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## Markers for carcinogenicity among butadiene-polymer workers in China

R.B. Hayes<sup>a,\*</sup>, L. Zhang<sup>b</sup>, J.A. Swenberg<sup>c</sup>, S.-N. Yin<sup>d</sup>,  
L. Xi<sup>b,d</sup>, J. Wiencke<sup>e</sup>, W.E. Bechtold<sup>f</sup>, M. Yao<sup>d</sup>,  
N. Rothman<sup>a</sup>, R. Haas<sup>g</sup>, J.P. O'Neill<sup>h</sup>, J. Wiemels<sup>b</sup>,  
M. Dosemeci<sup>a</sup>, G. Li<sup>c</sup>, M.T. Smith<sup>b</sup>

<sup>a</sup> Occupational Epidemiology Branch, National Cancer Institute, EPS 8114, Bethesda, MD, USA

<sup>b</sup> School of Public Health, University of California, Berkeley, CA, USA

<sup>c</sup> School of Public Health and Medicine, University of North Carolina, Chapel Hill, NC, USA

<sup>d</sup> Chinese Academy of Preventive Medicine, Beijing, PR China

<sup>e</sup> School of Medicine, University of California San Francisco, San Francisco, CA, USA

<sup>f</sup> Inhalation Toxicology Research Institute, Albuquerque, NM, USA

<sup>g</sup> California State Health Department, Berkeley, CA, USA

<sup>h</sup> Genetics Laboratory, University of Vermont, Burlington, VT, USA

### Abstract

We examined a spectrum of genotoxic and other outcomes in 41 butadiene-polymer production workers and 38 nonexposed controls, in China, to explore the role of butadiene in human carcinogenesis. Among butadiene-exposed workers, median air exposure was 2 ppm (6-h TWA), due largely to intermittent high-level exposures. Compared to unexposed subjects, butadiene-exposed workers had greater levels of hemoglobin N-(2,3,4-trihydroxybutyl)valine (THBVal) adducts ( $P < 0.0001$ ), and adduct levels tended to correlate, among butadiene-exposed workers, with air measures ( $P = 0.03$ ). Butadiene-exposed workers did not differ, however, from unexposed workers with respect to frequency of uninduced or diepoxybutane-induced sister chromatid exchanges, aneuploidy as measured by fluorescence

*Abbreviations:* BD, 1,3-butadiene; EB, 3,4-epoxy-1-butene; DEB, 1,2,3,4-diepoxybutane; EBD, 3,4-epoxy-1,2-butanediol; DMF, dimethyl formamide; GPA, glycophorin-A; GST, glutathione S-transferase; hprt, hypoxanthine-guanine phosphoribosyl transferase; M-1, mercapturic acid butanediol; M-2, mercapturic acid butenol; SCE, sister chromatid exchanges; THBVal, N-(2,3,4-trihydroxybutyl)valine; WBC, total leukocyte count.

\* Corresponding author. Tel.: +1-301-4353973; fax: +1-301-4021819.

E-mail address: hayesr@mail.nih.gov (R.B. Hayes).

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in situ hybridization of chromosomes 1, 7, 8 and 12, glycoprotein A variants or lymphocyte *hprt* somatic mutation. Also among the exposed, greater THBVal levels were not associated with increases in uninduced sister chromatid exchanges, aneuploidy, glycoprotein A, or *hprt* mutations. Butadiene-exposed workers had greater lymphocyte ( $P=0.002$ ) and platelet counts ( $P=0.07$ ) and lymphocytes as a percent of white blood cells were moderately correlated with greater THBVal levels (Spearman's  $\rho = 0.32$ ,  $P = 0.07$ ). Among butadiene-exposed workers, several serum cytokines correlated with THBVal adduct levels. Overall, the study demonstrated exposure to butadiene in these workers, by a variety of short-term and long-term measures, but did not show specific genotoxic effects, at the chromosomal or gene levels, related to that exposure. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* Butadiene; Carcinogenesis; China

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## 1. Introduction

1,3-butadiene (BD) is commonly used in the production of rubber and thermoplastic resins. BD has also been found in automobile exhaust, cigarette smoke, and in community air at the perimeter of manufacturing plants. It was first produced in large volumes during World War II for use in the production of synthetic rubber. Currently, more than 6 million tons of BD are produced annually worldwide. Approximately 50 000 workers in the US and 30 000 workers in Europe are potentially exposed to BD. Following the US and Japan, China is the third major producer of BD, producing more than 250 000 tons in 1990 [1].

