# Hemoglobin Adducts and Sister Chromatid Exchanges in Hospital Workers Exposed to Ethylene Oxide: Effects of *Glutathione S-Transferase T1* and *M1* Genotypes

**Lee C. Yong,[1] Paul A. Schulte, John K. Wiencke, Mark F. Boeniger, L. Barbara Connally, James T. Walker, Elizabeth A. Whelan, and Elizabeth M. Ward**

Division of Surveillance, Hazard Evaluations and Field Studies [L. C. Y., M. F. B., L. B. C., J. T. W., E. A. W., E. M. W.], Education and Information Division [P. A. S.], National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226, and Laboratory for Molecular Epidemiology, Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California 94143 [J. K. W.]

## Abstract

**Ethylene oxide (EtO) is a genotoxic carcinogen with widespread uses as an industrial chemical intermediate and sterilant. We examined the effects of *glutathione S-transferase T1* (*GSTT1*) and *M1* (*GSTM1*) genotypes on the levels of *N*-(2-hydroxyethyl)valine (HEV) adducts in the erythrocytes and sister chromatid exchange (SCE) in lymphocytes from a group of 58 operators of sterilizers that used EtO and nonexposed workers from nine hospitals in the United States and one hospital in Mexico City. Cumulative exposure to EtO was estimated during the 4-month period before the collection of blood samples. Results showed that EtO exposure was significantly associated with the levels of HEV adducts and SCE after adjusting for cigarette smoking and other potential confounders. A significantly higher HEV adduct level (0.17 ± 0.03 *versus* 0.08 ± 0.01, mean ± SE; $P = 0.02$) but lower SCE frequency (5.31 ± 0.39 *versus* 6.21 ± 0.17; $P = 0.04$) was observed in subjects with homozygous deletion of the *GSTT1* gene (null genotype) as compared with those with at least one copy of the gene (positive genotype). In multiple regression analysis, the *GSTT1*-null genotype was associated with an increase in HEV adduct level ($\beta = 1.62$; $P = 0.02$) and a decrease in SCE frequency ($\beta = -1.25$; $P = 0.003$) after adjusting for age, gender, race, education, cigarette smoking, and EtO exposure status. The inverse SCE-*GSTT1* relationship remained unchanged when SCE was further examined in relation to HEV adducts as an indicator of the internal EtO dose. The *GSTM1* genotype was not associated with the level of either HEV adduct or SCE. These data indicate that the *GSTT1*-null genotype is associated with increased formation of EtO-hemoglobin adducts in relation to occupational EtO exposure, suggesting that individuals with homozygous deletion of the *GSTT1* gene may be more susceptible to the genotoxic effects of EtO. The unexpected finding of decreased SCEs, which is less clear, may be attributed to the nonchemical specificity of this end point and the lack of expression of the GSTT1 enzyme in lymphocytes.**

## Introduction

EtO[2] is an important industrial chemical with widespread uses, mainly as an intermediate in the manufacture of ethylene glycol and other products (1). It is also used directly in the gaseous form for the fumigation and sterilization of a variety of heat-sensitive materials such as agricultural and medical products and hospital equipment. It is estimated that 224,000 hospital workers in the United States are exposed to EtO (2). Other than occupational sources, EtO is also formed metabolically from ethylene, a constituent of polluted urban air and cigarette smoke as well as a product of lipid peroxidation and endogenous metabolic processes (3–5). The genotoxicity of EtO in somatic and germ cells has been extensively reviewed (6), and occupational exposures to EtO have been observed to be associated with increased frequencies of SCE, chromosomal aberrations, and micronuclei (1). Although there is limited epidemiological evidence for an increased risk of cancer among workers exposed to EtO, the IARC has classified it as a human carcinogen based on supporting mechanistic data (1).

