*). Sister chromatid exchanges were also induced in Chinese hamster ovary cells when the chemical was added to the medium together with a metabolic activation system (*8*). The mono- and diepoxide derivatives of butadiene are directly genotoxic in a variety of short-term test systems (*5,6,8*). These derivatives are thought to be the intermediates largely responsible for the genetic toxicity of butadiene. *In vivo* studies have also demonstrated that butadiene is genotoxic to cells in the bone marrow of mice and rats (*9–13*).

Several epidemiological evaluations of workers in butadiene production facilities and in styrene–butadiene rubber (SBR) plants have been published and recently

critiqued (14). Recent studies (15–17) have shown a consistent increase in mortality from lymphatic and hematopoietic malignancies. Variability has been observed in the specific types of cancers and in the response in relation to the duration of exposure. However, it has been concluded that there is at least limited evidence that butadiene is carcinogenic to man (14).

Although the permitted exposure level (PEL) established by the Occupational Safety and Health Administration (OSHA) for 1,3-butadiene is 1000 ppm, current levels of occupational exposure are much lower (2). The existing epidemiological studies reflect previous exposures to higher levels of butadiene than are now typically experienced. It would take many years to determine the carcinogenic risks associated with current levels of exposure if mortality studies were used as the basis for determining effects. Rodent studies of butadiene could be used to estimate the carcinogenic risk to humans; however, the apparent differences between the sensitivities of rats and mice to butadiene make this type of assessment difficult. Species differences in the carcinogenic response to butadiene may be accounted for, at least in part, by parallel differences in rates and patterns of metabolism in mice, rats, and monkeys (18,19). These differences suggest that the order of sensitivity to the toxic effects of butadiene appears to be mice>rats> cynamologous monkeys. However, the interpretation of metabolic and pharmacokinetic data for cancer risk has been the subject of recent debate (20,21).

How, then, can the safety of current levels of occupational butadiene exposure be assessed if mortality data for recent exposure levels are not available, and the applicability of the animal data is debatable? This dilemma can be addressed by evaluating potentially exposed workers for biological markers of the effects of exposure (22). The process of carcinogenesis involves many steps, including the fixation of mutations in oncogenes and suppressor genes as exemplified in the progression of colorectal carcinoma in man (23). In the past decade, several assays for monitoring the frequency of somatic cell mutations in human blood cells have been developed for use as biomarkers of the carcinogenesis process (24–26). One of these assays, which determines the frequency of mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus in peripheral blood lymphocytes, was originally developed by Strauss and Albertini (27). It has been refined into a useful tech-

nique for screening populations for exposure to mutagenic agents (28–30).

Advances in chemical analytical techniques have also led to the development of sensitive methods for detecting, in body fluids, the biotransformation products of specific chemicals. Glutathione conjugation products of butadiene have been quantitatively detected in the urine of animals (31) and humans (32).

In this article we report the results of a preliminary study conducted to determine whether both the biochemical evidence of exposure to 1,3-butadiene and the possible mutagenic effects of exposure could be detected in a population of workers in a butadiene extraction plant where exposure levels are around 1 ppm. We have used a chemical biomarker specific for butadiene exposure (excretion of a known metabolite of butadiene in the urine) and a biomarker for the mutagenic effect of butadiene (hprt gene mutation). Elevated levels of the butadiene metabolite were detected in the urine of workers in the production area, as compared to workers in other areas of the plant. A correlated increase in the frequency of hprt mutants was observed in the lymphocytes of the exposed workers.

Materials and Methods

Study Design

This study was designed as a pilot project using a single sample to evaluate the frequency of hprt mutant lymphocytes in three groups of subjects: a) a group exposed to 1,3-butadiene in a chemical production plant, b) a group working in the same plant but in areas where exposures to butadiene were lower, and c) an outside, nonexposed group consisting of employees in the Department of Preventive Medicine and Community Health at The University of Texas Medical Branch (UTMB) (Galveston, TX). Lymphocytes obtained from the study subjects were assayed to determine the frequency of hprt mutants (referred to as the variant frequency or Vf) (29,30,33).

