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Genotoxic markers among butadiene polymer workers in China

R.B.Hayes[1,10], L.Zhang[2], S.Yin[3], J.A.Swenberg[4], L.Xi[2,3], J.Wiencke[5], W.E.Bechtold[6], M.Yao[3], N.Rothman[1], R.Haas[7], J.P.O'Neill[8], D.Zhang[9], J.Wiemels[2], M.Dosemeci[1], G.Li[3] and M.T.Smith[2]

[1]Occupational Studies Branch, National Cancer Institute, Bethesda, MD, USA, [2]School of Public Health, University of California, Berkeley, CA, USA, [3]Chinese Academy of Preventive Medicine, Beijing, China, [4]School of Public Health and Medicine, University of North Carolina, Chapel Hill, NC, USA, [5]School of Medicine, University of California San Francisco, San Francisco, CA, USA, [6]Inhalation Toxicology Research Institute, Albuquerque, NM, USA, [7]California State Health Department, Berkeley, CA, USA, [8]Genetics Laboratory, University of Vermont, Burlington, VT, USA and [9]Institute of Occupational Medicine, Yanshan Petrochemical Products Corporation, Yanshan, People's Republic of China

[10]To whom correspondence should be addressed
Email: hayesr@exchange.nih.gov

While 1,3-butadiene is carcinogenic in rodents, cancer causation in humans is less certain. We examined a spectrum of genotoxic outcomes in 41 butadiene polymer production workers and 38 non-exposed controls, in China, to explore the role of butadiene in human carcinogenesis. Because *in vitro* studies suggest that genetic polymorphisms in glutathione *S*-transferase enzymes influence genotoxic effects of butadiene, we also related genotoxicity to genetic polymorphisms in *GSTT1* and *GSTM1*. Among butadiene-exposed workers, median air exposure was 2 p.p.m. (6 h time-weighted average), due largely to intermittent high level exposures. Compared with 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 *in situ* hybridization of chromosomes 1, 7, 8 and 12, glycophorin 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, glycophorin A or *hprt* mutations. Butadiene-exposed workers had greater lymphocyte ($P = 0.002$) and platelet counts ($P = 0.07$) and lymphocytes as a percentage of white blood cells were moderately correlated with greater THBVal levels (Spearman's $\varphi = 0.32$, $P = 0.07$). Among butadiene-exposed workers, neither *GSTM1* nor *GSTT1* genotype status predicted urinary mercapturic acid butanediol formation, THBVal adducts, uninduced sister chromatid exchanges, aneuploidy or mutations in the glycophorin A or *hprt* genes. 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.

Abbreviations: BD, 1,3-butadiene; B-diol, 3-butene-1,2-diol; BDO, 3,4-epoxy-1-butene; BDO_2, 1,2,3,4-diepoxybutane; BDO-diol, 3,4-epoxy-1,2-butanediol; CE, cloning efficiencies; DMF, dimethyl formamide; FBS, fetal bovine serum; FISH, fluorescence *in situ* hybridization; GPA, glycophorin A; M-1, mercapturic acid butanediol; M-2, mercapturic acid butenol; PFPTH, pentafluorophenyl thiohydantoin; SCE, sister chromatid exchange; THBVal, *N*-(2,3,4-trihydroxybutyl)valine; WBC, total leukocyte count.

Introduction

The highly reactive flammable gas 1,3-butadiene (BD) was first produced in large volumes during World War II for use in the production of synthetic rubber. Currently, >5 000 000 tons of BD are produced annually world wide, ~1 500 000 tons of which are used in the USA (1). Approximately 65 000 US workers may be exposed annually to BD (2). While it 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 (3).

BD forms three major electrophiles: 3,4-epoxy-1-butene (BDO), 1,2,3,4-diepoxybutane (BDO_2) and 3,4-epoxy-1,2-butanediol (BDO-diol). BDO-diol, BDO and BDO_2 vary by ~200-fold in increasing genotoxicity as measured by mutagenicity at the *hprt* and *tk* loci in human TK6 lymphoblastoid cells (4) and by micronuclei induction in mouse erythrocytes (5). BD is carcinogenic in rodents, but mice are substantially more sensitive than rats (6,7). Following exposure to BD, mice show greater levels of genotoxic BD metabolites (including BDO, BDO_2 and their derivatives; 8–12) than rats and more frequent genotoxic events (13–16), suggesting that species differences in metabolism contribute to differential susceptibility to BD-induced cancer.

In a large study of workers in styrene/BD rubber plants, leukemias increased with increasing exposure to BD (17–19). Leukemia, however, was not in excess among workers in BD monomer production, whereas excesses were found for lymphosarcoma and reticulosarcoma, but a dose–response relationship was not evident (20,21). Excesses of lymphohematopoietic malignancies have also been reported among tire manufacturers, but a direct link with BD was not assessed (22,23). BD was recently classified by the International Agency for Research on Cancer as a probable human carcinogen (Group 2A), due to limited evidence in humans but sufficient evidence in animals (24).

Genotoxicity has been evaluated in studies of workers exposed to BD, however, the results have not been consistent (25–32). *In vitro* studies suggest that genetic polymorphisms in glutathione *S*-transferase enzymes predict genotoxic effects (33–42), but few human studies have been done (31,43). In workers at a Chinese BD polymerization facility, we recently demonstrated elevated levels of *N*-(2,3,4-trihydroxybutyl)valine (THBVal) adducts, which are formed from BDO-diol in its reaction with an N-terminal valine (44). Here, we assess the genotoxic effects of BD in these workers and relate genetic variation in *GSTT1* and *GSTM1* to these outcomes *in vivo*.

Table I. Study subjects, Yanshan, China

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

Materials and methods

Workers were studied at a polybutadiene rubber production facility, Yanshan, China. The purification of BD from an initial hydrocarbon stream (mainly alkanes and alkenes with 3–5 carbon atoms) occurred at two sites: the DMF facility, where initial distillation and extraction occurred using a proprietary dimethyl formamide (DMF) process (>95% pure BD), and the recovery facility, where final distillation occurred (>99.9% purity). BD was then transported to polymerization vats for synthesis of polybutadiene. Any BD remaining after the polymerization process was returned to the recovery unit, where it was mixed with incoming BD from the DMF recovery facility, repurified and repolymerized.

Study subjects

On an initial visit to the facility, three groups of workers with high potential exposure were identified. DMF process analysts sample process lines and analyze the product by gas chromatography in the DMF unit, while polymer process analysts carry out these tasks in the recovery and polymerization units. All analysts in these operations who were on duty during the days allocated for sample collection were eligible for study. A third group of exposed workers selected for study were process operators at the recovery facility who carry out routine minor maintenance and, as needed, major repair operations. All subjects in this work unit who would be involved in these activities during the study period were eligible for study.

After the purposes of the study and procedures were explained and informed consent was obtained, 41 of 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 the analysis. The study groups are shown in Table I.