EtO is reactive and alkylates cellular macromolecules (DNA and proteins) directly without requiring metabolic activation to form adducts (7). EtO is metabolized by hydrolysis and by conjugation with GSH, resulting in its conversion to less reactive and more soluble products and its subsequent excretion (8, 9). The GSH conjugation pathway is mediated by GSTs, a multigene family of enzymes that function primarily in the detoxification, but in some instances, also in the activation of a wide variety of electrophilic compounds of both exogenous and endogenous origin (10). Of the four main classes of GSTs that have been identified in humans ($\alpha$, $\mu$, $\pi$, and $\theta$), the isoenzymes of GSTM1 within the $\mu$ class and GSTT1 within the $\theta$ class are the most studied and have been shown to be polymorphic (11). The polymorphism is due to the inherited homozygous deletion of the respective *GSTM1* (12) and *GSTT1* (13, 14) gene, resulting in a null genotype and absence of enzymatic activity. The frequencies of both *GSTM1*-null and *GSTT1*-

Received 6/21/00; revised 2/7/01; accepted 2/26/01.
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[1] To whom requests for reprints should be addressed, at Industrywide Studies Branch, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, MS R15, Cincinnati, OH 45226.

[2] The abbreviations used are: EtO, ethylene oxide; SCE, sister chromatid exchange; GSH, glutathione; GST, glutathione *S*-transferase; HEV, *N*-(2-hydroxyethyl)valine; TWA, time-weighted average; HB, hemoglobin.

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null genotypes have been found to vary geographically as well as by ethnicity, with wide ranges reported in different studies (11, 12, 15).

In recent years, there has been considerable interest in the role that the polymorphic GSTM1 and GSTT1 enzymes may play in influencing individual susceptibility to the adverse effects of environmental exposure, particularly cancer risk, as has been reviewed by Strange and Fryer (16). Thus far, there is accumulating evidence that the *GSTM1*-null genotype is associated with increased risk of cancers of the bladder, skin, and gastrointestinal tract and particularly cancer of the lung, although conflicting findings have also been reported. In contrast, the *GSTT1* genotype-cancer risk relation is less clear, although increased risks have been shown for cancer of various sites, including the brain. It is generally assumed that the potential substrates of these GST enzymes include a wide range of environmental carcinogens, but few have been shown to be influenced by these polymorphic enzymes in *in vivo* studies in humans. Thus, the modifying role of these enzymes with respect to the causative substrate remains unclear.

The GSTM1 enzyme is best known for its potential role in the detoxification of epoxides and other reactive metabolites of polycyclic aromatic hydrocarbons, a group of carcinogenic compounds derived from dietary, environmental, and occupational sources, including cigarette smoke (11). In addition to carcinogenic effects, a higher frequency of SCEs has been observed in heavy smokers with the *GSTM1*-null genotype than in those with the positive genotype, suggesting that the *GSTM1* genotype-lung cancer association may be explained by the increased smoking-induced cytogenetic damage in the *GSTM1*-null smokers (17).

Based on laboratory investigations, several industrial chemicals, including EtO, have been identified as potential substrates for the GSTT1 enzyme. Fost *et al.* (18) have demonstrated that the amount of radioactivity bound to the erythrocyte fraction of the blood of "non-conjugators," was significantly higher than that of the "conjugators" after the incubation of blood samples with radiolabeled EtO. In another *in vitro* study in which blood samples were incubated with EtO, there was a marked induction of SCEs in the lymphocytes of non-conjugators but not in the lymphocytes of conjugators (19), which was later shown to correspond with the absence and presence of the *GSTT1* gene, respectively (13). These studies suggest that the GSTT1 enzyme may play a role in the *in vivo* detoxification of EtO, and we hypothesize that individuals with the *GSTT1*-null genotype have a higher risk of genotoxicity when exposed to EtO.

The objective of the current study was to examine the effect of the *GSTT1* genotype on the levels of two biological markers of genotoxicity, the EtO-hemoglobin adducts, *i.e.*, HEV, and SCE in a group of operators of sterilizers that used EtO and nonexposed workers from nine hospitals in the United States and one hospital in Mexico City, Mexico. Similar analyses were performed for the *GSTM1* genotype, and special attention was paid to cigarette smoke as a potential source of substrates for both the GSTM1 and GSTT1 enzyme.