BD was recently classified by the International Agency for Research on Cancer as a probable human carcinogen (Group 2A), based upon sufficient evidence for carcinogenicity in animals and limited evidence in humans [1]. Although several smaller investigations were carried out, the evidence of carcinogenicity in humans is based largely on one epidemiologic study in the US and Canada styrene-butadiene rubber industry. The study showed excesses of leukemia in these workers and that those with apparently higher BD exposure had higher risk than those with lower exposure [2,3].

To provide a mechanistic underpinning for the human carcinogenicity studies, we [4–6] and others [1,7–12,20] have investigated genotoxic effects in workers exposed to BD. In our study at a polybutadiene production facility in China, we examined a broad spectrum of genotoxic and other potential effects of BD.

## 2. Materials and methods

As described in detail elsewhere [4], workers were studied at a polybutadiene rubber production facility at Yanshan, China. The purification of BD from an initial hydrocarbon stream (mainly alkanes and alkenes with three–five carbon atoms) occurred at two sites: the DMF facility, where initial distillation and extraction occurred using a proprietary dimethyl formamide (DMF) process, and the recovery facility, where final distillation occurred (Fig. 1). Butadiene was then

transported to polymerization vats for synthesis of polybutadiene. Any butadiene remaining after the polymerization process was returned to the recovery unit, where it was mixed with incoming butadiene from the DMF recovery facility, repurified, and repolymerized.

### 2.1. Study subjects

Three groups of workers with high potential exposure were identified for study (1) DMF process analysts sampled process lines and analyzed the products by gas chromatography at the DMF unit; (2) polymer process analysts carried out these tasks at the recovery and polymerization units; and, (3) operators at the recovery facility who carried out routine minor maintenance and, as needed, major repair operations.

After the purposes of the study and procedures were explained and informed consent was obtained, 41 of the 42 exposed workers were included for study. For comparison, 40 unexposed subjects were selected for study from non-exposed work units. The unexposed subjects were age (5-year) and gender matched in groups to the exposed. Upon review of occupational histories, two controls, who were determined to have worked with BD in the past, were excluded from analysis. The study groups are shown in Table 1.

### 2.2. Material collection

Subjects completed a brief questionnaire, administered by study staff, regarding work history, selected medical conditions, and tobacco use. During the 6-h work shift, personal samplers were used for collecting air at the breathing zone, by drawing atmospheres through charcoal tubes using individual pumps (flow rate 35 ml/min). Two traps were combined in series to ensure that all butadiene was

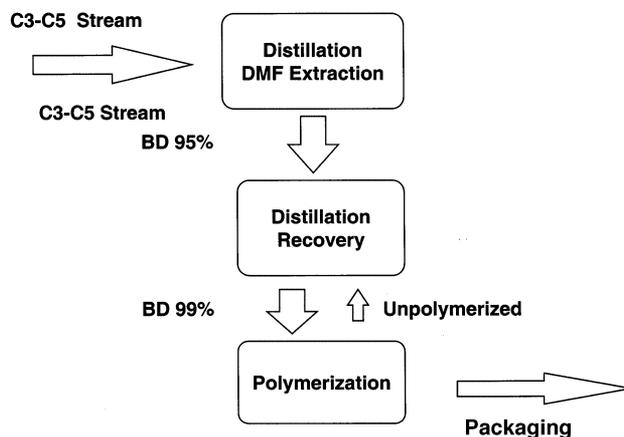


Fig. 1. Butadiene production process, Yian Shan, China.