Materials 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 to collect air in the breathing zone, by drawing the ambient atmosphere through a charcoal tube using an individual pump (flow rate 35 ml/min). Two traps were combined in series to ensure that all BD was retained. During the study, numerous grab samples in 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 (8 ml) was collected, from which mononuclear cells were isolated in a LeucoPREP® tube with Ficoll density gradient liquid and polyester gel (Becton and Dickinson, Lincoln Park, NJ). Mononuclear cells were washed in RPMI medium (UCSF, San Francisco, CA) with 10% fetal bovine serum (FBS) (Gemini Bioproducts), frozen (cells:cryosolution 1:1) in RPMI with 42% FBS and 8% dimethylsulfoxide under rate-controlled (1°C/min) conditions and stored as viable cells in the gas phase above liquid N_2. Blood samples (19 ml) were fractionated (serum, plasma, red blood cells and buffy coat) and stored. Whole blood cultures were established for cytogenetic studies. Lymphocytes were stimulated with phytohemagglutinin (PHA) and harvested at 72 h after culture initiation. Within 0.5 h of phlebotomy the MN blood type was determined using rabbit typing serum (Ortho Diagnostics). MN heterozygous blood was kept for 1–2 h at 4°C until formalin fixation. Spherical, formalin-fixed erythrocytes were prepared according to the method of Langlois *et al*. (45). The fixed specimens were stored at 4°C until analysis. A differential blood count was carried out with a Coulter blood counter on fresh whole blood within 2 h of collection. Absolute numbers ($\times 10^3$/μl blood) of granulocytes and lymphocytes were derived from a total leukocyte (WBC) count and the lymphocyte percentage. Urine samples were collected during work (0–3 and 4–6 h of a 6 h shift). During the work shift, urine samples were kept on ice. Within 2 h of the end of the work shift, the urine samples were aliquoted and frozen.

Air measurements

BD in air was collected by personal samplers and analyzed at the CAPM using an adaptation of NIOSH method 1024 (46). The analyte was desorbed with methylene chloride and analyzed by GC/FID, using a 12 foot×1/8 inch packed column (17%) of dibutylphthalate and 8.5% oxidipropionitrile on chromosorb 6201. Breathing zone air samples were analyzed on site within 30 min of collection with a Photovac 10S Plus (photoionization detector) using a 10 m×0.54 mm CP-Sil capillary column, calibrated with a 215 p.p.m. BD standard. Canister air samples were analyzed following the USEPA TO-14 guidelines. The instrument used was an HP 5890/5970 GC/MSD fitted with a flame ionization detector. The thermal desorption apparatus was a Tekmar 5010. The column eluant was split to flame ionization and mass selective detectors. The mass selective detector was operated in the selective ion monitoring mode to quantify 42 targeted organic compounds with re-analysis in the total ion chromatogram monitoring mode to confirm the identity of species. The level of detection for these compounds was estimated to be 1–2 p.p.b.

Urine measurements

Mercapturic acid butanediol (M-1) and mercapturic acid butenol (M-2) metabolites of BD were measured in urine samples by GC/GC/MS as previously described (47). Briefly, 5 μl injections of the sample were made into a 15 m×0.53 mm i.d. Restek Rtx-1 (1.0 mm film) column and the peaks were captured in a liquid nitrogen cooled loop and analyzed on a Restek Rtx-200 30 m×0.25 mm i.d. (0.25 mm film) capillary column. The MS was operated in selected ion monitoring mode, with monitoring for ions 129/132, 228/232, 377/382, 452 and 457 for M-1 and its deuterated analog, while ions 362/368 and 287/292 were monitored for M-2 and its deuterated analog. Standard curves were used to calculate absolute amounts of analytes in urine. Values were reported relative to mg creatinine, using standard methods.

Hemoglobin adducts

THBVal hemoglobin adducts were determined as previously described (48). In brief, globin was isolated from the red blood cell fraction (49) and derivatized with pentafluorophenyl isothiocyanate (Fluka, Buchs, Switzerland) to the pentafluorophenyl thiohydantoin (PFPTH) based on Törnqvist's modified Edman degradation for specific cleavage of *N*-alkylated terminal valines of the four chains in hemoglobin (50). A synthesized, derivatized external standard, THB($^{13}C_5$)Val-PFPTH was added to the sample. Samples were further processed by ultrafiltration with Centricon 30s (Amicon, Beverly, MA). The filtrate was extracted with diethylether (Fluka), washed on C18 columns (Alltech, Deerfield, IL) and eluted with acetonitrile (HPLC grade; Mallinckrodt, Paris, KY). The eluate was acetylated with 25% triethylamine (Aldrich, Milwaukee, WI) in acetonitrile (v/v) and 25% acetic anhydride (Mallinckrodt) in acetonitrile (v/v), dried, redissolved in pentane (Aldrich), washed with 40% aqueous methanol (HPLC grade; J.T. Baker, Phillipsburg, NJ), followed by GC/HRMS quantitation for THBVal-PFPTH at m/z 534.1084 for the analyte and 539.1254 for the external standard. Quantitation was based on the ratio of the peak area of the analyte to the peak area of the external standard.

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) (45). Sphered erythrocytes were incubated with anti-M (biotinylated 6A7) and anti-N (fluoresceinated BRIC 157) antibodies and prepared with avidin–phycoerythrin for flow cytometry. Singlet erythrocytes were selected for analysis using a polygonal gate based on forward scatter versus side scatter plots. The NØ and NN windows were 24 channels wide out of 256 channels and were individually adjusted using the MN mean peak for each specimen.

For the *hprt* mutations, mutation frequency (M_f) was determined by the T cell cloning assay (51). Briefly, viable cells were thawed and incubated in medium containing 1 μg/ml PHA (HA17; Wellcome Diagnostics) for 36–40 h to achieve mitogen stimulation. Washed cells were then placed in growth

medium (RPMI 1640 containing 20% nutrient medium HL-1, 5% defined supplemented bovine calf serum, 10–20% LAK supernatant containing 0.125 μg/ml PHA and 1×10^4 irradiated human lymphoblastoid feeder cells/well. After 10–16 days incubation, growing colonies were determined by use of an inverted phase contract microscope. 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.

Cytogenetic analysis

Sister chromatid exchange (SCE) was assessed in cultures, with and without exposure to BDO_2. Whole blood (0.5 ml) was cultured for 72 h at 37°C in 5% CO_2 with 98% humidity, in growth medium (4.5 ml), in 1 oz glass prescription bottles. For BDO_2-treated samples, treatment was carried out for 21 h, to a final concentration of 6 μM, and all samples were treated with 50 μM bromodeoxyuridine for 24 h. BDO_2 (Aldrich) was diluted in sterile water and a fresh stock solution was prepared for each experiment. Two hours before fixation, colcemid (2×10^{-7} M, final concentration; CIBA Pharmaceuticals, Summit, NJ) was added. Upon harvesting the cultured lymphocytes, microscopic slides were prepared and differentially stained, as described earlier (52). To estimate baseline SCE frequencies, 50 second division metaphases were scored per point; for BDO_2-treated cultures, 50 second division metaphases were scored. SCE frequency is expressed as the mean SCE/cell. For each subject, the mean BDO_2-induced SCE/cell frequency was calculated by subtracting the mean value for BDO_2-induced SCEs from the SCE culture result without BDO_2 treatment for that individual.