## Materials and Methods

**Study Subjects.** The study subjects were derived from a group of 73 previously studied workers from nine hospitals in the United States and one hospital in Mexico City, Mexico (20). These workers were generally employed in the central sterile supply department and were involved in the unloading of sterilizers, a job that provided the greatest opportunity for EtO exposure. In each hospital, workers with the lowest and highest potential cumulative exposure to EtO were selected based on records of the number of times they unloaded sterilizers during the previous 4 months. In addition, a group of nonexposed workers who handled no unloadings or worked in departments where EtO was not used was included in the study. Before the collection of a blood sample, each subject completed an interviewer-administered questionnaire. The questionnaire data included demographic characteristics; cigarette smoking history; intake of coffee, tea, and alcohol; and occupational and health history with information on exposure to X-rays, immunizations, history of cancer and viral infections, and medication use.

**Estimation of 4-month Cumulative EtO Exposure.** Details concerning the estimation of the 4-month cumulative EtO exposure of the study subjects have been presented previously (20). Briefly, exposure to EtO was estimated based on measurements of the personal breathing zone, stationary area, and grab air samples collected in each of the 10 hospitals over a 2–4-day period. For each subject, cumulative exposure to EtO was estimated over the 4-month period before the date of blood sampling to reflect the average 4-month life span of erythrocytes. The 4-month cumulative exposure for each subject was computed based on the measured mean exposure concentration for each of the primary tasks performed in his/her hospital, individual usage of the gas sterilizer equipment based on hospital records, personal interviews, questionnaires, and the professional judgement of an industrial hygienist. Each task, such as the unloading of sterilizers, taking inventory, or wrapping instrument packages, was considered a discrete activity. Each subject's 4-month cumulative EtO exposure (in ppm-h) was estimated by an algorithm that is the product of the measured mean exposure concentration for each job task in his/her hospital and the number of hours worked in that task, and this was totaled over all job tasks performed. Therefore, each worker's estimated cumulative exposure largely reflects the duration of time spent in the performance of the various tasks over the 4-month period. Consequently, workers who performed essentially the same tasks with similar duration due to equal job rotation within the same hospital, *i.e.*, with similar potential exposure, have the same cumulative exposure. In addition, the mean 8-h TWA was calculated for each subject based on his/her job tasks and weighted by the duration of each task at his/her hospital (20). For the nonexposed workers, the lack of exposure to EtO was confirmed based on measurements of the personal breathing zone and stationary area air samples. Because the EtO exposure concentrations of these measurements were below the lowest limit of detection, the 4-month estimated cumulative exposure and 8-h TWA of the nonexposed workers were arbitrarily assigned a value of 0.

**Laboratory Assays.** The levels of the HEV adducts and SCE of both the exposed and nonexposed workers presented in this study were determined previously, as described previously in detail (20). Briefly, the laboratory assays were conducted on heparinized venous blood samples drawn from each subject during the 2–4-day exposure monitoring period. The HEV adducts were determined by the radioimmunoassay method developed by Wraith *et al.* (21). The assay of SCE was performed using the method of Carrano and Moore (22) on lymphocytes separated from whole blood. At 68 h after the initiation of the cultures for SCEs, Colcemid (0.1 $\mu$g/ml) was added to arrest the cells in metaphase. The cells were harvested at 72 h, and 50 metaphases from each subject were examined for SCEs.

**Genotype Analysis.** The *GSTT1* and *GSTM1* genotypes of the subjects were determined by using the PCR method, as de-

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scribed in detail by Wiencke *et al.* (23), on DNA samples extracted from the whole blood stored at −70°C. For each gene, the subjects were classified as either null (homozygous gene deletion) or positive (at least one copy of the gene present).

**Statistical Analysis.** Comparisons of the crude levels of HEV adducts and SCE by EtO exposure and smoking status, *GSTT1* and *GSTM1* genotypes, and other variables of interest were conducted with the use of ANOVA, followed by analysis of covariance with adjustment for cigarette smoking. Separate linear regression models were used to examine the relationship between each biomarker and EtO exposure and to assess the effects of the *GSTT1* and *GSTM1* genotypes (coded as 0 for the positive genotype and 1 for the null genotype) on these relationships. Adjustment for cigarette smoking and other potential confounders was performed by including these variables in the multiple regression model.