Table 1  
Study subjects, Yanshan, China

	Butadiene-exposed			Total exposed	Total unexposed
	Analysts		Maintenance-recovery		
	DMF	Polymer			
Male	0	0	15	15	14
Female	10	15	1	26	24
Total	10	15	16	41	38

retained. During the study, numerous grab samples at the breathing zone were also taken, using 50-ml glass collection syringes. In addition, canister samples were collected at five locations.

A post-shift blood sample (8ml) was collected, from which mononuclear cells were isolated, frozen, and stored as viable cells in the gas-phase of liquid N<sub>2</sub>. Another blood sample was fractionated (serum, plasma, red blood cells, and buffy coat) and stored. Further, whole blood cultures were established for cytogenetic studies and MN blood type was determined by using rabbit typing serum (Ortho Diagnostics). MN heterozygous blood was kept for 1–2 h at 4°C until formalin fixation and preparation of spherical erythrocytes [13]. The fixed specimens were stored at 4°C until analysis. A differential blood count was carried out by Coulter blood counter on fresh whole blood within 2 h of collection. Urine samples were collected during work (0–3, 4–6 hours of a 6-h shift). Within 2 h of the end of the work shift, the urine samples were aliquoted and frozen.

### 2.3. Exposure measurements

Butadiene in air (full-shift) was collected by personal samplers and analyzed at the Chinese Academy of Preventive Medicine (CAPM) using an adaptation of the NIOSH method 1024 (National Institute of Occupational Safety and Health, 1987). Breathing zone (short-term grab) air samples were analyzed on site within 30 min of collection by Photovac 10S Plus (photoionization detector). Canister air samples were analyzed following USEPA TO-14 guidelines.

Mercapturic acid butanediol (M-1) and butenol (M-2) metabolites of BD were measured in urine samples by GC/GC/MS [14].

*N*-(2,3,4-trihydroxybutyl)valine (THBVal) hemoglobin adducts were determined based on Törnqvist's modified Edman degradation for specific cleavage of *N*-alkylated terminal valines of the four chains in hemoglobin [15,16]. Quantitation was based on the ratio of the peak area of the analyte to the peak area of the external standard.

#### 2.4. Somatic mutation assays

For the Glycophorin A (GPA) assay, formalin-fixed spherocytes of individuals heterozygous (MN) for GPA were analyzed to determine NN and NØ variant cell frequencies ( $V_f$ ) [13]. For the *hprt* mutations, mutation frequency ( $M_f$ ) was determined by the T-cell cloning assay [17]. The cloning efficiencies (CE) were calculated by the Poisson relationship,  $CE = -\ln P_0/x$ , where  $P_0$  is the fraction of wells negative for colony growth and  $x$  is the average number of cells originally inoculated per well. The thioguanine selected CE divided by the mean unselected CE yields the  $M_f$ .

#### 2.5. Cytogenetic analysis

Sister chromatid exchange (SCE) was assessed in cultures, with and without exposure to diepoxybutane (DEB) [18]. For fluorescence in situ hybridization (FISH), a total of four chromosomes were examined using probes for the centromeres of chromosomes one and seven and for the entire length of chromosomes eight and 12.

#### 2.6. Statistical analysis

Nonparametric procedures were used for statistical analysis, including the Spearman correlation test, the Wilcoxon test for independent samples, and the  $\chi^2$  test. For multivariate analyses of studied markers, linear regression analyses were carried after transformation to the natural log (ln). Analyses were carried out using the SPSS statistical package [19].

### 3. Results

Tobacco use was prevalent among exposed (86.7%) and unexposed men (78.6%); none of the women in either group smoked. Among cigarette smokers, the usual amount of cigarettes smoked and the cumulative amount smoked (pack-years) were similar for exposed and unexposed men. Among butadiene-exposed workers, duration of exposure was similar for men ( $8.6 \pm 6.1$  years) and women ( $6.5 \pm 5.5$  years).