Human Subject Recruitment

Study subjects were recruited from two areas of the production plant where significant exposures to butadiene had been clearly documented and from two areas where exposures to butadiene and other chemicals were low. Potential study subjects were not invited to participate if they reported that they were cigarette smokers or had been recently exposed to x-irradiation or mutagenic drugs. All subjects were

asked to complete a questionnaire that solicited information on the following subjects: sex, age, job title and work location, known chemical exposures at work, known chemical exposures outside of work, history of tobacco usage, current consumption of alcoholic beverages, use of coffee and other caffeinated beverages, general health, medications, and consumption of vitamin supplements. At the time that blood and urine samples were collected, a brief interview was conducted to clarify and confirm responses provided on the questionnaire.

Specimen Collection and Processing

Blood was collected by venipuncture into 15-ml vacuum tubes containing sodium heparin as an anticoagulant. Five tubes (approximately 65 ml of blood) were collected from each subject. Blood samples from the butadiene plant workers were stored at room temperature in insulated containers and returned to the UTMB laboratory by automobile within 12 hr of collection. Blood samples from the outside control group were collected at the laboratory. Urine specimens of 100 to 200 ml were collected in sterile plastic containers. Aliquots of about 30 ml were transferred to each of two 50-ml polypropylene centrifuge tubes. The tubes and remaining urine, in specimen containers, were frozen on dry ice within 1 hr after collection. The frozen specimens were transported to the laboratory and stored at −18°C. Approximately 1 week after collection, one tube of each sample was shipped to the Inhalation Toxicology Research Institute (ITRI) (Albuquerque, NM) for analysis to quantitate urinary metabolites of butadiene.

At the UTMB laboratory, blood samples were fractionated by layering on Histopaque (Sigma Chemical Co., St. Louis, MO) and centrifuging to separate lymphocytes, plasma, and erythrocytes. Lymphocytes were cryopreserved, at 10^7 cells/ml, in RPMI 1640 with 50% heat-inactivated fetal bovine serum (FBS) and 10% dimethylsulfoxide (DMSO). The cells, in 1-ml cryotubes, were stored in liquid nitrogen. Plasma and erythrocytes were stored at −80°C. Plasma samples were analyzed for cotinine levels, using a radioimmunoassay, in the laboratory of Dr. Helen Van Vunakis (Brandeis University, Waltham, MA). This cotinine assay is highly specific and has a limit of detection of 2 ng/ml (34).

The hprt Mutant Lymphocyte Assay

Cryopreserved lymphocytes were thawed and assayed for the frequency of

hprt mutant lymphocytes using an autoradiographic procedure, which has been previously described (30,35). Briefly, the lymphocytes were cultured in RPMI 1640 medium with 25 mM HEPES buffer, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM l-glutamine (all from GIBCO), 2% phytohemagglutinin (PHA) (HA-15, Burroughs Wellcome, Research Triangle Park, NC), 20% HL-1 medium (Ventrex, Costa Mesa, CA), 25% autologous plasma, and either 2×10^{-4} M 6-thioguanine (TG) in alkaline RPMI 1640 or an equal volume of alkaline RPMI 1640. Lymphocytes were suspended in culture medium at 1×10^6/ml, 5 ml per flask (Falcon no. 3013), and incubated for 24 hr at 37°C in a 5% CO_2 atmosphere. Tritiated thymidine ([^{3}H]TdR), 25 µCi per flask (ICN, specific activity 6.7 Ci/mmole) was added for an additional 18 hr. Free nuclei were released by the addition of 0.1 M citric acid. They were washed in fixative (7:1.5 methanol:acetic acid), resuspended in 0.25 ml of fixative and stored for at least 2 hr at 4°C. The nuclei were counted with a Coulter counter and the entire suspension from the TG cultures was placed on one or two 18 × 18 mm coverslips previously mounted on microscope slides. Approximately 2 to 3 × 10^5 nuclei from the nonselective cultures, (without TG) were placed on a coverslip in a similar manner. The slides were stained with 2% aceto-orcein (GIBCO), dipped in NTB-2 photographic emulsion (Kodak, Rochester, NY) and stored at 4°C in light-tight boxes for at least two days. They were then developed with Kodak D-19. Slides were coded to blind the scorer as to their identity and were then scored by scanning the entire coverslip microscopically to determine the number of labeled cells (M) on all the coverslips prepared from the TG-containing cultures from each subject. To determine the labeling index (LI) in nonselective cultures a differential count of 2500 cells was made and the numbers of labeled and unlabeled cells were counted. The number of evaluatable cells (N) is the number of cells that had the potential to become mutants. This term is calculated as the total number of cells recovered from TG cultures (as determined by the Coulter count) multiplied by the proportion of cells that could respond to PHA (the LI). The Vf is calculated as M/N.

Statistical Methods

Comparison of exposure groups was carried out by two methods to determine whether significant differences in Vf occurred among them. First, a weighted average

called a ratio estimator (Rf) (36,37) and its standard error [SE(Rf)] were calculated. In calculating the Rf, the Vf for each subject was given a weight proportional to the number of evaluatable cells obtained from that subject. A confidence interval (95 or 99%) was computed for each group's Vf as:

$$Vf=[Rf \pm t\, SE(Rf)]_{.95\ (or\ .99)}$$

where t is the Student's t corresponding to (n–1) degrees of freedom and the interval of 95% (or 99%). Details of the calculation of Rf and SE(Rf) have been published previously (29). A difference between groups at a particular level of significance is indicated by a lack of overlap of the confidence intervals for the groups' Vfs. Second, the mean Vfs among exposure groups were compared using analysis of variance. In this analysis, the square-root of the Vf was used as the dependent variable to better approximate normality and better stabilize intersubject variability.

To investigate the possibility that age or length of time working in this plant could explain all or part of the Vf results, we examined the distribution of ages and longevities in the exposure groups as well as the correlation between age or longevity and Vf. Similarly, to determine whether metabolite level, as a measure of exposure, was related to Vf we investigated the correlation of Vf and metabolite concentration in urine. This correlation was investigated using the square root of Vf and the logarithm of the metabolite concentration.

Results

Population Description

Samples of blood and urine were obtained from 20 subjects at the chemical plant and 9 subjects at UTMB. Of the 20 workers employed at the butadiene production plant, 7 worked in areas or at jobs that were considered likely to expose them to higher levels of butadiene than in other parts of the plant. These workers were process operators or rovers in the "north" and "south" butadiene production areas.

Ten individuals worked in areas where the likelihood of exposure to butadiene was considered to be low. These areas included the central control area for the production units, and the steam power and water plant with its control area. In addition, three individuals (process rovers) had duties which required that they spend time in both the production areas and the central control area. *hprt* Mutant lymphocyte assays were successfully conducted on six of the seven high-exposed individuals and two of the three individuals with variable exposure. At present, samples from five of the low-exposed and six of the nonexposed subjects have been analyzed. The subjects with variable exposure were grouped with the high-exposure individuals to total eight subjects in this group. The distributions of ages and years of work in the plant are summarized for each exposure group in Table 1. The ages of the high- and low-exposed workers had a similar distribution. In each of these two groups, one older worker (57 and 54 years of age, respectively) with many years of seniority (33 and 32.8 years) contributed to the dispersion of the ages and years of work of these groups. The median age was 35 and the median years of work was 3.8 for the high-exposed group, and the age was 40 and work years 3.4 for the low-exposed group. The median age in the nonexposed group was 36.

Exposure Description

Exposures to butadiene and other chemicals were estimated from an air sampling study conducted by the industrial hygiene department of the company and provided to the investigators by the workers' union. Between January 14 and July 21, 1991, 165 air samples were collected using Sensidyne no. 174 L low-range detector tubes, and the samples were analyzed by gas chromatography for butadiene, benzene, and methyl-tertiary-butyl ether (MTBE). Both area and personal samples were collected. All area samples were collected for 8 hr. Some of the personal samples were collected for only 15 to 20 min intervals, presumably to evaluate exposures

Table 1. Descriptive statistics for exposure groups.

| Exposure group | N | Sex | | Race | | | [a]Years | |
		M	F	W	B	H	Age ± SD	In plant ± SD
Nonexposure	6	6	0	4[b]	0	2	36.5(37)[b] ± 5.4	N/A
Low-exposure	5	4	1	3	2	0	41.7(42) ± 9.4	9.3 ± 13.18
Int-exposure	2	2	0	2	0	0	51 ± 8.5	17.7 ± 21.7
High-exposure	6	6	0	5	1	0	33.8(34) ± 3.8	3.3 ± 1.5

Abbreviations: W, white; B, black; H, hispanic. [a]Whites include two Asians; [b]Numbers in parentheses)indicate median values.

Table 2. Butadiene levels in workplace air. Area samples collected over approximately 8 hr.

Work area	N	Butadiene, ppm mean ± SD	Distribution,[a] ppm <1	1–10	>10
North unit	31	3.18 ± 7.13	23	4	4
South unit	29	3.89 ± 7.60	21	3	5
Central control	7	0.03 ± 0.03	7	0	0

[a] Distribution of numbers of samples falling in the indicated exposure ranges.

related to specific activities. The areas studied were the north and south production units, the MTBE production unit (which was not evaluated in this study), and the central control area. The steam power and water plant was not evaluated. Table 2 summarizes the results obtained for butadiene from the long-term area sampling. While the mean area and personal butadiene values obtained in the production areas were about 3.5 ppm, the majority of the individual samples showed concentrations below 1 ppm. The highest value obtained for an area sample in the production areas was 30 ppm. In central control the mean values were much lower (0.03 ppm) and the highest value obtained was 0.06 ppm. Levels for benzene were very low, averaging 0.002 ppm in the south area, 0.02 ppm in the north area, and 0.01 ppm in central

control. Levels for MTBE were also low, with none detected in the south area or central control, and 0.006 ppm in the North area.

hprt Mutant Lymphocyte Frequencies

The results of the analysis for hprt mutant lymphocyte frequencies are presented in Table 3. The frequency of mutant lymphocytes (the Vf) was significantly elevated in the high-exposed group as compared to both the low-exposed group and the nonexposed group. The weighted mean Vf (see "Statistical Methods") was $3.84(\pm\ 0.70) \times 10^{-6}$ per evaluatable cell in the high exposed group, $1.16(\pm\ 0.27) \times 10^{-6}$ in the low-exposed group, and $1.03(\pm\ 0.07) \times 10^{-6}$ in the nonexposed groups. As indicated by the confidence intervals presented in Table 3, the high-exposed group was significantly higher than both the nonexposed group ($p<0.01$) and the low-exposed group ($p<0.05$). The low-exposed and nonexposed groups were not significantly different from each other.

The same result was obtained when differences in exposure groups were investigated by analysis of variance. Differences among the three groups were significant ($p<0.05$) and the high-exposed group was significantly different from the low-exposed group ($p<0.03$) and the non-exposed group

($p<0.01$). The low-exposed and nonexposed groups were not significantly different from each other.

We found no correlations of hprt Vf with age or years of work in the plant. The nonsmoking status of the subjects was confirmed by analysis for plasma cotinine. The two individuals with high cotinine values (305 and 332 ng/ml of plasma) reported use of one-fourth to one-third of a 1 oz box of snuff daily, while the two subjects with low (31 and 72 ng/ml), but detectable, cotinines reported occasional snuff use. Nicotine in snuff should be readily absorbed resulting in elevated cotinine levels; however, the mutagenic combustion products present in cigarette smoke would be absent.

Urine specimens were analyzed for butadiene-specific metabolites at ITRI, (Albuquerque, NM). Details of this analysis are being reported separately (32). A single metabolite, 1,2 dihydroxy-4-(N-acetylcysteinyl)butane, was detected. The urinary concentrations of this butanediol conjugate for each subject are listed in Table 3 with the results from the lymphocyte samples analyzed for hprt mutants. The exposure group means (± SD) were 2927 ± 3995, 554 ± 152, and 320 ± 138 ng/ml for the high-, low-, and nonexposed groups respectively. By analysis of variance the intergroup differences were significant

Table 3. Frequencies of hprt mutant lymphocytes and butanediol metabolite levels in non–smoking butadiene plant workers and controls.