For the EtO exposure variable, subjects were categorized as nonexposed or were in the low (≤32 ppm-h) or high (>32 ppm-h) exposure group. The 32 ppm-h cut point between low and high exposure was selected after the inspection of an observable break in the data (a range of 3–32 and 74–869 ppm-h). The choice of this cut point was supported by the fact that exposed subjects were initially selected with potential for low and high EtO exposure (20). In addition, the ANOVA and regression results based on the 32 ppm-h cut point for the categorization of subjects with low and high exposure were similar to those based on other cut points selected, such as the median. Similar results were also obtained with the exposure variable entered in the regression model as either a continuous or indicator variable (low/high exposure based on either the median or 32 ppm-h cut point *versus* the nonexposed group). Thus, only the results based on the 32 ppm-h cut point for the exposed subjects are presented.

The potential confounders included in the multiple regression models were those that were identified in univariate analyses or have been reported previously in the literature. These included cigarette smoking, age, years of education, and gender (coded as 0 for male and 1 for female). Because data of the small sample of study subjects from the United States (whites and blacks) and Mexico (Hispanics) were combined in the analysis, race (as indicator variables: blacks or Hispanics *versus* whites as the reference group) was also included in the model to adjust for race and country differences. As previously reported in the literature, none of the other health (previous illnesses, medication use, and previous X-rays/immunizations) or lifestyle factors (intake of tea, coffee, or alcohol) was found to be a significant covariate in this group of subjects. These variables were correlated with age and education and did not make a further contribution to the variability of the biomarkers after age and education were included in the models.

For the cigarette smoking variable, subjects were categorized according to their reported lifetime smoking history before blood sampling as never smokers ($n = 32$) if they had smoked ≤100 cigarettes during their entire life or as smokers ($n = 26$). Among the smokers, 19 reported that they currently smoked (current smokers), and 7 had stopped smoking (former smokers). The former smokers as a group were not significantly different from current smokers with respect to the intensity and duration of smoking and were comparable in levels of HEV adduct and SCE. In addition, because smoking exposure was not verified but was based on self-reports, and the majority of the former smokers quit smoking within the 2-year period before blood sampling, data from both former and current smokers were combined in subsequent analysis. Consequently, smoking intensity (0 for never smokers and the average number of cigarettes smoked/day for both former and current smokers) was used to adjust for smoking in the analysis of HEV adducts to best reflect cigarette exposure during the life span of the erythrocytes, whereas pack-years (packs of cigarettes smoked/day multiplied by years of smoking) was used to reflect the longer relevant exposure period for SCE.

In addition to examining the independent effects of the *GSTT1* genotype on HEV adducts and SCE, effect modifications by the *GSTM1* genotype, cigarette smoking, and EtO exposure were examined by introducing interaction terms in the regression models and, if significant, by stratified analyses. Similar analyses were performed for the *GSTM1* genotype. Before the conduct of all analyses, the distribution of HEV adducts was normalized by $\log_e$ transformation. However, for ease of interpretation, we present results that have been exponentiated back to the original scale. Based on residual plots and several diagnostic tests, one subject was found to be a significant outlier for HEV adducts and was excluded from the analysis. For the final models of HEV adducts and SCE presented here, there was no indication of any violation of the linear regression assumptions. All analyses were performed by using the Statistical Analysis Systems software package (SAS Corp., Cary, NC), and a two-tailed $P < 0.05$ was considered statistically significant.

## Results

There were 59 subjects (53 exposed subjects and 6 nonexposed subjects) who had sufficient blood samples for the genotyping analysis and from whom prior informed consent had been obtained for inclusion in this component of the study. After the exclusion of one subject who was found to be a significant outlier for HEV adducts, 58 subjects were available for the current analysis. The ages of the subjects, all but four of whom were female, ranged from 22–70 years, with a mean age of 38 ± 2 (SE) years (Table 1). Twenty-two of the subjects were white (38%), 19 were black (33%), and 17 were Hispanic (29%). Never smokers comprised 55%, former smokers comprised 12%, and current smokers comprised 33% of the subjects.

