Biomonitoring of 1,3-Butadiene and Related Compounds

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The 1990 Clean Air Act Amendments list several volatile organic chemicals as hazardous air pollutants, including ethylene oxide, butadiene, styrene, and acrylonitrile. The toxicology of many of these compounds shares several common elements such as carcinogenicity in laboratory animals, genotoxicity of the epoxide intermediates, involvement of cytochrome P450 for metabolic activation (except ethylene oxide), and involvement of at least two enzymes for detoxication of the epoxides (e.g., hydrolysis or conjugation with glutathione). These similarities facilitate research strategies for identifying and developing biomarkers of exposure. This article reviews the current knowledge about biomarkers of butadiene. Butadiene is carcinogenic in mice and rats, which raises concern for potential carcinogenicity in humans. Butadiene is metabolized to DNA-reactive metabolites, including 1,2-epoxy-3-butene and diepoxybutane. These epoxides are thought to play a critical role in butadiene carcinogenicity. Butadiene and some of its metabolites (e.g., epoxynbutene) are volatile. Exhalation of unchanged butadiene and excretion of butadiene metabolites in urine represent major routes of elimination. Therefore, biomonitoring of butadiene exposure could be based on chemical analysis of butadiene in exhaled breath, blood levels of butadiene epoxides, excretion of butadiene metabolites in urine, or adducts of butadiene epoxides with DNA or blood proteins. Mutation induction in specific genes (e.g., HPRT) following butadiene exposure can be potentially used as a biomarker. Excretion of 1,2-dihydroxy-4-(N-acetylcysteiny1)-Sibutane or the product of epoxybutene with N-7 in guanine in urine, epoxybutene-hemoglobin adducts, and HPRT mutation have been used as biomarkers in recent studies of occupational exposure to butadiene. Data in laboratory animals suggest that diepoxybutane may be a more important genotoxic metabolite than epoxybutene. Biomonitoring methods need to be developed for diepoxybutane and other putative reactive butadiene metabolites. With butadiene and related compounds, the ultimate challenge is to identify useful biomarkers of exposure in which quantitative linkages between exposure and internal dose of the important DNA-reactive metabolites are established. — Environ Health Perspect 104(Suppl 5):907–915 (1996)

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Introduction

Human epidemiological studies of butadiene can benefit from data on biomarkers of this compound, particularly in the area of biomarkers of exposure. Biomonitoring of exposure to butadiene and related air toxics of low molecular weight could potentially be based on chemical analysis of exhaled breath, including parent chemical and epoxide metabolites; blood levels of parent compound and metabolites (e.g., epoxides; metabolites in excreta (e.g., mercapturic acids in urine); adducts of reactive intermediates (e.g., epoxides) with DNA or blood proteins; DNA adducts in urine; or analysis of a biological end point such as mutation induction in specific genes (e.g., HPRT). This article reviews the current knowledge about biomarkers of butadiene, discusses the applicability of biomarkers in field studies of this compound, and defines specific research directions for expanded biomarker use. Some of the advantages and limitations in biomarker studies of alkylating low-molecular weight agents are addressed.

1,3-Butadiene: Background

1,3-Butadiene is a flammable, colorless gas with a mildly aromatic odor. The boiling point of butadiene at 1 atmosphere is −4.4°C, and the vapor pressure is 2100 mm Hg at 25°C. Butadiene is used in the production of resins and plastics, including butadiene rubber, styrene rubber, adiponitrile, polychloroprene, nitrile rubber, styrene-butadiene latex, and acrylonitrile-butadiene-styrene (1). The butadiene used to manufacture man-made rubber is primarily for the production of automobile tires. In 1994, butadiene ranked in the top 20 of synthetic organic chemicals produced in the United States, with an annual production over 3 billion pounds (2). Butadiene is listed as one of 189 hazardous air pollutants under the 1990 Clean Air Act Amendments (3).