For fluorescence *in situ* hybridization (FISH), a total of four chromosomes were examined using two different types of probes purchased from Oncor Inc. (Gaithersburg, MD) and Vysis Inc. (Downers Grove, IL). The centromeres of chromosomes 1 and 7 were targeted by α-satellite DNA probes and chromosomes 8 and 12 were painted along their whole lengths with painting probes. The signals on chromosomes 1 and 8 were detected as green and those on 7 and 12 as red. A simplified denaturation and hybridization procedure was performed automatically by the HyBrite Denaturation/Hybridization system from Vysis Inc. The centromere and painting probes were mixed well, then applied to slides and coverslipped. The denaturation temperature was set at 72°C and the time at 7 min. Slides remained in the moist environment of the system at 37°C for 45–68 h in order to obtain optimal signals. Slides were then post-washed in 1× SSC at 70°C for 5 min and in phosphate buffer three times at room temperature. After detection and amplification of the hybridization signals, the signals were viewed under a Zeiss fluorescence microscope equipped with epifluorescent illumination, a 100× oil immersion lens and a triple bandpass filter for DAPI/FITC/Texas red. Here, we examine percent aneuploidy as a marker of genotoxic damage. Detailed analyses, including separate examination of hypoploidy and hyperploidy, will be described elsewhere (manuscript in preparation).

Classic karyotype analyses by G-banding were carried out to identify chromosomal abnormalities. Fifty metaphase spreads per subject were examined for structural and numerical changes. Detailed banding analyses will be described elsewhere (manuscript in preparation).

PCR analysis of GSTM1 and GSTT1 deletions

Target DNA (50–100 ng) was obtained from heparinized whole blood (Puregene; Gentra, Research Triangle Park, NC). PCR reactions were carried out in 50 μl volume containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1 mM 2-mercaptoethanol, 1% glycerol, 1 mM $MgCl_2$, 0.2 mM dNTPs and 2.5 U Amplitaq. For *GSTM1* the primers were 5′-GTGCCCTACTTGATTGATGGG-3′ and 5′-CTGGATTGTAGCAGATCATGC-3′. The primers for *GSTT1* were 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAGCA-3′. PCR products were electrophoresed on 2% agarose gels and the diagnostic bands were visualized using ethidium bromide staining. Control amplifications were run in all lanes using universal primers for actin.

Statistical analysis

Non-parametric procedures were used for statistical analysis, including the Spearman correlation test, the Wilcoxon test for independent samples and the χ^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 (53).

Results

BD-exposed workers were, on average, somewhat younger than the unexposed comparison group (Table II). None of the women in either group smoked. Tobacco use was prevalent among exposed (86.7%) and unexposed men (78.6%). Among

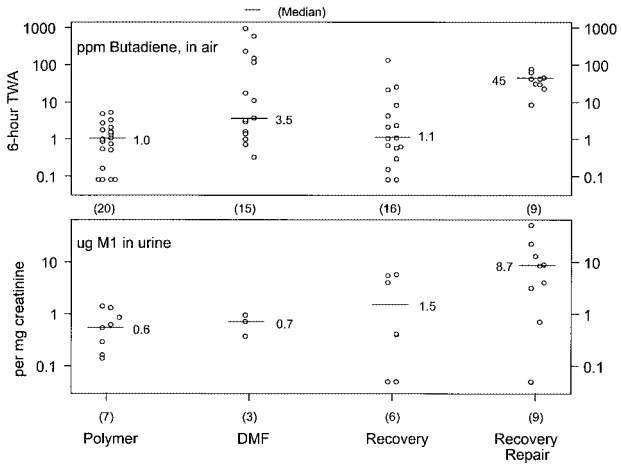

Fig. 1. Air and urine measures of butadiene exposure.

cigarette smokers, the usual number of cigarettes smoked and the cumulative amount smoked (pack-years) were similar for exposed and unexposed men (Table II). Among BD-exposed workers, men and women were exposed to BD for a similar number of years.

Full-shift individual exposures to BD varied widely, as measured in air and urine (M-1, corrected for creatinine) (Figure 1). Among DMF and polymer process analysts, median air levels of BD were 1.0 and 3.5 p.p.m., respectively. Recovery operators carrying out routine tasks were exposed to 1.1 p.p.m., however, recovery operators involved in pump repairs had median exposures of 45 p.p.m. Urinary M-1 levels were also substantially elevated in this group of highly exposed recovery operators. (Evidence for M-2 in urine was equivocal for one highly exposed worker.)

Short-term exposure to BD showed even greater extremes, as measured by breathing zone grab samples collected during BD-related operations (DMF analysts, $n = 50$, median BD 54 p.p.m., range 0–3090; polymerization analysts, $n = 41$, median 6.5 p.p.m., range = 0–1078; recovery operators, $n = 24$, median = 7.0 p.p.m. range 0–>12 000). Of the area samples taken by evacuated canister (DMF analysts laboratory, DMF process facility, recovery area, communications room and control area), measurable organics (including BD and several 4–6 carbon compounds) were found only in the recovery area. DMF was not detected in any of these samples. Air and urine monitoring for BD were carried out for a subset of unexposed workers. BD was undetectable in air ($n = 14$, limit of detection 0.08 p.p.m.). Measurable levels of M-1 were found, however, in three of four unexposed subjects (0.79, 0.79 and 0.32 μg/mg creatinine, respectively), indicating that this analyte may not be appropriate for assessment of low level BD exposure.