Full-shift individual air measurements established that workers in BD operations were exposed to butadiene ( $n = 39$ , median BD = 2.0 ppm, range BD = 20.6) and that study controls were not exposed ( $n = 14$ , median = 0, range = 0) ( $P < 0.0001$ ). Urinary M-1 levels were also substantially elevated in BD workers ( $n = 17$ , median = 1.3 g M1/mg creatinine, range = 5.2), however, measurable levels were also found among the controls ( $n = 4$ , median = 0.6, range = 0.7) ( $P = 0.16$ ). Short-term measures of BD by real-time breathing-zone grab samples showed great extremes in exposure [DMF analysts: samples = 50, median butadiene (ppm) = 54, range = 0–3090; Polymerization analysts: samples = 41, median = 6.5, range = 0–1078; Recovery operators: samples = 24, median = 7.0, range = 0–> 12 000].

THBVal hemoglobin adducts were significantly more common in butadiene exposed workers than controls ( $P < 0.0001$ ). The greatest adduct levels were found in DMF operators and in younger butadiene-exposed workers. Adduct levels did not vary among controls by age or tobacco use (Fig. 2). Among butadiene-exposed, air and urine measures of butadiene were correlated ( $n = 15$ , Spearman's  $\rho = 0.51$ ,  $P = 0.05$ ), while THBVal hemoglobin adducts correlated with air ( $n = 31$ , Spearman's  $\rho = 0.40$ ,  $P = 0.03$ ) and weakly with urinary butadiene ( $n = 12$ , Spearman's  $\rho = 0.37$ ,  $P = 0.24$ ).

Butadiene exposed workers had greater absolute lymphocyte counts and more lymphocytes as a proportion of total WBC. Platelet counts tended also to be greater in butadiene-exposed than unexposed workers. However, *hprt* mutations ( $M_f$ ), erythrocyte glycoprotein A mutations (NO and NN), SCEs (with and without DEB induction), and percentage aneuploidy did not differ significantly between butadiene-exposed and unexposed workers (Table 2). SCEs without DEB induction were unrelated to DEB SCE inducibility among butadiene-exposed workers (Fig. 3) or in unexposed controls (data not shown). Tests of statistical significance for these comparisons were similar when calculated by linear regression, with adjustment for age and sex.

Table 2  
Exposure to butadiene and hematologic and genotoxic effects, Yanshan, China

	Unexposed			Exposed			$P^b$
	$n$	Median	Range <sup>a</sup>	$N$	Median	Range	
<i>Hematologic effects</i>							
WBC	38	6.7	2.2	40	7.0	2.6	0.52
Granulocytes ( $\times 10^3/\mu\text{l}$ blood)	38	4.6	1.6	40	4.6	1.5	0.70
Lymphocytes ( $\times 10^3/\mu\text{l}$ blood)	38	1.8	0.8	40	2.4	0.9	0.002
Lymphocyte % (% of total WBC)	38	28.4	12.3	40	32.8	9.9	0.005
Erythrocytes ( $\times 10^6/\mu\text{l}$ blood)	38	4.3	0.9	40	4.4	0.7	0.74
Platelets ( $\times 10^3/\mu\text{l}$ blood)	38	212	79.5	40	225	89.0	0.07
<i>Genotoxic effects</i>							
GPA: <sup>c</sup> NO	18	6.0	5.8	23	3.9	4.4	0.12
GPA:NN	18	7.2	4.7	23	6.0	5.6	0.11
<i>hprt</i> <sup>d</sup>	28	17.2	18.1	32	16.8	14.4	0.76
SCEs <sup>e</sup>	37	10.3	1.6	40	9.7	5.0	0.20
SCEs (DEB-induced)	37	98.4	56.4	38	103.0	97.1	0.82
Aneuploidy <sup>f</sup>	36	11	11	39	10	8	0.30

<sup>a</sup> Interquartile range.

<sup>b</sup> Wilcoxin test for independent samples.

<sup>c</sup> GPA, variant frequency ( $V_f$ )  $\times 10^{-6}$ .