Labeling index	Total cells, ×10^6	Cells ×10^6	Evaluatable labeled cells	hprt Vf, ×10^6	Plasma cotinine[a], ng/ml	Dihydroxybutane urinary metabolite	Weighted mean[b] Vf ± SE, × 10^-6 [95 or 99% confidence intervals]
Exposed workers							
0.149	7.170	1.060	1	0.94	<2	564	3.84 ± 0.7
0.235	6.540	1.537	2	1.30	<2	514	
0.133	8.635	1.148	3	2.61	<2	1633	
0.169	19.380	3.275	10	3.05	305	1179	
0.128	14.692	1.881	7	3.72	<2	2002	
0.178	12.572	2.238	9	4.02	31	3879	CI^{99} = 1.74, 4.05
0.141	11.641	1.641	12	7.31	<2	1193	CI^{95} = 2.39, 5.79
0.135	7.424	1.002	9	8.98	<2	12,456	
Low-exposed worker controls							
0.171	15.023	2.569	2	0.78	<2	297	1.16 ± 0.27
0.104	9.744	1.013	1	0.99	332	661	
0.147	6.508	0.957	1	1.04	2	533	
0.079	11.439	0.904	1	1.11	<2	595	CI^{99} = 0.19-2.03
0.181	7.930	1.435	3	2.09	<2	640	CI^{95} = 0.64, 1.68
Outside controls							
0.206	18.217	3.753	3	0.80	75	186	1.03 ± 0.07
0.151	19.761	2.984	3	1.01	<2	286	
0.200	14.688	2.938	3	1.02	<2	149	
0.183	15.148	2.772	3	1.08	<2	357	
0.057	16.314	0.930	1	1.08	<2	487	$[CI^{99}$ = 0.81-1.24]
0.364	14.036	5.109	6	1.17	<2	455	

[a] Plasma cotinine determination: Detection limit is 2 mg/ml, two subjects in whom >20 mg/ml cotinine was detected reported moderate use of snuff. Two subjects with cotinine values of 305 and 332 reported using 1/4 of a 1 oz box of snuff daily. [b] See definition in Statistical Methods. [c] Significantly higher than the low- and the nonexposed groups, p<0.05.
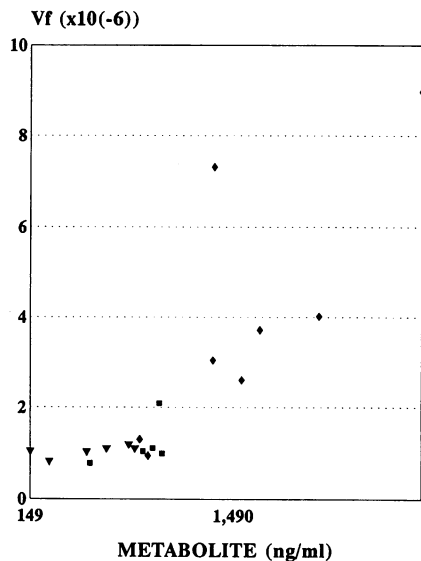
Vf (x10(-6))

METABOLITE (ng/ml)

Figure 1. Correlation between levels of 1,2-dihydroxy-4-(n-acetylcysteinyl-S)butane metabolite in urine and the frequency of hprt-deficient mutants per million evaluatable lymphocytes (Vf) in study subjects. Metabolite concentrations are plotted on a log scale. Exposure groups: nonexposed controls (▼); low-exposed (■); high-exposed (◆). See text for description of exposure groups.

($p<0.002$) with the high exposed group significantly different from the lowexposed group ($p<0.03$) and the nonexposed group ($p<0.002$). The low-and nonexposed groups were not significanlty different. The log of the concentration of the metabolite correlated strongly with the square root of the frequency of hprt mutants ($r = 0.85$). Figure 1 shows the relationship between hprt Vf and butadiene metabolite concentration.

Discussion

This investigation was designed as a pilot study to evaluate the feasibility of conducting a larger study to determine whether current levels of occupational exposure to butadiene are capable of inducing increased frequencies of single gene mutations or other types of genetic damage. The sample size was small, but we were fortunate in having detailed air sampling data available from the worksite, and we also had the opportunity to compare the results of the hprt mutant lymphocyte assay with levels of a butadiene-specific metabolite excreted in the urine.