THBVal hemoglobin adducts were significantly more common in BD-exposed workers than controls (Table III). BD-exposed workers had greater absolute lymphocyte counts and greater lymphocytes as a proportion of total WBC. Platelet counts also tended to be greater in BD-exposed than unexposed workers. However, *hprt* mutations (M_f) (as previously reported; 28), erythrocyte GPA mutations (NØ and NN), SCEs (with and without BDO_2 induction) and the frequency of total aneuploidy of chromosomes 1, 7, 8 and 12 did not differ significantly between BD-exposed and unexposed workers. As measured by classical cytogenetics, the frequency of structural (χ^2 test, $P = 0.39$) and numerical abnormalities (χ^2 test,

Table II. Selected characteristics of study subjects

	Male		Female	
	n	Mean ± SD	*n*	Mean ± SD
Age				
Exposed	15	28.5 ± 6.0	26	27.2 ± 6.0
Unexposed	14	31.6 ± 4.8	24	30.8 ± 6.1
Among smokers				
Cigarettes per day				
Exposed	13 (86.7%)	8.6 ± 7.8	0	
Unexposed	11 (78.6%)	10.4 ± 7.0	0	
Pack-years				
Exposed	13	6.1 ± 8.4	0	
Unexposed	11	7.1 ± 5.2	0	
Among butadiene exposed				
Years of butadiene exposure				
Exposed	15	8.6 ± 6.1	26	6.5 ± 5.5

Table III. Exposure to butadiene and genotoxic effects, Yanshan, China

Measure	Unexposed (n = 38)			Exposed (n = 41)			P[a]
	n	Median	Range[b]	*n*	Median	Range[b]	
Measures of exposure							
Butadiene in air	14	0		39	2.0	20.6	<0.0001
M-1 in urine	4	0.6	0.7	17	1.3	5.2	0.16
THBVal adducts	25	37.6	9.2	33	74.0	30.9	<0.0001
Hematological measures							
WBC	38	6.7	2.2	40	7.0	2.6	0.52
Granulocytes	38	4.6	1.6	40	4.6	1.5	0.70
Lymphocytes	38	1.8	0.8	40	2.4	0.9	0.002
Lymphocyte %	38	28.4	12.3	40	32.8	9.9	0.005
Erythrocytes	38	4.3	0.9	40	4.4	0.7	0.74
Platelets	38	212	79.5	40	225	89.0	0.07
Measures of genotoxicity							
Glycophorin A							
NØ	18	6.0	5.8	23	3.9	4.4	0.12
NN	18	7.2	4.7	23	6.0	5.6	0.11
hprt	28	17.2	18.1	32	16.8	14.4	0.76
SCEs	37	10.3	1.6	40	9.7	5.0	0.20
SCEs (BDO_2-induced)	37	98.4	56.4	38	103.0	97.1	0.82
Aneuploidy	36	11	11	39	10	8	0.30

Units: butadiene in air, p.p.m.; M-1 in urine, μg M-1/mg creatinine; THBVal adducts, pmol/g; leukocytes, granulocytes, lymphocytes and platelets, $\times 10^3$/μl blood; lymphocyte %, % of total WBC; erythrocytes, $\times 10^6$/μl blood; glycophorin A, variant frequency $(V_f)\times 10^{-6}$; *hprt*, mutation frequency $(M_f)\times 10^{-6}$; SCEs, per metaphase spread; aneuploidy %, per metaphase spread of chromosomes 1, 7, 8 and 12.
[a]Wilcoxon test for independent samples.
[b]Interquartile range

P = 0.36) also did not differ between exposed and unexposed (data not shown). Tests of statistical significance for these comparisons were similar when calculated by linear regression, with adjustment for age and sex.

Among the BD-exposed, air and urine measures of BD were correlated (n = 15, Spearman's φ = 0.51, P = 0.05) (Table IV), while THBVal hemoglobin adducts correlated with air (n = 31, Spearman's φ = 0.40, P = 0.03) and more weakly with urinary BD (n = 12, Spearman's φ =0.37, P = 0.24). There was no evidence, however, of a direct correlation between THBVal adducts and genotoxic markers, rather there was a tendency towards inverse correlations with GPA NN (φ = –0.43, P = 0.06) and uninduced SCEs (φ = –0.39, P = 0.03). THBVal adducts did correlate, however, with lymphocytes as a percentage of total WBC (φ = 0.32, P = 0.07).

Air and urine measurements of BD were also not associated with increases in genotoxic events; rather, the correlations for BD in air with GPA NN (P = 0.01), *hprt* (P = 0.06) and SCEs (P = 0.02) were in the negative direction. Increases in lymphocyte numbers (for air P = 0.001; for urine P = 0.004) and as a percentage of total WBC (for air P = 0.003; for urine P = 0.07) were noted. Other associations included a positive correlation between BDO_2-induced SCEs and granulocyte count ($P < 0.001$), although the biological relevance of this finding is unclear.

Among BD-exposed workers, levels of exposure did not differ substantially with respect to *GSTT1* (Table V) or *GSTM1* status (Table VI). Neither genotype predicted urinary M-1 or THBVal adduct formation, *hprt* or GPA mutations or SCEs among exposed workers. Aneuploidy as measured by FISH (Tables V and VI) and chromosomal aberrations, measured by classical cytogenetics (data not shown), were also not associated with genotype. Granulocyte levels were greater and

Table IV. Correlation of butadiene exposure estimates, genotoxic markers and peripheral blood lymphocytes among butadiene-exposed workers

		Butadiene exposure			Genotoxic markers						Hematological measures		
		Air	Urine	Adducts	Glycophorin A		*hprt*	SCEs	BDO_2 SCEs	Aneuploidy	Lymphocytes		Granulocytes
					NØ	NN					No.	Percent	No.
Air[a]	φ[b]		0.51	0.40	–0.29	–0.50	–0.33	–0.37	–0.19	–0.17	0.52	0.46	–0.05
	P[c]		*0.05*	*0.03*	*0.18*	*0.01*	*0.06*	*0.02*	*0.26*	*0.31*	*0.001*	*0.003*	*0.74*
Urine[d]	φ			0.37	0.13	–0.43	–0.16	–0.23	–0.51	0.07	0.66	0.44	–0.04
	P			*0.24*	*0.71*	*0.18*	*0.60*	*0.38*	*0.04*	*0.81*	*0.004*	*0.07*	*0.86*
Adducts[e]	φ				0.06	–0.43	–0.29	–0.39	–0.21	0.02	0.13	0.32	–0.27
	P				*0.80*	*0.06*	*0.16*	*0.03*	*0.24*	*0.90*	*0.49*	*0.07*	*0.13*
Glycophorin A													
NØ	φ					0.50	0.13	0.16	0.02	0.32	–0.18	0.03	–0.09
	P					*0.02*	*0.60*	*0.46*	*0.93*	*0.15*	*0.42*	*0.90*	*0.68*
NN	φ						0.22	0.47	–0.17	0.36	–0.58	–0.15	–0.25
	P						*0.37*	*0.03*	*0.46*	*0.10*	*0.004*	*0.50*	*0.24*
hprt	φ							0.20	0.10	–0.003	0.05	0.002	0.09
	P							*0.27*	*0.58*	*0.99*	*0.80*	*0.99*	*0.62*
SCEs	φ								0.05	0.13	–0.12	–0.03	–0.002
	P								*0.77*	*0.43*	*0.47*	*0.87*	*0.99*
BDO_2 SCE[f]	φ									–0.10	–0.08	–0.51	0.59
	P									*0.54*	*0.62*	*0.001*	*<0.001*
Aneuploidy	φ										–0.14	0.10	–0.18
	P										*0.41*	*0.56*	*0.26*
Lymphocyte no.	φ											0.51	0.251
	P											*0.001*	*0.12*
Lymphocyte %	φ												–0.664
	P												*<0.001*

[a]Butadiene in air (p.p.m.).
[b]Spearman's φ.
[c]*P* value.
[d]M-1 in urine (mg M-1/mg creatine).
[e]THBVal adducts (pmol/g).
[f]BDO_2-induced sister chromatid exchange.