<sup>d</sup> *hprt*, mutation frequency ( $M_f$ )  $\times 10^{-6}$ .

<sup>e</sup> SCEs (per metaphase spread).

<sup>f</sup> Aneuploidy % (per metaphase spread) of chromosomes 1, 7, 8 and 12.

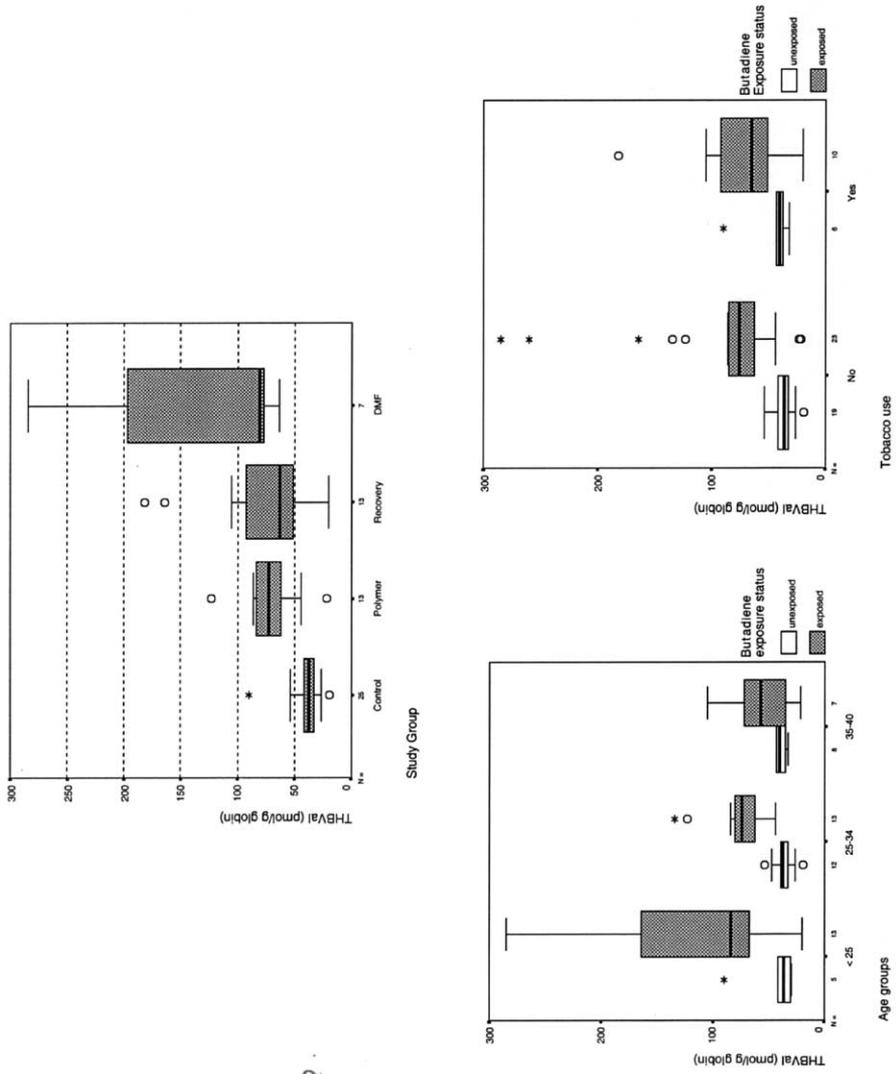


Fig. 2. THBVal adducts, by study group, age, and tobacco use.