The selection of work areas from which to recruit high- or low-exposed subjects was based initially on discussions with plant personnel who were familiar with the operations of the facility. The selections

were supported by the air sampling data, which indicated that the mean butadiene levels in the north and south production areas were about 100 times higher than in the low-exposed central control area. A similar selection of work areas had formed the basis for defining higher and lower exposure areas in earlier mortality studies of workers in the same facility (17,38).

The results of the air sampling survey appear to be generally consistent with other sampling studies of butadiene production plants (2). In general, the area results indicate that exposure levels in production areas were typically at or below 1 ppm, with occasional samples indicating higher exposures. In both the north and south production areas, three of four samples and three of five samples over 10 ppm (Table 2) were obtained during single periods of about 2 weeks rather than being evenly distributed throughout the period of the survey. The air sampling data were collected between January and July 1991, while the blood and urine samples were obtained at the end of October 1991. Consequently, the air sampling results cannot be used to determine actual levels of exposure during the periods of time that are significant for the mutation and metabolite analyses. Thus, the air sampling data indicate the patterns of exposure typically encountered in the plant within a few months of the time at which blood and urine specimens were collected for this study.

It should be noted that all of the samples were far below the current OSHA permitted exposure limit (PEL) of 1000 ppm (29 CFR 1910.1000) and that almost all samples were within the in-house standard of 10 ppm currently used by the plant. A majority of the samples were below the proposed OSHA PEL of 2 ppm (39).

The frequencies of hprt mutant lymphocytes were significantly elevated in the high-exposed group as compared to either the low-exposed or the nonexposed group. The correlation between exposure group assignment and Vf (using the square root transformation) was $r=0.72$ ($p<0.001$). Although the number of subjects was low, the Vfs for the high-exposed group appear to fall into three categories. Two subjects had values that were not distinguishable from the low-exposed or nonexposed groups. Four subjects had Vfs which were elevated about 2- to 4-fold over the nonexposed control mean, and two subjects had elevations of 7- to 8-fold over the control group. The individual with the highest Vf (and the highest butadiene metabolite level) (Figure 1) was assigned to a job with

a higher risk of exposure than normal day-to-day job assignments during part of the 6 weeks preceding blood sample collection. It is likely that his exposure level was higher than is typical for normal activities in the production areas. We are not aware of any specific activities that might explain the high Vf of the other individual with an elevated Vf. Of the two process rovers who spend time in both high- and low-exposure areas, one had a Vf of 0.94×10^{-6} and a metabolite level of 564 while the other had a Vf of 3.05×10^{-6} and a metabolite level of 1179. These values may reflect the variety of exposures that individuals in this job assignment may experience. It appears probable that the middle group of elevated Vf values are reflective of typical day-to-day exposures in the production areas.

The high correlation between the Vfs and the levels of urinary metabolites strengthens the impression that the elevation in Vf is a result of butadiene exposure rather than being due to some other undefined factor. The strength of the correlation is greater than we expected in that the significant periods of exposure for producing elevated hprt mutant lymphocyte frequencies and elevated levels of urinary metabolites are somewhat different. In earlier studies of patients receiving mutagenic drugs or radiation treatments (29,30) we found that the time interval between the onset of a mutagen exposure and the appearance of increased frequencies of mutant lymphocytes was about 2 weeks. We also found that mutant frequencies declined to near pretreatment levels by about 6 weeks following the termination of acute exposures. Based on these observations, the critical time period for the induction of mutant lymphocytes by exposure to mutagens would begin about 6 weeks prior to the time of blood sample collection, and exposures occurring within 1 or 2 weeks of sampling would not significantly affect the Vf. In contrast, metabolites of butadiene should be excreted rapidly, so their levels in urine would be a reflection of exposure only during the previous 24 hr. Since the urine specimens were collected at the end of the work shift, the metabolite levels are probably reflections of exposures during that shift. The fact that the metabolite levels and Vfs are so highly correlated suggests that patterns of exposure were probably fairly consistent over the 6-week period ending with the day of sample collection.