Table V. *GSTT1* and genotoxic effects among butadiene-exposed workers

Measure	Null (n = 15)			Present (n = 24)			P[a]
	n	Median	Range[b]	*n*	Median	Range[b]	
Measures of exposure							
Butadiene in air	15	1.6	4.6	22	2.2	30.0	0.66
M-1 in urine	7	1.3	3.7	9	5.4	15.0	0.27
THBVal adducts	13	74.0	31.7	19	72.8	47.2	0.89
Hematological measures							
WBC	15	7.6	2.7	24	6.6	2.3	0.04
Granulocytes	15	5.2	1.7	24	4.0	1.8	0.009
Lymphocytes	15	2.5	1.0	24	2.4	0.9	0.72
Lymphocyte %	15	29.8	8.4	24	35.9	12.3	0.02
Erythrocytes	15	4.3	0.8	24	4.4	0.6	0.51
Platelets	15	218	126	24	225	76	0.94
Measures of genotoxicity							
Glycophorin A							
NØ	9	3.9	5.0	14	4.0	4.2	0.90
NN	9	5.3	3.4	14	7.0	7.2	0.31
hprt	12	18.9	21.5	19	16.7	11.5	0.92
SCEs	15	9.6	1.0	24	9.8	2.0	0.78
SCEs (BDO_2 induced)	15	138.9	14.3	22	86.2	15.8	<0.0001
Aneuploidy (%)	14	11	6	24	8	10	0.43

Units: butadiene in air, p.p.m.; M-1 in urine, μg M-1/mg creatinine; THBVal adducts, pmol/g; leukocytes, granulocytes, lymphocytes and platelets, $\times 10^3$/μl blood; lymphocyte %, % of total WBC; erythrocytes, $\times 10^6$/μl blood; GPA, variant frequency $(V_f)\times 10^{-6}$; *hprt*, mutation frequency $(M_f)\times 10^{-6}$; SCEs, per metaphase spread; aneuploidy %, per metaphase spread of chromosomes 1, 7, 8 and 12.
[a]Wilcoxon test for independent samples.
[b]Interquartile range.

Table VI. *GSTM1* and genotoxic effects among butadiene-exposed workers

Measure	Null (n = 21)			Present (n = 19)			P[a]
	n	Median	Range[b]	n	Median	Range[b]	
Measures of exposure							
Butadiene in air	21	3.2	43.0	17	1.7	5.9	0.47
M-1 in urine	9	1.3	12.9	8	1.1	5.1	0.85
THBVal adducts	17	72.8	55.1	16	76.0	31.8	0.83
Hematological measures							
WBC	21	7.0	3.2	19	6.7	1.3	0.70
Granulocytes	21	4.6	2.2	19	4.7	1.6	0.55
Lymphocytes	21	2.4	1.1	19	2.4	0.7	0.98
Lymphocyte %	21	32.4	9.9	19	35.5	9.9	0.61
Erythrocytes	21	4.4	0.9	19	4.4	0.6	0.88
Platelets	21	230	120	19	221	68	0.59
Measures of genotoxicity							
Glycophorin A							
NØ	14	3.7	5.1	9	5.5	4.4	0.59
NN	14	6.2	5.4	9	5.1	8.9	0.88
hprt	19	17.0	10.3	13	16.7	23.8	0.70
SCEs	21	9.7	1.2	19	9.8	2.9	0.56
SCEs (BDO_2 induced)	20	95.1	54.6	18	107.6	48.4	0.85
Aneuploidy (%)	20	9	6	19	10	10	0.72

Units: butadiene in air, p.p.m.; M-1 in urine, μg M-1/mg creatinine; THBVal adducts, pmol/g; leukocytes, granulocytes, lymphocytes and platelets, $\times 10^3$/μl blood; lymphocyte %, % of total WBC; erythrocytes, $\times 10^6$/μl blood; GPA, variant frequency $(V_f)\times 10^{-6}$; *hprt*, mutation frequency $(M_f)\times 10^{-6}$; SCEs, per metaphase spread; aneuploidy %, per metaphase spread of chromosomes 1, 7, 8 and 12.
[a]Wilcoxon test for independent samples.
[b]Interquartile range.

lymphocytes as a percentage of total WBCs were less in BD-exposed workers with the *GSTT1* null genotype. After adjustment for age, sex and THBVal level, weaker associations remained for *GSTT1* status with granulocytes (P = 0.06) and lymphocyte percent (P = 0.05) (data not shown).

Subjects with the *GSTT1* null genotype had substantially greater frequency of BDO_2-induced SCEs, which is consistent with earlier reports of a strong correlation between *GSTT1* genotype and the BDO_2-induced SCE phenotype (35–37). This relationship of *GSTT1* genotype and BDO_2-induced phenotype was also observed among unexposed workers (data not shown). BDO_2-induced SCEs also did not vary in frequency between exposed and unexposed when examined within strata of the *GSTT1* genotype (genotype null P = 0.26; genotype present P = 0.92). *GSTM1* genotype was not associated with any of the blood count or genotoxic markers (Table VI).

Discussion

Using several approaches to air monitoring for BD, by determination of the M-1 metabolite in urine and by determination of THBVal hemoglobin adducts, we established that workers in a BD polymer production facility were exposed to BD. As earlier shown in the USA (11), M-1 was found in the urine of BD-exposed workers, but M-2 was not detected.

BD-exposed workers did not have increased levels of somatic mutations as measured by the GPA and *hprt* mutation frequency assays. SCE frequency and chromosomal abnormalities were also not increased in BD-exposed workers. The assays chosen for this study encompassed measures of genotoxicity in erythrocyte precursors (45) and T cells (51) and included outcomes found after exposure to BD metabolites *in vitro* (4,33,42,52) and in animal studies (16). Our negative results are relevant, however, only for exposures in the BD 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 BD, but the results have not been consistent. Among US BD/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 (25). Increased frequency of mutations in *hprt* were also observed in US BD workers (26,27) but excesses were not found in our study, as reported earlier (28), or in the Czech Republic (32). Increased frequency of chromosomal aberrations and SCE were reported in the Czech Republic (31,32); an earlier investigation of these subjects and workers in Portugal showed no excesses (29,30). We found increases in lymphocytes in BD-exposed workers with levels tending to increase with increasing BD exposure, suggesting a role for BD in lymphocyte proliferation and increased cell turnover. However, the mechanism and significance of this modest increase are unknown and earlier studies showed no hematological effects (54,55).