Table 1 presents the sociodemographic characteristics of the study subjects according to the EtO exposure status. Overall, there were 5 subjects (9%) in the nonexposed group, 36 subjects (62%) in the low exposure (≤32 ppm-h) group, and 17 subjects (29%) in the high exposure (>32 ppm-h) group. The mean 4-month cumulative exposure for the low and high exposure group was 12.4 and 226.9 ppm-h, respectively, both of which were lower than the United States 8-h TWA permissible level of 1 ppm (Table 1). Subjects are comparable with respect to age, education, length of employment in current job, and smoking status distribution among the exposure groups. The major difference is in the distribution of race, as shown by a higher percentage of the Hispanic subjects in the high exposure group and a lower percentage of Hispanic subjects in the low exposure group as compared with the white and black subjects.

The prevalence of the *GSTT1*-null genotype was 17% in the overall group of subjects, with the following distributions among races: 2 in the white (20%) and 4 (40%) each in the black and Hispanic subjects (Table 2). The subjects did not differ significantly in age, education, length of employment, and the distributions of race, exposure, and smoking status by the *GSTT1* genotype status. The 10 subjects with the *GSTT1*-null genotype were equally distributed between the low and high exposure groups, but

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*Table 1* Selected characteristics of study subjects (overall and by EtO exposure status)

| Variable | Exposure status | | | |
|---|---|---|---|---|
| | Total ($n$ = 58) | Nonexposed ($n$ = 5)[a] | ≤32 ppm-h ($n$ = 36) | >32 ppm-h ($n$ = 17) |
| Age (yrs) | 37.74 ± 1.56[b] | 41.23 ± 5.38 | 37.64 ± 2.01 | 36.91 ± 2.92 |
| Education (yrs) | 12.81 ± 0.23 | 12.80 ± 0.80 | 12.72 ± 0.30 | 13.00 ± 0.43 |
| Employment (yrs) | 5.19 ± 0.55 | 6.60 ± 1.83 | 4.51 ± 0.69 | 6.18 ± 0.99 |
| Cumulative chronic exposure (4-month); ppm-h)[c] | 74.22 ± 19.01 | 0 | 12.40 ± 17.88 | 226.94 ± 26.02 |
| 8-h TWA (ppm)[c,d] | 0.14 ± 0.03 | 0 | 0.06 ± 0.03 | 0.35 ± 0.04 |
| Race[e] | | | | |
| White | 22 (37.9)[f] | 1 (20) | 19 (52.8) | 2 (11.8) |
| Black | 19 (32.8) | 3 (60) | 10 (27.8) | 6 (35.3) |
| Hispanic | 17 (29.3) | 1 (20) | 7 (19.4) | 9 (52.9) |
| Gender | | | | |
| Male | 4 (6.9) | 0 | 3 (8.3) | 1 (6) |
| Female | 54 (93.1) | 5 (100) | 33 (91.7) | 16 (94) |
| Smoking status | | | | |
| Never | 32 (55.2) | 3 (60) | 21 (58.3) | 8 (47.1) |
| Former | 7 (12.1) | 0 | 3 (8.3) | 4 (23.5) |
| Current | 19 (32.8) | 2 (40) | 12 (33.3) | 5 (29.4) |

[a] Subjects with EtO exposure levels below the lowest limit of detection. These subjects were assigned a value of 0 ppm-h for the 4-month cumulative exposure and the 8-h TWA.
[b] Mean ± SE.
[c] Significant difference by exposure status (ANOVA, $P$ = 0.0001).
[d] The mean shown is the average of the 8-h TWAs for the job tasks of subjects in each designated exposure category.
[e] Significant difference by race (Fisher's exact test, $P$ = 0.013).
[f] Number (percentage is in parentheses).

*Table 2* Selected characteristics of study subjects by *GSTT1* and *GSTM1* genotypes

| Variable | *GSTT1* genotype | | *GSTM1* genotype[a] | |
|---|---|---|---|---|
| | Null ($n$ = 10) | Positive ($n$ = 48) | Null ($n$ = 23) | Positive ($n$ = 34) |
| Age (yrs) | 39.81 ± 3.78[b] | 37.31 ± 1.72 | 34.90 ± 2.46 | 39.86 ± 2.02 |
| Education (yrs) | 12.30 ± 0.55 | 12.92 ± 0.25 | 12.61 ± 0.34 | 12.79 ± 0.28 |
| Employment (yrs) | 5.88 ± 1.38 | 5.06 ± 0.60 | 5.04 ± 0.87 | 5.27 ± 0.73 |
| