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Biological monitoring for mutagenic effects of occupational exposure to butadiene

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Abstract

The use of biological markers in the evaluation of human exposure to hazardous agents has increased rapidly in recent years. Because 1,3-butadiene is a mutagenic carcinogen, existing occupational levels of exposure may be appropriately evaluated using somatic cell mutation as a biomarker. Previously, we have described a biomarker study of workers in a butadiene monomer plant (Ward et al., 1994). We now report results from a second study of the same group of workers, conducted after plant modernization, and present preliminary results from a study of exposures in a styrene butadiene rubber (SBR) plant. Air levels of butadiene were determined using either charcoal tubes with air pumps or passive badge dosimeters. The quantity of a butadiene metabolite in the urine was used as a biomarker of exposure and the mutagenic effects of exposure were measured using the autoradiographic *hprt* mutant lymphocyte assay. In all three studies, the frequencies of *hprt* mutants were significantly elevated in workers from the areas of highest exposure when compared to workers from lower exposure areas or non-exposed subjects. The concentration of the urinary metabolite was significantly increased in high-exposed workers in the first study of monomer plant workers but not in the second. In the first monomer plant study, historical air concentrations of butadiene were higher in the production units than in the central control unit. While concurrent determined air concentrations were not elevated in the second monomer plant study, they were elevated in high exposure areas in the SBR plant study. Mutant frequencies in the lower-exposure and the non-exposed groups were consistent with historical values for non-smoking individuals who were not exposed to known mutagens. The use of biomarkers, including the *hprt* mutant lymphocyte assay, may be of great value in determining an appropriate occupational exposure limit for butadiene.

Keywords: Biological monitoring; *hprt* mutation; Butadiene; Human; Occupational health

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1. Introduction

Recognition of the role and value of biological markers in environmental health research has increased rapidly over the last decade. In the context of evaluating exposure to chemical carcinogens, biological markers are early events in the continuum leading from exposure to clinical disease. The events that are used as biological markers permit the detection of recent exposure in humans or experimental animals, facilitate internal dosimetry, and identify effects of exposure that may be mechanistically relevant to disease (NRC, 1987). The use of biomarkers can address some of the major weaknesses of traditional epidemiologic studies in occupational cancer research. These weaknesses include the need to successfully link past exposure to more recent disease, and the need to study large populations because of the low incidence of specific cancers. Biological markers of exposure and effect can be observed within minutes to days of exposure. They occur with a relatively high incidence in exposed populations so that significant increases in biomarker frequencies can be observed in small populations. In the present study, we have utilized biomarkers of exposure and effect to evaluate contemporary conditions of occupational exposure to 1,3-butadiene in industrial plants in Texas.

At the time we began our studies, the carcinogenic effects of butadiene in rodents were well established (Melnick et al., 1990), and it was known that butadiene was mutagenic in in vitro assays (de Meester et al., 1980; Arce et al., 1990) and was capable of damaging chromosomes (Tice et al., 1987). Several human epidemiologic studies had been published (IARC, 1992), which consistently pointed to carcinogenic effects on the hematopoietic system, but they were apparently not convincing to all parties interested in the health effects of butadiene (Bond et al., 1995).

We initiated a series of studies of occupational exposure to butadiene using the quantitation of a butadiene metabolite in the urine as a marker of exposure, and mutation at the *hprt* gene locus in lymphocytes as a marker of effect. We have now carried out two studies of one population, and a study of a second population is in progress. In this paper we review the previously published results of the first study (Bechtold et al., 1994; Ward et al., 1994), report the results from the second study, and briefly describe some preliminary results from the third study.

2. Methods

2.1. *The hprt* mutant lymphocyte assay

The frequency of *hprt* mutant lymphocytes was determined using an autoradiographic assay (Albertini 1985; Ammenheuser et al., 1988, 1994; Ward et al., 1994). Previously cryopreserved lymphocytes were cultured in 6-thioguanine (TG) and labeled with [^{3}H]thymidine for the final 18 h of the 42 h culture period. Harvested cells were counted, and microscope slides were prepared for autoradiography and were scored after replacing the identifier with a code.

2.2. *Urine metabolite assay*

This assay quantitatively detects 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane by gas chromatography/mass spectroscopy. The method has been previously described (Bechtold et al., 1994; Kelsey et al., 1995).