Investigations in the USA (26,27), Europe (30) and China report similar average exposure levels of ~1–2 p.p.m. BD in air, but individual exposures vary and the approaches to exposure assessment have been limited. Because BD production is a closed operation, exposures are generally low. As we found in China, however, certain tasks are associated with strikingly high, short-term exposures (exceeding 1000 p.p.m.), while full-day high level exposures were found only among workers involved in major repair operations. When peak exposures are brief and intense, air measurements may not accurately reflect biological dose because exposure avoidance may limit the actual dose received, absorbtion and retention may be decreased (56) and technical limitations of the pump sampling methodology may bias results. Further limiting comparisons, historical episodes of high level exposure (e.g. accidents) were not recorded. Also, measurements of exposures have generally been over a limited number of days, while the genotoxic effects being assessed are probably related to exposures occurring over several months.

Assessment of hemoglobin adducts in our study provided an independent approach to BD exposure assessment that obviated some of the limitations of earlier studies. Because erythrocytes are relatively long lived (average ~120 days), adducts in hemoglobin tend to integrate biological dose over an extended period of time. Hemoglobin adducts were also moderately correlated with 1 day air and urine exposure measurements, suggesting that the 1 day measurements tended to reflect an ordinal ranking of exposures in the longer term. Further detailed calibration studies will be needed, however, to determine the quantitative relationship between level of BD exposure and THBVal adduct formation in occupational settings. It should also be noted that measurable levels of THBVal and urinary M-1 are found in subjects unexposed to BD. The sources for this are presently not understood.

In vitro exposure of human lymphocytes to BDO_2 characterizes individuals as 'sensitive' or 'resistant' to BDO_2-induced SCE (34), a phenotype that we and others have shown to correlate closely with the *GSTT1* genotype (35–37). Exposure *in vitro* of human lymphocytes to BDO also characterizes a 'sensitive' phenotype, which appears to be linked to *GSTM1* as well as *GSTT1* deficiency (38–40). BDO-diol also induces SCE in lymphocyte cultures, but results were not dependent upon *GSTM1* or *GSTT1* genotype (41). Although *in vitro* exposures may point to inter-individual differences in susceptibility to BD carcinogenicity, extrapolation to studies of humans occupationally exposed may be limited. The *in vitro* exposures are much higher than occur in humans and this may lead to saturation of this detoxification pathway. It appears that the *GSTT1* genotype does not confer an increased susceptibility under the conditions of this study. This lack of genotoxicity supports BDO-diol being the major metabolite of BD in humans (see below).

We found no evidence in our study in China that *GSTT1* or *GSTM1* genotype was related to BD genotoxicity. Other studies have not shown consistent relationships. In the Czech and Portuguese BD facilities, chromosomal aberration frequency tended to be greater among workers in monomer and polymer production who had the null *GSTT1* genotype, while the *GSTM1* genotype had no effect among the BD-exposed workers (43). A more recent investigation of BD monomer workers in the Czech facility found no association with *GSTT1* genotype, however, decreased chromosomal aberrations were associated with the null genotype of *GSTM1* (31). Our cytogenetic analyses showed no effects due to these genetic variants. An added complexity is that weak associations between genotype and chromosome aberrations were also found among non-exposed controls in these studies (31,43).

THBVal adducts can form from the highly genotoxic BDO_2 or BDO-diol, an epoxide with 1/200th the mutagenicity (4,5). Although the major pathway for THBVal formation may be through the BDO-diol (57,58), the relative ratio of these precursors is not directly determinable (48). In rodents, the relative contribution of BDO-diol to THB–guanine DNA adducts is estimated to be 95–98% (59). Thus, elevated THBVal levels indicate exposure to BD, but may not be a good marker for individual genotoxic risk. Glutathione transferases are involved in the detoxification of BDO_2 and its precursor (BDO) as well as in the detoxification of the BDO-diol precursors [BDO and 3-butene-1,2-diol (B-diol)], however, in our study THBVal adduct levels did not vary by *GSTT1* or *GSTM1* status among BD-exposed workers. Similarly, M-1, which is a glutathione conjugation product of B-diol, did not vary with respect to variants in these genes.

In summary, this investigation in China demonstrated exposure to BD, by a variety of short-term and long-term measures, but did not show specific genotoxic effects related to that exposure. Modest perturbations in blood count profiles were found. In contrast to *in vitro* investigations, studies of potential susceptibility groups also revealed no genotoxic effects.

References

1. Morrow,N.L. (1990) The industrial production and use of 1,3-butadiene. *Environ. Health Perspect.*, **86**, 7–8.
2. Fajen,J.M., Roberts,D.R., Ungers,L.J. and Krishnan,E.R. (1990) Occupational exposure of workers to 1,3-butadiene. *Environ. Health Perspect.*, **86**, 11–18.
3. International Agency for Research on Cancer (1992) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.* Vol. 54, *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals*. IARC, Lyon.
4. Cochrane,J.E. and Skopek,T.R. (1994) Mutagenicity of butadiene and its epoxide metabolites: I. Mutagenic potential of 1,2-epoxybutene 1,2,3,4-diepoxybutane and 3,4-epoxy-1,2-butanediol in cultured human lymphoblasts. *Carcinogenesis*, **15**, 713–717.
5. Adler,I.-D., Kliesch,U., Nylund,L. and Peltonen,K. (1997) *In vitro* and *in vivo* mutagenicity of the butadiene metabolites butadiene diepoxide, butadiene monoepoxide and diepoxybutane. *Mutagenesis*, **12**, 339–345.
6. Melnick,R.L., Huff,J., Chou,B.J. and Miller,R. (1990) Carcinogenicity of 1,3-butadiene in C57BL/6×C3H F1 mice at low exposure concentrations. *Cancer Res.*, **50**, 6592–6599.
7. Owen,P.E. and Glaister,J.R. (1990) Inhalation toxicity and carcinogenicity or 1,3-butadiene in Sprague-Dawley rats. *Environ. Health Perspect.*, **86**, 19–25.
8. Csanady,G.A., Guengerich,F.P. and Bond,J.A. (1992) Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis*, **13**, 1143–1153 [Erratum (1993) *Carcinogenesis*, **14**, 784].
9. Sabourin,P.J., Burka,L.T., Bechtold,W.E., Dahl,A.R., Hoover,M.D., Chang,I.Y., Henderson,R.F. and Hallenbeck,W.H. (1992) Species differences in urinary butadiene metabolites; identification of 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane, a novel metabolite of butadiene. Cancer risk assessment for the inhalation of 1,3-butadiene using physiologically based pharmacokinetic modeling. *Bull. Environ. Contam. Toxicol.*, **49**, 66–70.
10. Himmelstein,M.W., Turner,M.J., Asgharian,B. and Bond,J.A. (1994) Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. *Carcinogenesis*, **15**, 1479–1486.
11. Bechtold,W.E., Strunk,M.E., Chang,I.-Y., Ward,J.B. and Henderson,R.F. (1994) Species differences in urinary butadiene metabolites: comparisons of metabolite ratios between mice, rats and humans. *Toxicol. Appl. Pharmacol.*, **127**, 44–49.
12. Thorton-Manning,J.R., Dahl,A.R., Bechtold,W.E., Griffith,W.C. and Henderson,R.F. (1995) Disposition of butadiene monoepoxide and butadiene diepoxide in various tissues of rats and mice following low-level inhalation exposure to 1,3-butadiene. *Carcinogenesis*, **16**, 1723–1731.
13. Melnick,R.L. and Kohn,M.C. (1995) Mechanistic data indicate that 1,3 butadiene is a human carcinogen. *Carcinogenesis*, **16**, 157–163.
14. Bond,J.A., Recio,L. and Andjelkovich,D. (1995) Epidemiological and mechanistic data suggest that 1,3-butadiene will not be carcinogenic to humans at exposures likely to be encountered in the environment or workplace. *Carcinogenesis*, **16**, 165–171.
15. Cunningham,M.J., Choy,W.N., Arce,G.T., Rickard,L.B., Vlachos,D.A., Kinney,L.A. and Sariff,A.M. (1986) *In vivo* sister chromatid exchange and micronucleus induction studies with 1,3-butadiene in B6C3F1 mice and Sprague-Dawley rats. *Mutagenesis*, **1**, 449–452.
16. Meng,Q., Recio,L., Reilly,A.A., Wong,B.A., Bauer,M. and Walker,V.E. (1998) Comparison of the mutagenic potency of 1,3-butadiene at the *hprt* locus of T-lymphocytes following inhalation exposure of female B6C3F1 mice and F344 rats. *Carcinogenesis*, **19**, 1019–1027.
17. Macaluso,M., Larson,R., Delzell,E., Sathiakumar,N., Hovinga,M., Julian,J., Muir,D. and Cole,P. (1996) Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology*, **113**, 190–202.
18. Delzell,E., Sathiakumar,N., Hovinga,M., Macaluso,M., Julian,J., Larson,R., Cole,P. and Muir,D.C. (1996) A follow-up study of synthetic rubber workers. *Toxicology*, **113**, 182–189.
19. Sathiakumar,N., Delzell,E., Hovinga,M., Macaluso,M., Julian,J., Larson,R.,

Cole,P. and Muir,D.C. (1998) Mortality from cancer and other causes of death among synthetic rubber workers. *Occup. Environ. Med.*, **55**, 230–235.
20. Divine,B.J. and Hartman,C.M. (1996) Mortality update of butadiene production workers. *Toxicology*, **113**, 169–181.
21. Ward,E.M., Fajen,J.M., Ruder,A.M., Rinsky,R.A., Halperin,W.E. and Fessler-Flesch,C.A. (1995) Mortality study of workers in 1,3-butadiene production units idenitified from a chemical worker cohort. *Environ. Health Perspect.*, **103**, 598–603.
22. McMichael,A.J., Spirtas,R., Gamble,J.F. and Tousey,P.M. (1976) Mortality among rubber workers: relationship to specific jobs. *J. Occup. Med.*, **18**, 178–185.
23. Andjelkovich,D., Taulbee,J., Symons,M. and Williams,T. (1977) Mortality of rubber workers with reference to work experience. *J. Occup. Med.*, **19**, 397–405.
24. International Agency for Research on Cancer (1998) *1,3-Butadiene*. IARC, Lyon.
25. Hallberg,L.M., Bechtold,W.E., Grady,J., Legator,M.S. and Au,W.W. (1997) Abnormal DNA repair activities in lymphocytes of workers exposed to 1,3-butadiene. *Mutat. Res.*, **383**, 213–221.
26. Ward,J.B., Ammenheuser,M.M., Bechtold,W.E., Whorton,E.B.Jr and Legator,M.S. (1994) hprt mutant lymphocyte frequencies in workers at a 1,3-butadiene production plant. *Environ. Health Perspect.*, **102** (suppl. 9), 79–85.
27. Ward,J.B., Ammenheuser,M.M., Whorton, Jr. E.B., Bechtold,W.E., Kelsey,K.T. and Legator,M.S. (1996) Biological monitoring for mutagenic effects of occupational exposure to butadiene. *Toxicology*, **113**, 84–90.
28. Hayes,R.B., Xi,L., Bechtold,W.E., Rothman,N., Yao,M., Henderson,R., Zhang,L., Smith,M.T., Zhang,D., Wiemels,J., Dosemeci,M., Yin,S. and O'Neil,J.P. (1996) *hprt* mutation frequency among workers exposed to 1,3-butadiene in China. *Toxicology*, **113**, 100–105.
29. Sorsa,M., Autio,J., Demopoulus,N.A., Jarventaus,H., Rossner,P., Sram,R.J., Stephanou,G. and Vlachodimitropoulos,D. (1994) Human cytogenetic biomonitoring of occupational exposure to 1,3-butadiene. *Mutat. Res.*, **309**, 321–326.
30. Sorsa,M., Peltonen,K., Anderson,D., Demopoulus,N.A., Neuman,H.-G. and Osterman-Golkar,S. (1996) Assessment of environmental and occupational exposures to butadiene as a model for risk estimation of petrochemical emissions. *Mutagenesis*, **11**, 9–17.
31. Sram,R.J., Rossner,P., Peltonen,K., Podrazilova,K., Mrackova,G., Demopoulus,N.A., Stephanou,G., Vlachodimitropoulos,D., Darroudi,F. and Tates,A.D. (1998) Chromosomal aberrations, sister chromatid exchanges, cells with high frequency of SCE, micronuclei and comet assay parameters in 1,3-butadiene-exposed workers. *Mutat. Res.*, **419**, 145–154.
32. Tates,A.D., van Dam,F.J., de Zwart,F.A., Darroudi,F., Natarajan,A.T., Rossner,P., Peterkova,K., Peltonen,K., Demopoulus,N.A., Stephanou,G., Vlachodimitropoulos,D. and Sram,R.J. (1996) Biological effect monitoring in the industrial workers from the Czech Republic exposed to low levels of butadiene. *Toxicology*, **113**, 91–99.
33. Xi,L.Q., Zhang,L.P., Wang,Y.X. and Smith,M.T. (1997) Induction of chromosome-specific aneuploidy and micronuclei in human lymphocytes by metabolites of 1,3-butadiene. *Carcinogenesis*, **18**, 1687–1693.
34. Wiencke,J.K., Christiani,D.C. and Kelsey,K.T. (1991) Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. I. Correlation with chromosomal aberrations. *Mutat. Res.*, **248**, 17–26.
35. Kelsey,K.T., Christiani,D.C. and Wiencke,J.K. (1991) Sensitivity to cytogenetic damage induced by diepoxybutane is bimodal in humans and is associated with baseline SCEs and familial factors. *Environ. Mol. Mutagen.*, **17**, 35–35.
36. Wiencke,J.K., Pemble,S., Ketterer,B. and Kelsey,K.T. (1995) Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol. Biomarkers. Prev.*, **4**, 253–259.
37. Norppa,H., Hirvonen,A., Jarventaus,H., Uuskula,M., Tasa,G., Ojajarvi,A. and Sorsa,M. (1995) Role of GSTT1 and GSTM1 genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. *Carcinogenesis*, **16**, 1261–1264.
38. Wiencke,J.K. and Kelsey,K.T. (1993) Susceptibility to induction of chromosomal damage by metabolites of 1,3-butadiene and its relationship to 'spontaneous' siter chromatid exchange frequencies in human lymphocytes. In Sorsa,M., Peltonen,H., Vainio,H. and Hemminki,K. (eds) *Butadiene and Styrene: Assessment of Health Hazards*. IARC, Lyon, pp. 265–273.
39. Uuskula,M., Jarventaus,H., Hirvonen,A., Sorsa,M. and Norppa,H. (1995) Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. *Carcinogenesis*, **16**, 947–950.