Sources of exposure to butadiene include cigarette smoke and emissions from butadiene production, storage, transport, and end use. The most likely mode of exposure to butadiene occurs in the industrial setting or in the environment, where inhalation of butadiene vapor is the route of uptake. Few data exist on concentrations of butadiene in ambient air. Concentrations of butadiene in urban air have been reported to range from 1 to 10 ppb (4,5). A mean concentration of 1.39 µg butadiene/m³ (range 0.11–6.94 µg/m³ or 0.62 ppb, range 0.05–3.1 ppb) was reported for 24 hr ambient air samples taken in 19 U.S. cities in 1987 to 1988 (6). Studies in Sweden on urban air pollution show an average level of 0.3 ppb butadiene (7). The U.S. Environmental Protection Agency (U.S. EPA) estimated that butadiene is emitted in automobile exhaust at 8.9 to 9.9 mg/mile (5.6–6.1 mg/km) and makes up roughly 0.85% of total hydrocarbons in exhaust emissions (8). Neligan (4) reported a concentration of butadiene in automobile exhaust of 20 to 60 ppb. Löffroth et al. (9) reported 0.4 mg butadiene/cigarette in sidestream smoke, and levels in smoky indoor environments were reported to range from 10 to 20 µg/m³ (4.5–8.9 ppb). Bruunemann et al. (10) reported an average

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Abbreviations used: GSH, glutathione; GC, gas chromatography; HPLC, high-performance liquid chromatography; M1, 1,2-dihydroxy-4-(N-acetylcysteiny1)-Sibutane; M2, 1-hydroxy-2-(N-acetylcysteiny1)-Sibutane; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PFPTH, pentfluorophenylthiodyantoin.
amount of butadiene in sidestream cigarette smoke of 205 to 361 µg/cigarette and 16 to 75 µg/cigarette in mainstream smoke. Typical occupational levels of butadiene are less than 2 ppm (11).

The carcinogenicity of butadiene in rodents is well established. Although both sexes of B6C3F1 mice and Sprague-Dawley rats developed tumors in 2-year inhalation rodent bioassays (12–14), the most striking aspect of butadiene-induced carcinogenicity in rodents is the high sensitivity of mice compared with rats. Rats developed tumors from exposures to butadiene concentrations (1000–8000 ppm) as much as three orders of magnitude higher than those that caused cancer in mice (6.25–1250 ppm). Furthermore, tumor sites differed in rats and mice. The carcinogenicity data for rats and mice were used for a butadiene risk assessment by the U.S. Occupational Safety and Health Administration (15). For the most part, risk estimates of cancer deaths for workers exposed to 10 ppm butadiene were higher when based on mouse tumor incidence data compared to estimates derived from rat tumor data.

The genotoxicity of butadiene has been studied using a variety of in vitro and in vivo mutagenicity assays [see review by de Meester (16)]. For example, butadiene displayed mutagenic activity in bacteria (Ames test), but only in the presence of liver S9 fractions (17). Butadiene causes genetic damage in bone marrow cells and peripheral blood lymphocytes in B6C3F1 mice (18) but not in Sprague-Dawley rats (19,20), observations that correlate with species differences in carcinogenicity.

The butadiene metabolites epoxycarputene and diepoxycarputene are also carcinogenic and genotoxic in vivo (21–25). Diepoxycarputene is a more potent carcinogen in mice than epoxycarputene (21) and is nearly 100 times more mutagenic on a molar basis than epoxycarputene in mammalian systems (26). Diepoxycarputene also induces genetic damage in vitro in mammalian cells (Chinese hamster ovary cells and human peripheral blood lymphocytes) at lower concentrations than epoxycarputene (27,28).

**Metabolism of 1,3-Butadiene**

Knowledge of quantitative differences in butadiene metabolism among species is critical because metabolism is likely to be a key factor for butadiene carcinogenicity. Some pathways of butadiene metabolism are illustrated in Figure 1. Three enzymes appear to play major roles in the overall metabolism of butadiene: cytochrome P450 monoxygenase, epoxide hydrolase, and glutathione (GSH) S-transferase. The metabolic activation of butadiene proceeds by cytochrome P450-mediated oxidation to epoxycarputene (29–33). Cytochrome P4502E1 is the major P450 isozyme for the metabolism of butadiene (32), although P4502A6 can also oxidize butadiene to epoxycarputene (34). Epoxycarputene is metabolized further by cytochrome P450 to diepoxycarputene, and recent studies indicate that both P4502E1 and P4503A4 catalyze this oxidation step (35). Epoxide hydrolase and GSH S-transferase are the two enzymes responsible for the metabolic inactivation of epoxycarputene (32,36–38) and diepoxycarputene (39,40).