2.3. *Statistical methods*

The autoradiographic *hprt* mutant frequency assay measures the variant frequency (Vf), which is determined by dividing the number of labeled mutant cells from TG-selection cultures by the number of evaluatable cells. The evaluatable cells are the total number of cells recovered from the TG cultures after correcting for the proportion of dividing cells as determined by a non-selection culture (the labeling index, LI) and expressing the result as a rate per million labeled cells. The Vf values (per subject) were analyzed using two way analysis of variance methods. Pooled intersubject variability was the error variance used for significance testing. This analysis allows an assessment of Vf differences between the three butadiene exposure groups, and of differences between smokers and non-smokers. It also allows evaluation of the smoker by exposure interaction. Vf

data were analyzed using both Vf values, as well as transformed Vf values re-expressed as $Y = (\sqrt{Vf} + \sqrt{(Vf + 1)})/2$, the average square root. Non-parametric analyses were also used to corroborate the inferences, since the Vf values in the two highest exposure groups were more variable than those in the lower and non-exposed groups. Thus outliers or extreme Vf values were not responsible for significance of differences between butadiene exposure groups. Urinary metabolite values were converted to the log(10) scale for analysis. Data from studies one and two were analyzed separately. The effect on Vf of exposure to butadiene was controlled for smoking using the two-way analysis and there was no indication of an interaction of smoking and butadiene exposure.

3. Results

3.1. Study design

Three evaluations of occupational exposure to butadiene were conducted. The first two evaluated workers in a butadiene production plant in Port Neches, TX and the third examined workers in a styrene-butadiene rubber (SBR) plant in the same area. In the first study, three groups were evaluated: workers in butadiene production units (high-exposure, $N = 10$), workers in the central control facility and steam, power, and water utilities (low-exposure, $N = 10$), and participants from The University of Texas Medical Branch with no occupational exposure to butadiene ($N = 9$). None of these subjects smoked cigarettes by personal report or plasma cotinine analysis. The second study was a follow-up 8 months after the first one. This study was carried out in conjunction with a study on the responsiveness of lymphocytes from butadiene-exposed subjects to an in vitro challenge by diepoxybutane (Kelsey et al., 1995). Workers were categorized as experiencing high or low exposure on the same basis as in Study I; however, there were enough participants who fell in an intermediate category to place them in a separate exposure group. No outside controls were evaluated in this second study. In the third study, which is still in progress, workers were recruited from traditionally defined areas of higher exposure (reactor, recovery, tank farm and laboratory) and lower exposure (blend, coagulation, bailers, shipping, utilities, and shops). To date, 16 high-exposed and 9 low-exposed subjects have been evaluated for *hprt* Vf. Of these subjects, five high-exposed and three low-exposed were cigarette smokers.

In the first study, exposures were estimated from an exposure survey conducted by the company 9 to 3 months prior to sample collection. In the second study, exposures were determined from 8-h personal breathing zone air samples collected on the day of sampling using charcoal tubes and air pumps. In the third study, exposures were determined using passive dosimeters (3M 3500) worn in the breathing zone by the workers for the 8-h shift on the date of sample collection. Both charcoal tubes and passive dosimeters were analyzed by gas chromatography by a commercial laboratory (Natlsco, Long Grove, IL). Samples of blood and urine for analysis were collected at the end of the work shift.

3.2. Butadiene production plant

The results of the first two studies in the butadiene monomer plant are summarized in Table 1. The estimated exposures preceding Study 1, based on area 8-h samples, averaged about 3.5 ± 7.25 ppm. If samples collected in high exposure areas, in which workers spend little time, are ignored the average exposure is estimated to be about 1 ppm. Exposure in the Central Control Area averaged 0.03 ± 0.03 ppm. In Study II, the average exposures measured using personal samples were 0.30 ± 0.59, 0.21 ± 0.21, and 0.12 ± 0.27 ppm in the high, intermediate, and low areas, respectively. In both studies the Vf of the high-exposure group was significantly elevated in relation to either of the comparison groups ($P < 0.05$) even when one outlier with a very high Vf in the high exposure group was deleted from the analysis to improve the stability of the variances of the transformed variable. The low-exposed and non-exposed groups in Study I and the intermediate- and low-exposure groups in Study II were not signifi-

Table 1
Evaluation of butadiene exposure in a monomer production plant

Exposure group[a]	n	*hprt* Vf[b] ($\times 10^{-6} \pm$ S.D.)	Urine metabolite (ng/mg creatinine $\pm$ S.D.)
Study I (10/91)			
High	8	3.99[c] $\pm$ 2.81	1690[d] $\pm$ 201.3
Low	5	1.20 $\pm$ 0.51	355 $\pm$ 250
None	6	1.03 $\pm$ 0.12	580 $\pm$ 191
Study II (6/92)			
High	7	5.33[c] $\pm$ 3.76	761 $\pm$ 245
Intermediate	7	2.27 $\pm$ 0.99	596 $\pm$ 155
Low	8	2.14 $\pm$ 0.97	684 $\pm$ 176