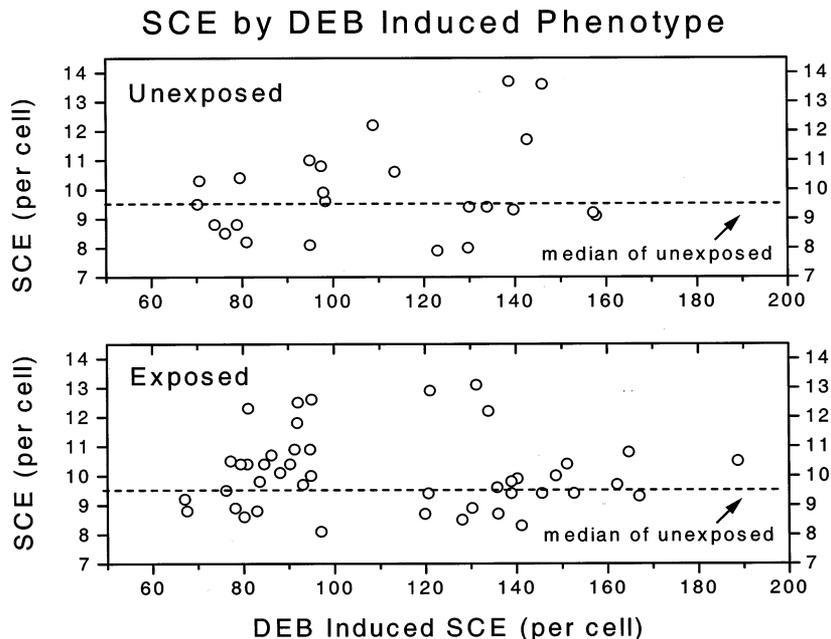


Fig. 3. In vivo and in vitro (DEB-induced) sister chromatid exchange, by butadiene exposure group.

#### 4. Discussion

We established, by air monitoring for butadiene, by determination of the M-1 metabolite in urine, and by determination of THBVal hemoglobin adducts, that workers in the Yanshan BD facility were exposed to 1,3-butadiene. These workers, however, did not have increased levels of somatic mutations, as measured by the GPA and *hprt* mutation frequency assays, or SCEs. Also, analysis of specific aneuploidic events on four chromosomes showed no effects of BD (Zhang, HEI conference). Our negative results are relevant, however, only for exposures in the butadiene exposure range studied. Also, the relatively small size of the study sample limits our ability to detect modest effects.

Other studies have shown genotoxic outcomes in workers exposed to butadiene, but the results have not been consistent. Among US butadiene-styrene workers, dicentrics were significantly correlated with urinary M-1 and there was evidence of deficiencies in DNA repair by the CAT-host cell reactivation assay [7]. Increased frequency of mutations in *hprt* were also observed in the US workers [11,12], but excesses were not found in our study [5] or in the Czech Republic [20].

Increased frequency of chromosomal aberrations and sister chromatid exchange were reported in the Czech Republic [10,20]; an earlier investigation of these subjects and workers in Portugal showed no excesses [8,9]. A more recent study of European butadiene-exposed workers which assessed similar outcomes as in our investigation in China, also found no substantial cytogenetic effect of BD (Albertini

et al., this volume). We found increases of lymphocytes in butadiene-exposed workers with levels tending to increase with increasing butadiene exposure, suggesting a role for butadiene in lymphocyte proliferation and increased cell turnover. However, the mechanism and significance of this modest increase are unknown and earlier studies showed no hematologic effects [21,22].

Earlier, we found [4], as did others [23], that in vitro DEB-induced SCE frequency is greatest among subjects who carry the *GSTT1* null polymorphism, suggesting a role for this enzyme in DEB detoxification. However, we found no relation in China between the *GSTT1* genotype [4] or the DEB induction phenotype and the frequency of in vivo SCE in butadiene-exposed workers or controls, in contrast to findings in the US [24]. Our finding of negative results for a spectrum of cytogenetic and other intermediate outcomes, suggests that the relatively low BD exposures in our study may have been insufficient to produce an in vivo DEB response, although similar low levels of BD exposure also occurred in the US study [24]. Alternatively, DEB may not be a significant metabolite in humans, relative to other pathways such as EBD formation [16].

In summary, this investigation in China demonstrated exposure to butadiene, by a variety of short-term and long-term measures, but did not show specific genotoxic effects related to that exposure.

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