40. Bernardini,S., Hirvonen,A., Pelin,K. and Norppa,H. (1998) Induction of sister chromatid exchange by 1,2-epoxy-3-butene in cultured human lymphocytes: influence of *GSTT1* genotype. *Carcinogenesis*, **19**, 377–380.
41. Bernardini,S., Pelin,K., Peltonen,K., Jarventaus,H., Hirvonen,A., Neagu,C., Sorsa,M. and Norppa,H. (1996) Induction of sister chromatid exchange by 3,4-epoxybutane-1,2-diol in cultured human lymphocytes of different GSTT1 and GSTM1 genotypes. *Mutat. Res.*, **361**, 121–127.
42. Vlachodimitropoulos,D., Norppa,H., Autio,K., Catalan,J., Hirvonen,A., Tasa,G., Uuskula,M., Demopoulus,N.A. and Sorsa,M. (1997) GSTT1-dependent induction of centromere-negative and -positive micronuclei by 1,2,3,4-diepoxybutane in cultured human lymphocytes. *Mutagenesis*, **12**, 397–403.
43. Sorsa,M., Osterman-Golkar,S., Peltonen,K., Saarikoski,S.T. and Sram,R.J. (1996) Assessment of exposure to butadiene in the process industry. *Toxicology*, **113**, 77–83.
44. Swenberg,J.A., Christova-Gueorguieva,N.I., Upton,P.B., Ranasinghe,A., Yin,S., Xi,L., Zhang,L., Smith,M.T., Rothman,N., Dosemeci,M. and Hayes,R.B. (1999) Molecular epidemiology of *N*-(2,3,4-trihydroxybutyl)valine in hemoglobin of Chinese butadiene workers. In *Proceedings of the AACR* (abstract), American Association for Cancer Research, Philadelphia, PA.
45. Langlois,R.G., Nisbet,B.A., Bigbee,W.L., Ridinger,D.N. and Jensen,R.H. (1990) An improved flow cytometric assay for somatic mutations at the glycophorin A locus in humans. *Cytometry*, **11**, 513–521.
46. National Institute of Occupational Safety and Health (1994) *NIOSH Manual of Analytic Methods*, 4th edn, US DHHS, Washington DC.
47. Bechtold,W.E., Strunk,M.R., Thornton-Manning,J.R. and Henderson,R.F. (1995) Species differences in the metabolism of 1,3-butadiene: appearance of butadiene diepoxide in the blood of mice, but not rats, exposed to butadiene. *Chem. Res. Toxicol.*, **8**, 182–187.
48. Swenberg,J.A., Christova-Gueorguieva,N.I., Upton,P.B., Ranasinghe,A., Scheller,N., Wu,K.-Y., Yen,T.-Y. and Hayes,R.B. (1999) Hemoglobin adducts as biomarkers of butadiene exposure and metabolism. Health Effects Institute Research Report, Health Effects Institute, Cambridge, MA (in press).
49. Mowrer,J., Tornqvist,M., Jensen,S. and Ehrenberg,L. (1986) Modified Edman degradation applied to hemoglobin for monitoring occupational exposure to alkylating agents. *Toxicol. Environ. Chem.*, **11**, 215–257.
50. Tornqvist,M., Mowrer,J., Jensen,S. and Ehrenberg,L. (1986) Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. *Anal. Biochem.*, **154**, 255–266.
51. Albertini,R.J., Nicklas,J.A. and O'Neill,J.P. (1990) Studies of DNA alterations in *in vivo* somatic cell mutations in humans. In Sutherland,B.M. and Woodhead,A.D. (eds) *DNA Damage and Repair in Human Tissues*. Plenum Press, New York, NY, pp. 315–328.
52. Wiencke,J.K., Wrensch,M.R., Milke,R. and Petrakis,N.L. (1991) Individual susceptibility to induced chromosome damage and its application for detecting genotoxic exposures in human populations. *Cancer Res.*, **51**, 5266–5269.
53. SPSS Inc. (1993) *SPSS for Windows*, *Release 6.0*. SPSS Inc., Chicago, IL.
54. Checkoway,H. and Williams,T.M. (1982) A hematology survey at a styrene-butadiene synthetic rubber manufacturing plant. *Am. Ind. Hyg. Assoc. J.*, **43**, 164–169.
55. Cowles,S.R., Tsai,S.P., Snyder,P.J. and Ross,C.E. (1994) Mortality, morbidity and haematological results from a cohort of long term workers involved in 1,3-butadiene monomer production. *Occup. Environ. Med.*, **51**, 323–329.
56. Bond,J.A., Dahl,R.F., Henderson,R.F., Dutcher,J.S., Mauderly,J.L. and Birnbaum,L.S. (1986) Species differences in the disposition of inhaled butadiene. *Toxicol. Appl. Pharmacol.*, **84**, 617–627.
57. Perez,H.L., Lahdetie,J., Landin,H.H., Kilpelainen,I., Koivisto,P., Peltonen,K. and Osterman-Golkar,S. (1997) Haemoglobin adducts of epoxybutanediol from exposure to 1,3-butadiene or butadiene epoxides. *Chem. Biol. Interact.*, **105**, 181–198.
58. Cheng,X. and Ruth,J.A. (1993) A simplified methodology for quantitation of butadiene metabolites. Application to the study of 1,3-butadiene metabolism by rat liver microsomes. *Drug. Metab. Dispos. Biol. Fate Chem.*, **21**, 121–124.
59. Koc,H., Tretyakova,N.Y., Walker,V.E., Henderson,R.F. and Swenberg,J.A. (1999) Molecular dosimetry of N-7 guanine adduct formation in mice and rats exposed to 1,3-butadiene. *Chem. Res. Toxicol.*, **12**, 566–574.

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