[a]See study design for description.
[b]*hprt* Vf defined as mutants per million evaluatable cells (see text).
[c]Significant variation across groups ($P < 0.02$). Vf of high exposure group significantly higher than other groups ($P < 0.05$).
[d]Variance across exposure groups significant by ANOVA based on log of metabolite/mg creatinine ($P < 0.02$). Butadiene metabolite level in high exposure group significantly higher than low exposure group ($P < 0.05$).

cantly different from each other. The only butadiene metabolite detected in either study was the dihydroxybutane mercapurate. In both studies the mean concentration of metabolite, in ng/mg creatinine, was highest in the high-exposure group. However, the difference was only statistically significant ($P < 0.05$) between the high- and low-exposure groups. In Study I but not Study II, the metabolite level (normalized for creatinine) was significantly correlated with the Vf ($r = 0.71$; $P < 0.001$). The levels of metabolite in the high-exposure group were lower in Study II than in Study I. This finding was consistent with a decrease in the air concentrations of butadiene between Study I and Study II. The plant was undergoing a modernization program designed to reduce fugitive emissions associated with leaks and chemical sample collection. The success of this program apparently accounted for the reduced exposures.

Table 2
Hprt Vfs in lymphocytes of workers in a styrene-butadiene rubber plant, classified by exposure group and smoking status (preliminary data)

Exposure group[a]	Cigarette smoking			
	n	No (*hprt* Vf)[b]	n	Yes (*hprt* Vf)[b]
High[c]	11	7.47 $\pm$ 5.69	5	6.24 $\pm$ 4.37
Low	6	1.68 $\pm$ 0.85	3	3.42 $\pm$ 1.57

[a]High exposure areas: reactor, recovery, tank farm, laboratory. Low-exposure areas: blend, coagulation, bailers, shipping, water plant, machine shop, electric shop.
[b]*hprt* Vf: number of mutant cells per million evaluatable cells $\pm$ S.D.
[c]By two-way analysis of variance, effect of exposure significant ($P < 0.01$ and interaction between smoking and exposure not significant.

3.3. Styrene-butadiene rubber plant

Preliminary data is now available on exposure levels and *hprt* Vf. The passive badge dosimeters used had a detection limit for butadiene of approximately 0.25 ppm over an 8-h period. Of 40 samples collected in the high-exposure areas, 20 exceeded the detection limit and 11 exceeded 1 ppm. In the low-exposure areas none of 26 samples exceeded the detection limit. Styrene levels were measured simultaneously. Only one sample in the high-exposure area exceeded the reporting limit of 1 ppm. Where quantitative measures of both butadiene and styrene were made, the styrene levels averaged 25% of the butadiene levels. To date, 16 *hprt* Vfs have been determined in workers in high-exposure areas and nine in low-exposure areas. Of the 16 high-exposure subjects, five smoke cigarettes while of the nine low-exposure subjects, three smoke. The results, classified by exposure and smoking, are shown in Table 2. A 2-factor analysis of variance found a highly significant effect of exposure ($P < 0.01$), but no significant interaction between the effects of exposure and smoking. The Vfs in non-exposed, non-smokers were consistent with those in Studies I and II in the monomer plant. The effect of butadiene exposure on Vf appeared to be greater in the SBR plant than in the

monomer plant. Additional samples are being evaluated from workers in both high- and low-exposure areas in this plant, and urine samples are being evaluated for metabolites.

3.4. Cigarette smoking

These results may be compared to the results of the effect of cigarette smoking on Vf in a study that is being conducted concurrently with the SBR plant study. To date 11 cigarette smokers and 11 non-smokers recruited from among The University of Texas Medical Branch employees and students have been studied. The self-reported tobacco use of all subjects was confirmed by plasma cotinine determination. The Vf ($\pm$S.D.) among non-smokers was 1.74 ($\pm$0.78) $\times 10^{-6}$ evaluatable cells while the Vf among smokers was 7.24 ($\pm$3.71) $\times 10^{-6}$. The difference was significant at $P < 0.001$. The Vfs in non-smokers were similar to those found in non-smoking non-exposed workers, and the difference between smokers and non-smokers was similar to the differences between butadiene-exposed and non-exposed workers.