Significant species differences exist in metabolism of butadiene [reviewed in Himmelstein et al. (41)]. Comparison of butadiene metabolism in liver and lung microsomes indicates that the rate of metabolism is greater in mice compared with rats and humans (mice > rats ≈ humans) (32). Additionally, mice have a faster rate of GSH conjugation with diepoxycarputene in lung tissues than rats or humans, and humans have faster rates of epoxycarputene hydrolysis by epoxide hydrolase compared to rats or mice (32). The rate of cytochrome P450-mediated epoxidation of epoxycarputene to diepoxycarputene in liver microsomes is highest in mice, while rats and humans have a similar rate (35). Enzyme-mediated liver GSH conjugation with diepoxycarputene indicates that the rate of conjugation is greater in mice compared with rats and humans (mice > rats > humans) (39).

Enzyme-mediated liver hydrolysis of diepoxycarputene indicates that the rate of hydrolysis was greater in humans than in mice and rats (humans > rats > mice) (40).

Numerous in vivo data substantiate the in vitro metabolism studies showing that butadiene undergoes oxidation to epoxycarputene and that there are significant species differences in metabolism [reviewed in Himmelstein et al. (41)]. In studies by Himmelstein et al. (42,43), peak concentrations of epoxycarputene in mice compared to those in rats were 4- to 8-fold higher in blood, 13- to 15-fold higher in lung, and 5- to 8-fold higher in liver following inhalation of 62.5, 625, 1250, and 8000 ppm (rats only) ppm butadiene for up to 6 hr. The concentration of diepoxycarputene was greatest in the lungs of mice. Diepoxycarputene could not be detected in livers of mice or lungs and livers of rats. Thornton-Manning et al. (44) recently reported that concentrations of epoxycarputene were 3 to 74 times greater in tissues of mice compared with rats following exposure to 62.5 ppm butadiene for 4 hr. Levels of diepoxycarputene in blood and tissues of rats were 40- to 163-fold lower than in corresponding mouse tissues.

**1,3-Butadiene Biomarkers**

**Urinary Metabolites**

Osterman-Golkar et al. (45) investigated the excretion of epoxybutene mercapturic acid in the urine of Wistar rats exposed to butadiene concentrations of 0, 250, 500, and 1000 ppm (6 hr/day, 5 days/week for 2 weeks). The samples were analyzed using the method developed by Gérin and Tardif (46) for the mercapturic acid of ethylene.
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oxide. This method involves deacylation to give a cysteine adduct, derivatization with phthalaldialdehyde, and high-performance liquid chromatography (HPLC) with fluorescence detection. The product analyzed in samples of rat urine was identical to the main synthetic product of the reaction between epoxybutene and cysteine; the authors assumed that the product was S-(2-hydroxy-3-butenenyl)cysteine; however, the structure was not verified. The urinary concentration of this product increased proportionally with butadiene exposure concentration; levels were about 6, 13, and 18 pmol/day for 250, 500, and 1000 ppm, respectively. Analysis of other excretion products was not attempted. The authors concluded that further improvements are needed before the analytical methods can be applied to biomonitoring in humans (45).

Sabourin et al. (36) examined the urinary excretion of mercapturic acids in mice, rats, hamsters, and monkeys. All four species produced two metabolites, 1,2-dihydroxy-4-((N-acetylcysteinyl)-S)butane (M1) and 1-hydroxy-2-(N-acetylcysteinyl)-S)-butene (M2). M2 is formed by conjugation of GSH with epoxybutene, and M1 appears to be formed by GSH conjugation with butenediol, the product of hydrolysis of epoxybutene. When comparing the four species, the ratio of M1 to the sum of M1 + M2 was linearly related to hepatic epoxide hydrolase activities for each species. Bechtold et al. (38) developed an assay based on isotope-dilution gas chromatography and mass spectrometry (GC-MS) for the quantification of M1 and M2. The assay was applied to urine samples of employees at a butadiene production plant and one outside control group that had no known exposure to butadiene. This study extended the findings of Sabourin et al. (36) by showing that humans are similar to the monkey in that M1 is the predominant metabolite in urine. The findings are consistent with the higher ratio of M1/M1 + M2 in humans compared to rats or mice and the higher rate of epoxide hydrolase activity in the livers of humans compared to rats and mice (i.e., humans > rats > mice) (32). The average values of M1 for exposed, immediately exposed, nonexposed, and outside control employees were 3200 ± 1600, 1390 ± 550, 630 ± 190, and 320 ± 70 ng/ml (mean ± SE), respectively. Exposed and immediately exposed employees were exposed to 3 to 4 ppm butadiene, while nonexposed and outside control employees were exposed to <0.1 ppm butadiene. Values for exposed subjects were statistically different from the outside control group (p < 0.05). M1 was also detected in urine of outside control workers; the implications of this observation are unknown. The method of Bechtold et al. (38), although limited to assessment of recent exposure (the last day; days since last exposure), is probably the most sensitive method available at this time to detect butadiene exposure in humans.

**Hemoglobin Adducts**

The dose response for formation of adducts of epoxybutene with N-terminal valine in hemoglobin has been investigated in butadiene-exposed mice and rats (45,47,48) using the N-alkyl Edman method (49) and GC-MS or GC and MS-MS (tandem mass spectrometry) for adduct determination. Male B6C3F1 mice and Sprague-Dawley rats were exposed to 0, 2, 10, and 100 ppm butadiene (6 hr/day, 5 days/week for 4 weeks) (47). The adducts were detectable after 1 week of exposure at all butadiene exposure concentrations (unpublished observation). In these studies, adduct levels in mice increased linearly with butadiene concentration. Adduct levels were about 3 to 8 times lower in rats than in mice, and the levels in rats began to plateau above 10 ppm. Studies of epoxybutene–hemoglobin adducts in Wistar rats exposed to higher butadiene concentrations (0, 250, 500, and 1000 ppm (6 hr/day, 5 days/week for 2 weeks) showed a proportional increase in adduct levels with increasing exposure concentration (about 0.05 pmol/g globin per ppm×hr). Albrecht et al. (48) exposed mice and rats at 0, 50, 200, 500, and 1300 ppm and showed a nonlinear dose response in mice at concentrations above 200 ppm and a linear dose response for rats. The adduct data are in general agreement with concentrations of epoxybutene measured in blood and tissues that were lower in rats than in mice exposed to butadiene (42–44,50). The low epoxybutene–hemoglobin adduct levels in rats and mice suggest that some reactive metabolite other than epoxybutene, presumably diepoxbutyene, is the important genotoxic metabolite.

According to studies in experimental animals, the adducts of epoxybutene with N-terminal valine in hemoglobin are chemically stable (shown for N-(2-hydroxy-3-butenenyl)valine [(45,50]; and unpublished data). This implies that epoxybutene–hemoglobin adducts are suitable for monitoring prolonged butadiene exposures in humans.

Preliminary data on hemoglobin adduct levels in nine workers at a U.S. chemical production plant and a few outside controls were reported by Osterman-Golkar et al. (47). The workers, all nonsmokers, were divided into two groups on the basis of work location. Exposures to butadiene were estimated from an environmental sampling survey conducted between 3 and 9 months before the collection of blood samples. Increased adduct levels (1.1–2.6 pmol/g globin; estimate of adducts with both carbon 1 and carbon 2 of 1,2-epoxybutene) were recorded in workers in the butadiene production areas (n = 4). Time-weighted average 8-hr area samples showed a mean value of about 3.5 ppm, although most samples contained <1 ppm. Most of the samples with higher levels were collected in areas not frequented by workers, suggesting that the average exposure of workers to butadiene was <3.5 ppm. As control groups, five workers from two nonproduction areas—the plant (no exposure data available) and the central control area (about 0.03 ppm butadiene)—and two external controls were evaluated. Adduct levels in control samples were below the detection limit of 0.5 pmol/g globin. The authors noted that further research is needed to more clearly define a relationship between butadiene exposure and hemoglobin adduct levels.

van Sittert and van Vliet (51) compared the hemoglobin adduct level of N-(2-hydroxy-3-butenenyl)valine (formed by reaction at carbon 1 of 1,2-epoxybutene) in control and butadiene-exposed workers at a manufacturing site that produces butadiene by a naphtha cracking process. Control workers were from the same plant site but were not involved in butadiene manufacturing. The median (range) adduct levels were <2 (<1 to <4) pmol/g globin in nonsmoking (n = 17) and smoking subjects (n = 9) exposed occupationally to butadiene. Personal air samples taken during the study were generally <1 ppm (8-hr time-weighted average). The median concentrations of adducts were <4 (<1 to <7) pmol/g globin in nonsmoking control subjects (n = 15) and <3 (<2 to <8) pmol/g globin in smoking control subjects (n = 10). The method detection limit for N-(2-hydroxy-3-butenenyl)valine was 1 to 8 pmol/g globin, indicating that these adducts could not be detected in workers exposed to <1 ppm butadiene.
Blood samples from 26 employees at a butadiene manufacturing plant in Portugal were analyzed for \(N\)-(2-hydroxy-3-butenyl)valine using tandem mass spectrometry for adduct detection (52,53). The subjects included smokers and nonsmokers. Exposures to butadiene were estimated from an environmental sampling survey that was conducted in connection with the biomonitoring study (54). The adduct levels recorded in laboratory and maintenance workers (<0.1 pmol/g globin; \(n = 7\); median butadiene concentration 0.1 ppm) were not increased above the background found in the controls (<0.13 pmol/g globin, \(n = 9\)). Increased adduct levels (on average, 0.16 pmol/g globin) were recorded in workers engaged in butadiene sampling and voiding in the butadiene production areas (\(n = 10\); median butadiene concentration, 1 ppm).

Mainstream smoke contains about 0.06 mg butadiene/cigarette (range 0.016–0.075 mg butadiene/cigarette) (10). The amount of butadiene in the mainstream smoke of 30 cigarettes, about 1.8 mg, is similar to the amount (1.6 mg) that would be inhaled during 8 hr of work at an air concentration of 0.1 ppm butadiene. Thus, the contribution from smoking to the adduct level in hemoglobin of production plant workers (reported cigarette consumption 0–25 cigarettes/day) is expected to be smaller than the contribution from exposure to butadiene (median exposure level about 1 ppm).

The most sensitive analytical techniques used for adduct quantification are based on cleavage of the adduct from the protein chain (55). For low molecular weight epoxides with intermediate to high \(s\)-values (56) (e.g., ethylene oxide, epoxycyanate, and styrene oxide), the N-terminal valine in hemoglobin is an important binding site. Adducts with the N-terminal amino acid can be released by a modified Edman method (49) involving derivatization with pentafluorophenyl isothiocyanate, extraction and purification of the thiodyantoin (PFPTH) derivative of the modified amino acid, and mass spectrometric quantification. For example, a modified globin or peptide containing adducts of deuterated ethylene oxide can serve as an internal standard. The limit of detection of the N-alkyl Edman method is about 1 to 10 pmol/g globin using GC and MS and about 0.05 to 1 pmol/g globin using GC and MS-MS.

The analytical conditions are critical, and different detection limits have been achieved for the analysis of epoxycyanate adducts in different studies (47,51,52). The method used by Osterman-Golkar et al. (52) for adduct determination (derivatization of large globin samples (200–300 mg)) according to the N-alkyl Edman method and detection of the alkylvaline-PFPTH by GC and MS-MS) can be used for monitoring of exposure to butadiene at the level of parts per million or higher.

**DNA Adducts and Modified Nucleic Acid Bases in Urine**

-Methods being developed to monitor DNA adducts of low molecular weight compounds include immunological assays (57), GC-MS (58,59), HPLC with electrochemical detection (60) or fluorescence detection (61), and the \(^{32}\)P-postlabeling technique (62).

Peltonen et al. (63) investigated the potential for using modified purine bases as urinary markers of butadiene exposure. \(N\)-(2-Hydroxy-3-buten-1-yl)guanine and \(N\)-7-(1-hydroxy-3-buten-2-yl)guanine were identified in calf thymus DNA incubated with epoxycyanate and in urine of one worker exposed to butadiene. The concentration of butadiene to which the worker was exposed was not reported. The authors used HPLC with electrochemical detection; the limit of detection was 0.2 to 0.3 pmol guanine adduct. Further research is needed to correlate guanine adducts in urine with the exposure concentration of butadiene.

Sorsa et al. (53) exposed Wistar rats and CB6F1 mice to 0, 50, 200, 500, and 1300 (mice only) ppm, 6 hr/day for 5 consecutive days. The \(^{32}\)P-postlabeling technique was used for analysis of adenine-N\(^6\) adducts of epoxycyanate. DNA was digested to 3'-nucleotides; the adducts were enriched by HPLC and were then labeled. The labeled samples were purified by thin-layer chromatography, and the final analysis was based on HPLC with radioactivity detection. The adduct levels in lung samples of mice were slightly higher than the adduct levels in rats. Adduct levels in rat liver (about 2, 6, 18, and 26 fmol adduct/100 nmol 3' dAMP for 0, 50, 200, and 500 ppm, respectively) were about 10-fold higher than in rat livers. Adduct levels in mouse liver were not analyzed. The reported sensitivity of the assay was 200 to 500 attomol depending on the amount of DNA used in the analysis.

**Biological End Points**

Three human population monitoring studies for genotoxic end points in individual workers from butadiene production facilities have been reported in the literature (64–66). In a small pilot study at a butadiene production facility (64), an increase in hprt variant frequency \((HPRT \ V_f)\) was detected in workers in the high exposure group \((n = 8)\) compared to the low exposure group \((n = 5)\) and outside-facility controls \((n = 6)\). There was a correlation between an increase in the \(HPRT \ V_f\) and increased levels of a butadiene metabolite (M1) in urine. The \(HPRT \ V_f\) assay, which uses autoradiographic detection of hprt variants, was used. Therefore, the increased \(HPRT \ V_f\) cannot be confirmed as an increased frequency of in vivo \(HPRT\) mutation. Studies using the \(HPRT\) mutant T-lymphocyte cloning assay combined with T-cell receptor pattern and DNA sequence analysis (67,68) are required to confirm the observations of Ward et al. (64) on butadiene-exposed human populations.

In a separate study, Au et al. (65) reported no significant increase in chromosome aberrations in peripheral blood lymphocytes isolated from the butadiene-exposed group [individuals used for the Au et al. (65) study were the same as those reported in the Ward et al. (64) study]. Following in vitro X-ray irradiation of lymphocytes isolated from these individuals, however, the number of induced chromosomal alterations in the butadiene-exposed group was significantly increased relative to controls. According to these researchers, the increased frequency of chromosomal alterations following X-ray irradiation in the butadiene-exposed group may indicate a DNA repair deficiency in the butadiene-exposed population.

Peripheral blood lymphocytes from 40 exposed workers and 30 controls from two butadiene production plants were evaluated for chromosome damage (chromosome aberrations, micronuclei, and sister chromatid exchange in peripheral lymphocytes) (66). The ambient exposure levels of butadiene in the manufacturing plants were in the range of 1 to 3 ppm. No exposure-related increases in either of the cytogenetic end points assessed were observed in the butadiene-exposed workers.

**In Vivo Stability of Adducts**

The accumulation of stable hemoglobin adducts during prolonged exposure is the result of daily increments \((a)\) of the adduct level, and daily losses due to removal of the oldest fraction of the erythrocytes \((1/\tau_e; \tau_e\) is the erythrocyte lifespan) from circulation (70–72). After exposure for a period of
time exceeding $t_{1/2}$ (126 days in humans), a steady-state adduct level ($A_n$) is attained:

$$A_n = a \times t_{1/2}/2 = 63a$$

Thus, measurement of $A_n$ of stable adducts gives information on exposure during the months before blood sampling. Unstable adducts build up to a less extent and approach more rapidly the steady-state level (71,72).

For a serum protein or DNA adduct, the level approaches:

$$A_n = a/k$$

where $k$ includes constants for turnover of the macromolecule and instability of the adduct. Thus, the steady-state level of chemically stable serum protein adducts is approximately 30a ($k' = 0.035$ days) (73).

Several low molecular weight adducts with N-terminal valine in hemoglobin, including the adducts of epoxybutene, ethylene oxide (74), styrene oxide (75), and acrylonitrile (76), have been studied in experimental animals and appear to be chemically stable at low exposure levels. Tates (77) studied hydroxyethylvaline in hemoglobin of four workers accidentally exposed to high concentrations of ethylene oxide. The adduct levels decreased linearly over time and reached background values after approximately 110 days, as expected for chemically stable adducts.

Use of adducts for dosimetry purposes requires knowledge of the kinetics of adduct elimination. Contrary to protein adducts, DNA adducts are subjected to repair, and their stability varies considerably between type of adduct and cell type. In the absence of information on adduct stability, DNA adduct measurements give only qualitative information on exposure.

