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Sister-chromatid exchanges, glutathione S-transferase θ deletion and cytogenetic sensitivity to diepoxybutane in lymphocytes from butadiene monomer production workers ☆

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Abstract

The magnitude of health risks to workers associated with current and past exposures to butadiene has been the subject of considerable recent debate. Butadiene is metabolized in-vivo and in-vitro to the genotoxic intermediates 3,4-epoxybutene and diepoxybutane. Studies in animals and in in-vitro systems have clearly demonstrated that 1,3-butadiene is a genotoxin and a potent inducer of sister-chromatid exchanges (SCEs). Data on the genotoxicity of butadiene in humans is, however, limited. Epidemiologic data indicate that butadiene is a probable human carcinogen. Recent work has further demonstrated that cultured lymphocytes from the approximately 20% of the Caucasian population that lack the glutathione S-transferase class theta gene (*GSTT1*) are relatively sensitive to the induction of cytogenetic damage by butadiene metabolites. In order to test whether butadiene exposure was associated with increases in SCE frequencies in peripheral blood lymphocytes and whether any increase observed could be affected by the DEB sensitivity-*GSTT1* deletion, we studied 40 workers employed in the production of butadiene. In these workers baseline frequencies of SCEs, diepoxybutane-induced SCE frequencies and *GSTT1* deletion status were assessed. Questionnaires were administered to each worker and exposure to 1,3-butadiene was determined using three separate approaches. Industrial hygiene personal sampling was used to measure breathing zone butadiene exposure and urine was collected to use in measurement of the urinary butadiene metabolite 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*-)-butane (M1). Exposure to butadiene was generally below 2 ppm. The urinary metabolite M1 was found

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in all workers, but it did not correlate significantly with exposure. Six of 40 of the workers were GST theta-deleted DEB sensitive. No measure of acute or chronic exposure to butadiene was associated with an increase in SCE frequency. However, smoking and DEB sensitivity-*GSTT1* null status were each significantly associated with elevations in baseline SCE frequency.

1. Introduction

In 1987, 1,3-butadiene production was approximately 2.7 billion pounds in the United States with 11.9 billion pounds produced worldwide (American Chemical Society, 1987). Most of the world's 1,3-butadiene is made as a by-product of ethylene production. In the US there are 11 facilities involved in the extraction of 1,3-butadiene from mixed butenes. These facilities handle butadiene primarily as a liquefied, compressed gas because of its flammability. It has been estimated that there are 3050 to 4200 workers exposed to 1,3-butadiene in these plants (Morrow, 1990).

1,3-butadiene has been shown to be a potent carcinogen in animals, inducing cancers at multiple sites in both rats and mice (Melnick et al., 1990; Owen and Glaister, 1990). Inhalation exposure in mice has induced the formation of malignant lymphomas, hemangiosarcomas, bronchoalveolar cell lung cancers, breast cancers, and ovarian, stomach and hepatocellular carcinomas. At inhalation doses as low as 6.25 ppm, significant induction of malignancies have been observed in mice (Melnick et al., 1993). Much higher doses of 1,3-butadiene are needed to induce neoplastic disease in rats, compared to mice, and the target organs are somewhat different in rats.

Epidemiologic studies of 1,3-butadiene exposure have also suggested that it is a human carcinogen. A large study of styrene-1,3-butadiene rubber workers has reported increases in lymphatic and hematopoetic cancers (Matanoski and Schwartz, 1987; Matanoski, 1990). In addition, recent studies of butadiene monomer production workers have also shown an elevated mortality from lymphoma, and for lymphatic and hematopoetic neoplasms (Meinhardt et al., 1982; Downs et al., 1987; Devine, 1990).

Butadiene has been shown to be a genotoxin and a potent inducer of sister-chromatid exchange (SCE) in-vivo and in-vitro (Cunningham et al., 1986; Sharief et al., 1986; Shelby, 1990). Inhalation exposure induced significant increases in SCEs in mouse bone marrow at a dose of 6.25 ppm. (Shelby, 1990). The reason that butadiene may be such a potent inducer of cytogenetic damage may be attributable to its metabolism to highly reactive epoxides. Butadiene is metabolized to 3,4-epoxybutene and diepoxybutane (DEB) (Malvoisin and Roberfoid, 1979; Bolt et al., 1983). The bifunctional DNA alkylating agent diepoxybutane is at least a 50-fold more potent inducer of SCE compared to the monofunctional DNA alkylating agent monoepoxybutane (Sasiadek et al., 1991). These metabolites are well known DNA alkylating agents and potent mutagens (Kolmark and Kilbey, 1968; Olszewska and Kilbey, 1975; Perry and Evans, 1975; Wade et al., 1979; Dean and Hodson-Walker, 1979; Voegel et al., 1981). Interestingly, the population distribution of induced cytogenetic damage in human lymphocytes by one metabolite of butadiene, DEB, has recently been shown to be bimodal, and to reliably predict as much as 50% of the variation in background, 'baseline' SCE frequencies (Kelsey et al., 1991a; Wiencke et al., 1991a; Wiencke et al., 1991b; Wiencke and Kelsey, 1994). Approximately 20% of the Caucasian population is relatively sensitive to the cytogenetic effects of butadiene when this agent is added to lymphocyte cultures in-vitro (Kelsey et al., 1991a; Wiencke et al., 1991a; Wiencke et al., 1991b; Wiencke and Kelsey, 1994). This cytogenetic response is also inherited (Kelsey et al., 1991b), and has recently been shown to be attributable to a novel polymorphism in the glutathione S-transferase class theta (Wiencke et al., 1995). Recent work has also suggested that individuals sensitive to DEB who lack the *GSTT1* gene that codes for the GST theta may also be sensitive to 3,4-epoxybutene (Wiencke and Kelsey, 1994).

Importantly, there is a paucity of data on the genotoxicity in butadiene in humans. One recent study suggests that low-level exposure in production

plants is not associated with the induction of chromosome abnormalities (Sorsa et al., 1994). However, Ward et al. have reported data indicating that low levels of butadiene may pose a significant mutagenic risk to humans, inducing an elevated *hprt* variant frequency in lymphocytes from exposed workers (Ward et al., 1995). Recent work also reported a non-significant increase in chromosome aberration frequency in butadiene-exposed workers (Au et al., 1995). In order to determine if butadiene induces cytogenetic damage in lymphocytes of exposed workers, and to further investigate if individuals who are *GSTT1*-deleted and sensitive to DEB are at an increased risk of cytogenetic damage induction by occupational exposure to this compound, we studied 40 workers employed in the production of butadiene monomer, measuring exposure using 8 h TWA personal sampling and urinary metabolite measures over 2 shifts in a monomer production facility.

2. Materials and methods

2.1. Study population

All 46 of the production workers in a large butadiene monomer production facility who were members of the Oil, Chemical, and Atomic Workers Union were invited to participate in the study. The workers volunteered for the study and were given an interviewer-administered questionnaire that included an occupational history, medical history, history of medication use and questions concerning smoking status, coffee consumption, and drug and alcohol use. The questionnaire was reviewed at the time of blood draw. All of the workers were afebrile and had no history of recent viral illness at the time of study. Each worker wore personal sampling pumps for the entire shift prior to blood draw. They also donated a urine specimen for analysis of urinary metabolites of butadiene immediately after the cessation of their shift.

2.2. Butadiene air sampling

Workshift exposure to butadiene via inhalation was measured using charcoal tubes and small air pumps. Ambient butadiene was collected by personal sampling through the full work shift on charcoal tubes, using a method previously described in detail (Fajen et al., 1990). Briefly, samples were collected on tandem charcoal tubes with a forward 400-mg tube acting as the primary collection medium. The backup 200-mg tube acted to quantify any breakthrough that might occur. Samples were collected with low flow pumps. Two separate pumps and collection tubes were worn during the shift by several workers to compare the results of side by side collection. These samples were coded and blindly analyzed in concert with all of the other samples.

The samples were desorbed in methylene chloride and analyzed by the NIOSH approved method (method 1024) in accordance with the NIOSH Quality Assurance and Quality Control procedures (Lunsford et al., 1984, 1987; NIOSH, 1984). The gas chromatograph was a Hewlett Packard 5890A equipped with a flame ionization detector, the chromatographic column was a Supelcowax 10 Fused Silica Capillary Column, 60 m × 0.25 mm I.D. with 0.25 μm film thickness. The injector and detector temperatures were both 250°C. The initial oven temperature was 35°C for 3 min, followed by temperature ramp of 5°C/min to 60°C. This temperature was held for 0.5 min, followed by ramp of 20°C to 200°C. The column head pressure was 110 Kpa, with nitrogen carrier. Injections were split with a split vent flow of 20 cm^3/min. Injection volumes were 3 μl.

The accuracy of the method was examined by blind analysis of adsorbant tubes spiked with butadiene. Twenty adsorbant tubes were spiked with between 3 and 200 μg butadiene by procedures described in NIOSH method 1024, and analyzed as described above. The method proved accurate to within 4% (data not shown).

2.3. Butadiene urine metabolite determination

Urine samples were analyzed for M1 by first precipitating the analyte with $Ba(OH)_2$, followed by analysis using multidimensional gas chromatography–mass spectroscopy. Specifically, one ml of human urine (with 1.0 perdeuterated M1/M2 analogues in rat urine added as internal standards) was combined with an equal volume of 10 mM barium hydroxide octahydrate (Aldrich) in Milli-Q water.

The solution was mixed and centrifuged at 1000 rpm for 20 min. The resulting pellet was extracted 3 × 1 ml volumes of methanol. The methanol was combined and dried under N_2 gas. Samples were derivatized with 1:1 mixture of bis(Trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-Pierce, Rockford, IL) in acetonitrile (Burdick and Jackson) at 60°C for 30 min.

Samples were analyzed in two dimensions using a Hewlett Packard 5890/5979B GC/MSD modified with an interface capable of column switching with in-line cryofocusing (Bechtold et al., 1994). Five μl injections of the sample were made onto a 15 m × 0.53 mm I.D. Restek Rtx-1 (1.0 μm film) column. Peaks were captured onto a liquid nitrogen cooled loop, thermally desorbed, and analyzed on Restek Rtx-200 30 m × 0.25 mm I.D. (0.25 μm film) capillary column. The initial oven temperature of 100°C was held for 1 min followed by an increase of 20°C/min to 300°C with a hold of 1 min. Peaks eluting between 10.2 and 12 min were captured from the first column. The oven temperature was decreased at a rate of 50°C/min to 100°C. After desorption of captured peaks onto the analytical column, the temperature was increased at 20°C/min to 300°C. Flow rates were approximately 15 ml/min through the first column and 1 ml/min through the second. The MSD was operated in selected ion monitoring mode acquiring ions 129, 228, 377, and 452 amu for the analyte and ion 132,232,382, and 457 for the deuterated analog. The M1 eluted from the second column at 33.4 min.

2.4. Sister-chromatid exchange

Although blood was drawn from 46 workers, only 40 cytogenetic preparations were suitable for analysis.

Venous blood was drawn from donors into sodium-heparinized Vacutainers. For cell cultures, 0.5 ml of whole blood was added to a final volume of 5 ml of RPMI 1640 tissue culture medium containing 10% fetal calf serum, 0.1 ml of phyto-hemagglutinin (PHA-M) (Difco Laboratories, Detroit, MI), penicillin (100 units/ml), and streptomycin (100 μg/ml), in 1-oz glass prescription bottles. Lymphocytes were treated with DEB ((±)-1,3-butadiene diepoxide, Aldrich, Milwaukee, WI) at 21 h of culture. DEB was diluted in sterile water and a fresh stock solution was prepared for each experiment. At 24 h of culture, 50 μM bromodeoxyuridine (BrdUrd) was added to each culture. Cells were cultured for 72 h at 37.5°C in 5% CO_2 with 98% relative humidity. 2 h before fixation, colcemid (2 × 0–7 M, final concentration; Ciba Pharmaceuticals, Summit, NJ) was added. Cells collected by centrifugation were exposed for 8 min to 0.075 M KCl at 37°C to spread the chromosomes and fixed 3 times in methanol/acetic acid (3:1). The resulting suspension was dropped onto microscope slides and differentially stained by a modification of the fluorescence-plus-Giemsa technique (Perry and Wolff, 1974). The slides were immersed for 15 min in a solution of 5 μg Hoechst 33258 (Riedel-De Haen AG, Hannover, Germany) per ml in Sorensen's buffer, pH 6.8, then washed, dried, mounted with buffer under the coverslip, and exposed for 8 min to black light 2 cm from 2 BLB GE tubes at 55°C. The slides were then stained for 4 min in a 3% Giemsa solution made in the same Sorensen's buffer. To estimate baseline SCE frequencies, 50 2nd-division metaphases were scored per point; for DEB-treated cultures 30 2nd-division metaphases were scored.

2.5. Statistical analysis

Analysis of the sources of variation in SCE means were examined using an analysis of variance. Independent variables, as indicated, were included in the analysis to determine if they were significantly associated with changes in baseline SCE (the dependent variable). DEB-induced SCE frequency was also included in the model, both as a dichotomous variable ('sensitive' or 'resistant') and as the integer SCE value. Similar analyses were performed on high SCE frequency cells (HFCs, the mean of the highest 5 cells).

3. Results

The demographic characteristics of the study population are shown in Table 1. Only 4 of the workers were smokers at the time of the study. They had a mean of 18.6 years in the trade, and 11.0 years in the plant studied. Personal breathing zone air sampling

Table 1
Demographic characteristics of the study population

All plant employees, $n = 46$ (8 women, 38 men)	
Current smokers	$n = 4$
Mean age (±S.D.) in years	41.5±9
Range	24–60
Mean years in the trade (±S.D.)	18.6±9
Range	4–40
Mean years in the plant (±S.D.)	11.0±12
Range	1–40
Current coffee use	$n = 36$
Current alcohol use	$n = 27$

was done on all of the workers. Eleven workers wore two sets of sampling equipment. As is seen in Table 2, the mean butadiene exposure level was well below 1 ppm. The range of the samples was from non-detectable (19 workers) to a maximum of 8.53 ppm. The duplicate sample for the 8.53 ppm measurement was 1.76 ppm, the maximal level observed in the tandem sampling. When the data from the two separate samplings was compared (including the maximally discrepant measurement), the Pearson's correlation coefficient was 0.86 ($p < 0.001$).

Urine was collected and analyzed for the presence of 1,2-dihydroxy-4-(*N*-acetylcysteine-*S*-)-butane (M1). The mean value of M1 per mg creatinine was 1206.6 ± 2604.1. The median was 483. The values of M1 ranges from 119.6 to 16708.7. M1 was consistently measured in urine samples from all individuals studied, including all of the workers with non-detectable exposure, measured in the breathing zone.

In the 40 workers where SCE could be analyzed,

Table 2
Personal monitoring data from concurrent breathing zone samplings of butadiene in monomer production workers

Personal sampling 1, $n = 44$ workers monitored	
Arithmetic mean	0.22±0.38 ppm
Range	< 0.02–1.76 ppm
No. of samples at or below the limit of detection (0.02 ppm) = 19	
Personal sampling 2, $n = 13$	
Arithmetic mean	1.04±2.28 ppm
Range	< 0.02–8.53 ppm
No. of samples at or below the limit of detection (0.02 ppm) = 4	

Pearson's correlation coefficient for association of the two samplings = 0.86; $p < 0.001$.

Table 3
Analysis of variance for possible determinants of SCE frequency in butadiene-exposed workers

Source of variation	df	*F* Statistic	*P*
Dependent variable-baseline SCE frequency			
Smoking	1	15.2	0.001
DEB-induced SCE	1	6.8	0.01
TWA butadiene exposure	1	1.1	0.31
Dependent variable-high SCE frequency cells (HFCs)			
Smoking	1	15.5	0.0004
TWA butadiene exposure	1	2.4	0.13
DEB-induced SCE	1	1.9	0.18
Non-significant sources of variation (for both analyses)			
Coffee consumption			
Alcohol consumption			
Years in trade			
Years in plant			

34 were relatively 'resistant' to induction of SCEs by DEB, while 6 were relatively 'sensitive'. The median value of SCE in the lower mode of the distribution was 67 SCE per cell. The median SCE frequency in the upper mode was 102 SCE per cell. Thus, 15% of this population was DEB sensitive. All DEB-sensitive individuals were *GSTT1* null.