4. Discussion

These biomonitoring studies evaluated a butadiene monomer plant on two occasions 8 months apart, and they also provide preliminary information on a third study of occupational exposures in an SBR plant. The three studies show a consistent elevation in frequencies of *hprt* mutant lymphocytes in workers in the higher areas of exposure in both facilities. In the monomer plant, a modernization program appears to have been successful in reducing fugitive emissions, and, thus, occupational exposure to butadiene was reduced during the time interval between the two studies. This is reflected in lower air levels of butadiene and reduced concentrations of the dihydroxybutane mercapturate urinary metabolite in Study II. Although the numerical differences in *hprt* Vf between the high- and low-exposure areas in Studies I and II were similar, a difference in the distribution of Vfs was seen in the high-exposure groups. In Study I, six of eight high-exposed workers had Vfs greater than the workers with the highest Vfs in the low-exposure group. In contrast, in Study II only three of seven high-exposed workers had Vfs greater than the highest values in the intermediate and low groups. At the present time, the duration of persistence of high Vfs after reduction of exposure, in subjects receiving a chronic low-dose exposure to mutagens, is not known. Although Vfs decline rapidly following cessation of acute treatment with high doses of mutagenic agents (Ammenheuser et al., 1988, 1991), they may persist for longer periods after chronic exposures are reduced. Earlier exposures to other mutagens or other unknown factors also might account for the persistence of elevated frequencies of mutant lymphocytes in some of the Study II workers.

The results from the SBR plant, while preliminary, are consistent with the results from the monomer plant. Exposure levels appear to be somewhat higher in the SBR plant, with dosimeter readings ranging up to about 5 ppm. The mean *hprt* Vf of the high-exposure SBR group was greater than the mean Vf of the high-exposure group from the monomer plant, and a two-way analysis of variance indicates that butadiene exposure and not cigarette smoking accounted for the increased Vf.

Results with the autoradiographic *hprt* mutant lymphocyte assay in these three studies of butadiene exposure and in both our previous (Ammenheuser et al., 1994) and concurrent evaluations of the effects of cigarette smoking demonstrate a high degree of consistency in baseline values and response to exposure. In all of these studies, healthy, non-smoking, non-exposed individuals had Vfs that never exceeded 3.5×10^{-6}. The magnitude of increase in Vf is similar for butadiene-exposed non-smokers and for cigarette smokers who were not exposed to butadiene. The Vfs for workers in low-exposure areas of both plants were not significantly elevated over those of non-exposed non-smokers. This suggests that significantly elevated Vfs occur only in individuals who work in areas where

butadiene exposures are greatest. The relationship between air concentrations of butadiene and *hprt* Vf should be interpreted cautiously since the air concentrations were measured in different ways and over different time periods in the three studies. In no case was a comprehensive evaluation of air concentration made over the time period when detectable *hprt* mutants were likely to form (probably 2–6 weeks prior to sample collection).

Assays based on the growth of clones of thioguanine-resistant cells have detected differences in mutant frequency between smokers and non-smokers of less than 2-fold (Cole et al., 1988; Burkhart-Schultz et al., 1993) and have not found significant differences between butadiene exposed and non-exposed subjects (Hayes et al., this issue; Tates and Sram, this issue). The reasons for the differences in response of the clonal and autoradiographic assays are not clear at this time. It is likely that *hprt* mutants are selected from somewhat different subsets of lymphocytes in the two types of assays. Statistical factors may also be important since the large number of evaluatable cells and consistent baseline Vfs seen in the autoradiographic assay would contribute to relatively high sensitivity in detecting change. Baseline frequencies in the clonal assay are typically higher and more variable (Cole et al., 1988; O'Neill et al. 1989; Burkhart-Schultz et al., 1993).

It is difficult to escape the conclusion that chronic butadiene exposures in the range of 1 ppm cause significant elevations in *hprt* Vf. While a specific degree of long-term health risk cannot be determined from these data, the induction of mutations by current occupational levels of butadiene raises obvious concerns. With the strengthening of the evidence for elevated incidences of leukemia in SBR workers, as reported by Delzell et al. (this issue), the need to establish an acceptably safe level of butadiene exposure becomes more urgent. In combination with comprehensive assessments of current exposure levels, biomarkers, including *hprt* mutant frequencies, may be more useful for determining appropriate exposure standards than attempts to develop current risk estimates by extrapolating from past exposures.

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