**Comparison of Adduct Formation with 1,3-Butadiene and Some Other Low Molecular Weight Compounds**

The daily adduct increment, $a$, given, for example, in pmol adduct/g hemoglobin, can be recalculated to adduct increment per ppm×hr and used as a binding index (HBI) for epoxybutene. This binding index is directly proportional to the concentration in erythrocytes integrated over time (AUC; area under the concentration curve) of the adduct-forming reactive metabolite:

$$HBI = k_{HB} \times AUC$$

The constant, $k_{HB}$, is the second-order rate constant for adduct formation in hemoglobin. $k_{HB}$ can be determined in *in vitro* experiments and, accordingly, the relationship between AUC and resulting adduct levels can be established.

Table 1 presents data on hemoglobin adduct levels from exposure to butadiene and some related compounds (styrene, ethylene, ethylene oxide, and acrylonitrile) in nonsmokers, smokers, and subjects with occupational exposure (52,53,78-82). The HBI of butadiene, on the order of $1 \times 10^{-3}$ pmol/g globin per ppm×hr, is low when compared to the value calculated for rats ($50 \times 10^{-3}$), indicating a lower overall efficiency of butadiene in humans than in rats. However, the adducts measured are those of the primary metabolite, epoxybutene. The HBI estimated for butadiene is considerably lower than the HBI for ethylene in humans.

**Background Levels: A Limitation and an Opportunity**

The possibility of detecting chemical exposures by adduct measurements is in some cases limited by the presence of high background levels. This background may be of artificial origin (i.e., introduced *in vitro* during handling of the blood sample). Calleman et al. (83) analyzed cysteine

### Table 1. Hemoglobin (N-terminal valine) adduct levels (pmol/g globin) from exposure to butadiene and some other low molecular weight compounds in referents (nonsmokers and smokers) and subjects with occupational exposure.

| Compound (reactive intermediate) | Background adduct level (average, range, or mean ± SD) | Type of exposure | Exposure concentration | Adduct level (average, range) | Binding index (pmol/g globin per ppm×hr unless otherwise stated)* | Methodology and reference |
|---------------------------------|-------------------------------------------------------|------------------|------------------------|-----------------------------|--------------------------|--------------------------|
| Butadiene (epoxybutene)         | $\leq 0.13^p$                                        | Occupational     | 0.1 (median value)     | $< 0.1^b$                   | $-0.5 \times 10^{-3}bc$  | GC/MS-MS (52,53)         |
|                                 |                                                       | Lab and maintenance work | 1 ppm (median value)    | $0.16 \leq 0.1-0.32^a$     | $-1 \times 10^{-3}c$     | GC/MS (78)               |
|                                 |                                                       | Process work      | 75 ppm (average)       | 28, 15-52                   |                          | GC/MS (78)               |
| Styrene (styrene 7.8-oxide)     | $< 10^2$                                              | Occupational     | 0.3 (0.1–1) ppm, 40 hr/week | 43, 22–65 (nonsmokers)       | 0.2 (0.0–0.06)           | GC/MS (79)               |
|                                 |                                                       | Occupational     | 1–25 cigarettes/day    | 146, 50–355                 | 9 pmol/g globin per cigarette/day | GC/MS (80)               |
|                                 |                                                       | Tobacco smoking  | $> 15$ cigarettes/day  | 361 ± 107 (smoking women; maternal blood) | 147 ± 68 (newborn babies’ blood) | GC/MS (81)               |
|                                 |                                                       | 42 ± 18 (newborn babies’ blood) |                      | 9 pmol/g globin per cigarette/day | GC/MS (82)               |
| Ethylene oxide                  | 14–26 (see also data for ethylene)                   | Occupational     | Low–28 ppm/week       | 84–2070^p                   | 9^                       | GC/MS (82)               |
| Acrylonitrile                   | $< 2$                                                  | Tobacco smoking  | 5–20 cigarettes/day    | 2.2–178                     | 9 pmol/g globin per cigarette/day | GC/MS-MS                 |

*Calculated assuming that the steady-state adduct level corresponds to exposure at work hours (8 hr/day, 5 days/week) during 9 weeks. *Only one of the two regioisomers measured. *Order of magnitude. *Current unpublished studies indicate that the background is below 0.1 pmol/g globin. *Based on data from Duus et al. (82), we estimate a steady-state adduct level of 3.4 nmol/g globin (Duus et al. (82) gives the value 2.4) for exposure to 1 ppm during work hours. *Data from Bergmark (personal communication).
adducts of ethylene oxide in globin hydrolysates and found a high background of hydroxethylcysteine when mercaptopethanol was added as an antioxidant during the hydrolysis. Metabolic or catabolic processes in blood samples in vitro may in some cases be of concern in monitoring studies because of artificial adduct formation. Törnqvist (84) demonstrated formation of hydroxyethylvaline, possibly due to ethylene production, during prolonged storage of samples of blood or erythrocytes. Immediate precipitation and storage of the globin eliminate this source of error.