As previously reported, urinary excretion of M1 correlated with butadiene exposure (Bechtold et al., 1994). However, there was no association between the SCE frequency in lymphocytes of the workers and either the 8 h TWA measured ambient exposure to butadiene or the urinary excretion of M1 (Table 3). Analysis of variance (ANOVA) was done with baseline SCE frequency as the dependent variable and including DEB sensitivity (measured as a dichotomous variable or as induced SCEs per cell) cigarette smoking status (current, ex- and never), coffee consumption (cups per day) and alcohol consumption (drinks per week), as well as either the 8 h TWA butadiene exposure measurement or the urinary excretion of M1 (ng/mg creatinine) as independent variables. In this analysis only cigarette smoking status and DEB sensitivity significantly predicted baseline SCE frequency. Since previous studies have suggested that high SCE frequency cells (HFCs) may reflect the cumulative effects of exposure over longer time periods, the ANOVA was repeated using HFCs as the dependent variable (Table 3). In this analysis only cigarette smoking was significantly associated with the mean HFC frequency. We also tested for

interaction between DEB sensitivity and exposure in this model, since we hypothesized that individuals who were DEB-sensitive might also be more susceptible to SCE induction by exposure to butadiene. No significant interaction was found.

Since the measured exposure pattern might not accurately reflect historical patterns of exposure within this group of workers we also attempted to model historical exposure to determine if this was significantly associated with baseline SCE frequency. To address this, additional models were constructed using tenure in the trade (years), tenure in the plant (years) or exposure group (based upon historical industrial hygiene data—both area and personal samples over approximately 10 years—and assessments by company industrial hygiene personal blinded to the current 8 h TWA measurement data and the SCE data) as independent predictor variable. These models also showed no association between any of these measures of exposure and SCE frequency in peripheral blood lymphocytes. Again, no significant interaction between DEB sensitivity and these measures of cumulative exposure was observed.

4. Discussion

Recent reviews of the health hazards of exposure to butadiene have stressed the importance of gaining further understanding of the mechanism by which butadiene induces genetic damage in humans (Heseltine et al., 1994). It is clear that occupational exposure to butadiene is strongly associated with a carcinogenic risk (Heseltine et al., 1994). However, we were unable to demonstrate any effect of butadiene exposure on the induction of cytogenetic damage. This may have been attributable to the current low level of exposure at this facility or to the lack of a comparable comparison group. Our findings are consistent with those reported by Sorsa et al. (1994) where exposure to butadiene in the production process was not associated with cytogenetic alterations. It also remains unclear if the DEB sensitivity trait confers a susceptibility to the induction of chromosomal damage by butadiene. Our study of only six sensitive workers exposed to very low levels of butadiene has little power to address this question.

It is interesting to note, however, that the one previous pilot study of butadiene monomer production workers was carried out in the same facility and reported a significant induction of *hprt* variants in peripheral blood from the same workers that we have studied (Ward et al., 1992). Of the workers studied previously, only one was *GSTT1* null and DEB-sensitive. This worker had the highest *hprt* variant frequency; this may have been due to higher levels of exposure (his level of M1 metabolite was also the highest in the group), or it could also be related to the intrinsic sensitivity of his cells to DEB. Further work will be necessary to determine if the DEB sensitivity trait is related to sensitivity to the induction of cytogenetic damage from butadiene.

In addition, since previous work in this plant has suggested that low levels of butadiene exposure are associated with the induction of genetic damage, it is important to continue to study this question in this, as well as other, butadiene-exposed populations. It is possible that low-level exposure to butadiene is capable of inducing detectable *hprt* variants, but not sister-chromatid exchanges. However, if the genotoxic metabolite is the mono- or di-epoxide, it seems likely that acute exposure to these compounds would result in efficient induction of SCEs. Hence, it may be that the *hprt* assay is detecting the mutagenic consequences of lesions in lymphocytes exposed to butadiene in the relatively distant past. In this case, DNA repair may have occurred prior to blood draw, leaving detectable mutations, but no DNA lesions capable of inducing SCEs. It is possible that further studies will clarify this situation.

References

American Chemical Society., Facts and Figures for the Chemical Industry, Chem. Eng. News, June 8 (1987) 27.

Au, W.W., Bechtold, W.E., Whorton, E.B., Jr. and Legator, M.S., Chromosome aberrations and response to gamma-ray challenge in lyphocytes of workers exposed to 1,3-butadiene, Mutation Res,. 334 (1995) 125–130.

Bechtold, W.E., Strunk, M.R., Chang, I.Y., Ward, J.B. and Henderson, R.F., Species differences in urinary butadiene metabolites: Comparisons of metabolite ratios between mice, rats, and humans, Toxicol. Appl. Pharmacol., 127 (1994) 44–49.

Bolt, H.M., Schmidel, G., Filser, F.G., Rolzhanser, H.P., Lieger, K., Wistuba, D. and Schurig, V., Biologic activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolite by exposed rats, J. Cancer Res. Clin. Oncol., 166 (1983) 112–116.

Cunningham, M.J., Choy, W.N., Arce, G.T., Rickard, L.B., Viachos, D.A., Kinney, L.A. and Sarrif, A.M., In vivo sister-chromatid exchange and micronucleus induction studies with 1,3-butadiene in B6C3F$_1$ mice and Sprague–Dawley rats, Mutagenesis, 1 (1986) 449–452.

Dean, B.J. and Hodson-Walker, G., An in vitro chromosome assay using cultured rat-liver cells, Mutat Res., 64 (1979) 329–337.

Devine, B.J., An update on mortality among workers at a 1,3-butadiene facility—preliminary results, Environ. Health Perspect., 86 (1990) 119–128.

Downs, T.D., Crane, M.M. and Kim, K.W., Mortality among workers at a 1,3-butadiene facility, Am. J. Ind. Med., 12 (1987) 311–329.

Fajen, J.M., Roberts, D.R., Ungers, L.J. and Krishnan, E.R., Occupational exposure of workers to 1,3-butadiene, Environ. Health Perspect., 86 (1990) 11–18.

Heseltine, E., Peltonen, K., Sorsa, M. and Vainio, H., Assessment of Health Hazards of 1,3-Butadiene and Styrene, J. Occup. Med., 35 (1994) 1089–1095.

Kelsey, K.T., Christiani, D.C. and Wiencke, J.K., Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. II. Relationship to baseline SCE frequency, Mutation Res., 248 (1991) 27–33.

Kelsey, K.T., Hirsch, B. and Wiencke, J.K., Sensitivity to cytogenetic damage induced by diepoxybutane is bimodal in humans and is associated with baseline SCEs and familial factors, Environ. Mol. Mutagen., 17 (suppl. 19) (1991) 35.

Kolmark, H.G. and Kilbey, B.J., Kinetic studies of mutation induction by epoxides in *Neurospora crassa*, Mol. Gen. Genet., 101 (1968) 89–98.

Lunsford, R., Gagnon, Y. and Palassis, J., 1,3-Butadiene (Method 1024) (1984, 1987) In: NIOSH Manual of Analytical Methods, 3rd ed., NIOSH, Cincinnati, OH, 1984, Second Supplement.

Malvoisin, E. and Roberfoid, M., Hepatic microsomal metabolism of 1,3-butadiene, Xenobiotica, 12 (1979) 137–144.

Matanoski, G.M., Mortality of a cohort of workers in the styrene-1,3-butadiene polymer manufacturing industry, (1948–1982), Environ. Health Perspect., 86 (1990) 107–112.

Matanoski, G.M. and Schwartz, L., Mortality of workers in styrene–butadiene polymer production, J.Occup. Med., 29 (1987) 675–680.

Meinhardt, T.J., Lemen, R.A., Crandell, M.J. and Young, R.J., Environmental epidemiologic investigation of the styrene-1,3-butadiene rubber industry—mortality problems with discussion of the hematopoietic and lymphatic malignancies, Scan. J. Work Environ. Health. 8 (1982) 2250–2259.