The autoradiographic assay for hprt mutant lymphocytes has both advantages and disadvantages when compared to the more complex version of the assay which is

based on the long-term growth of clones of mutant lymphocytes in culture (*40*). The clonal assay permits the further biochemical and genetic characterization of putative mutant clones to establish that they are true mutants. It is possible to sequence the DNA of the mutant cells to identify specific genetic changes and additional work can be done to determine whether individual clones are unique in origin or siblings from a clone of cells arising *in vivo* (*41*). While the autoradiographic assay does not provide viable mutant cells for such analyses, it is a more appropriate technique for screening populations. The autoradiographic method is less dependent on control of variables such as those related to longer-term lymphocyte cultures and it can be standardized more readily. Because cells are routinely cryopreserved prior to assay, they may be stored until a convenient time for analysis. In our experience, the Vfs of normal controls have been very consistent (*29,42,43*) when compared to the greater degree of variability observed in the clonal assay (*44*). This consistency, which was also observed in the present study, is critical to the detection of significant differences in Vf in small populations. The assay has also proven very sensitive in mice, detecting mutations induced by subacute inhalation exposures to benzene at concentrations as low as 40 ppb (*45*).

The use of two biologic markers and the availability of air sampling data permitted much more significant information to be obtained from a small population than would have been possible if only one end point had been evaluated. The two biological markers were strongly complimentary of each other because the butanediol conjugate was specific for the chemical of interest, butadiene, while the other detected a general, but important, biologic response, gene mutation. The analysis for urinary metabolites demonstrated that exposures to butadiene occurred, and provided useful information about human metabolism of butadiene (*32*). The observation of a correlated increase in *hprt* Vf indicates that these exposures are capable of inducing mutations, a biological response which connotes an increased risk of cancer.

The increase in *hprt* Vf observed in this study can be placed in context by comparing it to the effects of cigarette smoking and mutagenic medical treatments, which we have evaluated with the same assay. The increase in *hprt* Vf that we have previously observed in adult cigarette smokers, based on our results to date, has been similar to the increase related to butadiene exposure (Vf for nonsmokers 1.33 ($\pm$ 0.10) $\times$ 10^{-6}; n = 33; Vf for smokers 4.61($\pm$ 0.43) $\times$ 10^{-6}; n=15). Comparable proportionate increases in Vf were seen in cord blood lymphocytes from newborns born to smoking, as compared to nonsmoking mothers (*43*). In addition, baboons trained to smoke cigarettes showed a similar 3-fold increase in Vf at the end of a 4-month period of smoking as compared to a presmoking sample (*46*). In contrast, patients receiving radiation therapy had 5- to 15-fold increases in Vfs 2 to 3 weeks after onset of exposure to cumulative doses of about 900 cGy (*30*). These comparisons indicate that the increased *hprt* Vfs associated with current levels of occupational exposure to butadiene are similar to those associated with cigarette smoking.

The dihydroxy-butane conjugate found in human urine is one of two *N*-acetycysteine conjugates of butadiene excreted in the urine of other mammals exposed to high concentrations of butadiene. The proportion of this conjugate in the urine of mice, rats, hamsters, and monkeys increases in proportion to the levels of hepatic epoxide hydrotase (*31*). While the human metabolic pattern appears more like that of monkeys or rats than that of mice, the high correlation of butadiene metabolite excretion with *hprt* Vf indicates that genotoxic intermediates are being formed in proportion to the level of formation of this metabolite. The correlation of the metabolite excretion pattern and the induction of mutations in humans exposed to low levels of butadiene indicates that the role of butadiene metabolism and pharmacokinetics in susceptibility to butadiene toxicity (*19*) should be reevaluated.

Our results at this time indicate that, although current exposure levels are at or below the proposed OSHA PEL for butadiene, they may not be sufficiently low to protect workers from the health risks that are associated with mutagen exposures. We are currently conducting tests for other biomarkers of genetic damage using additional biological materials obtained during the sample collection on which the current results are based. Additional studies will be needed, and are in progress, to establish the consistency of these effects and to better define the relationship between *hprt* Vf, butadiene exposure level, and work activity. Furthermore, research to better define the relationship between mutant frequency and biologically effective dose is needed.

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