Background adducts may also originate from confounding exposures to chemicals that form the same adduct as the particular compound studied, from additional sources of this compound, or possibly from incorporation of modified amino acids in the biosynthesis of globin. Examples of compounds that may introduce the hydroxyethyl adduct are ethylene and ethylene oxide, 1,2-dibromoethane, and N-halogenoethyl-N-nitrosourea. The problem is still more pronounced for mercapturic acids. N-Acetyl-(2-hydroxyethyl)cysteine is a common urinary mercapturic acid metabolite of a large number of structurally different chemicals, including acrylonitrile and vinyl chloride (85).

The study of background blood protein adducts may provide an opportunity to identify cancer initiators of endogenous origin or from widespread external sources via their in vivo products of reaction.

### Detection of Low-level Environmental Exposures

The possibility of detecting environmental exposures by adduct measurements is determined by the exposure level, the binding index (Table 1), the analytical sensitivity, the in vivo stability of the adduct, and the background adduct level in nonexposed individuals. Which one of these factors will set the limit depends, in part, on the chemical. N-terminal valine adduct increments from exposures to ethylene, butadiene, styrene, or acrylonitrile estimated for an arbitrarily chosen air level of 10 ppb are presented in Table 2 and compared to background levels and increments from cigarette smoking. The adduct increments are based on binding indexes from Table 1. The adducts are assumed to be stable. The background level of adducts (hydroxyethylvaline) from ethylene, about 20 pmol/g globin, corresponds to a tobacco use of about two cigarettes per day and hampers the detection and quantitation of additional environmental, low-level sources of ethylene. The background of acrylonitrile adducts (<2 pmol/g globin) corresponds to less than one-quarter of a cigarette per day. The potential use of this adduct to quantify exposure to environmental tobacco smoke should be investigated. In the case of butadiene and styrene, the background levels of valine adducts are not known. The limiting factors are the low binding levels of these compounds and the sensitivity of the analytical methods. At present measurements of hemoglobin adducts of styrene and butadiene are limited to the monitoring of occupational exposures.

### Future Research Opportunities

In summary, research on biomarkers of exposure to butadiene is at the early stage of development. Blood doses of the primary reactive metabolite, epoxybutene, can be measured in globin samples of humans with occupational exposure to butadiene levels of about 1 ppm or higher. Recent exposure to the same occupational exposure levels can be detected by means of mercapturic acids in the urine. Further studies are required to determine the true background levels of these biomarkers and to evaluate the impact of smoking, both as a source of exposure to butadiene as well as its possible influence on butadiene metabolism. One of the key deficiencies of this work relates to the complete absence of biomarkers of diepoxybutane, an important metabolite of butadiene. Metabolism, toxicokinetic, and genotoxicity data clearly indicate that diepoxybutane should be the focus of further biomarker research.

### Table 2. Estimates of hemoglobin adduct increments from exposures to ethylene, butadiene, styrene, or acrylonitrile, at an air level of 10 ppb compared with background levels and increments from cigarette smoking (for references, see Table 1).

| Compound | Estimated adduct increment (pmol/g globin) | Background in nonsmokers (pmol/g globin) | Detection limitb | Increment from smoking (pmol/g globin per cigarette/day) |
|----------|-------------------------------------------|------------------------------------------|-----------------|------------------------------------------------------|
| Ethylene | 2                                        | 20 (mainly of endogenous origin)         | -0.05           | 9                                                    |
| Butadiene | 0.01b                                    | Low (< 0.3)                              | -0.05           | Increment indicated in a few heavy smokers           |
| Styrene  | 0.01b                                    | Low (< 0.1)                              | -0.1            | Not analyzed                                         |
| Acrylonitrile | 20b                                     | Low (< 2)                                | 2d              | 9                                                    |

aGC/MS-MS. *Crude estimate from measurements at higher exposure levels, assuming a linear dose response. bEstimate based on data for smoking. cA considerably lower limit could probably be achieved.

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