Melnick, R.L., Huff, J.E., Roycroft, J.H., Chow, B.J. and Miller, R.A., Inhalation Toxicology and Carcinogenicity of 1,3-butadiene in B6C3F mice following 65 weeks of exposure, Environ, Health Perspect., 86 (1990) 27–36.

Melnick, R.L., Shackelford, C.C. and Huff, J., Carcinogenicity of 1,3-butadiene, Environ. Health Perspect., 100 (1993)227–236.

Morrow, N.L., Industrial production and use of 1,3-butadiene, Environ. Health Perspect. 86 (1990) 7–8.

NIOSH, DPSE Laboratory Operating Procedures: Version 1.1 Alice C, Hamilton Laboratory, NIOSH, Cincinnati, OH, 1984.

Olszewska, E. and Kilbey, B.J., The mutagenic activity of diepoxybutane in yeast, Mutat, Res., 33 (1975) 383–390.

Owen, P.C. and Glaister, J.R., Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague–Dawley rats, Environ. Health Perspect., 86 (1990) 19–25.

Perry, P. and Evans, H.J., Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange, Nature, 258 (1975) 121–125.

Perry, P. and Wolff, S., New Giemsa method for the differential staining of sister chromatids, Nature, 251 (1974) 156–158.

Sasiadek, M., Norppa, H. and Sorsa M., 1,3-Butadiene and its epoxides induce sister-chromatid exchanges in human lymphocytes in vitro, Mutation Res., 261 (1991) 117–121.

Sharief, Y., Brown, A.M., Backer, L.C., Campbell, J.A., Westbrook-Collins, B., Stead, A.G. and Allen, J.A., Sister chromatid exchange and chromosomal aberration analysis in mice after in vivo exposure to acrylonitrile, styrene or butadiene epoxide, Environ. Mutagen., 8 (1986) 439–448.

Shelby, M.D., Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene and chloroprene, Environ. Health Perspect., 86 (1990) 71–73.

Sorsa, M., Autio, K., Demopoulos, N.A., Jarventaus, H., Rossner, P., Sram, R.J., Stephanou, G. and Vlachodimitropoulos, D., Human cytogenetic biomonitoring of occupational exposure to 1,3-butadiene, Mutation Res., 309 (1994) 321–326.

Voegel, C.E., van der Stel, I.J. and Jacobs, J.J., The mutagenic action of aliphatic epoxides, Mutation Res., 89 (1981) 269–282.

Wade, M.J., Moyer, J.W. and Hine, C.H., Mutagenic action of a series of epoxides, Mutation Res., 66 (1979) 367–371.

Ward, J.B., Jr., Ammenheuser, M.M. and Legator, M.S., Carcinogenicity of 1,3-butadiene, Lancet, 340 (1992) 1104.

Ward, J.B., Ammenheuser, M.M., Bechtold, W.E., Whorton, E.B. and Legator, M.S., Biological monitoring of workers exposed to 1,3-butadiene for mutation at the hprt locus in lymphocytes, Environ. Mol. Mutagen., 25 (1995) 56.

Wiencke, J.K. and Kelsey, K.T., Susceptibility to induction of chromosome damage by metabolites of 1,3-butadiene and its relationship to 'spontaneous' sister chromatid exchange frequencies in human lymphocytes, in: M. Sorsa, K. Peltonen, H. Vanio and K. Hemminki (Eds.), Butadiene Styrene: Assessment of Health Hazards, IARC Scientific Publication, 127, Lyon, 1994, 265–273.

Wiencke, J.K., Christiani, D.C. and Kelsey, K.T., Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes, I. Correlation with chromosomal aberrations, Mutation Res., 248 (1991) 17–26.

Wiencke, J.K., Wrensch, M.R., Maeke, R. and Petrakis, N.L., Individual susceptibility to induced chromosomal damage and its implications for detecting genotoxic exposures in human populations, Cancer Res., 51 (1991) 5266–5269.

Wiencke, J.K., Pemble, S., Ketterer, B. and Kelsey, K.T., Gene deletion of glutathione S-transferase Θ: Correlation with induced genetic damage and potential role in endogenous mutagenesis, Cancer Epidemiol., Biomarkers Prevention, 4 (1995) 253–260.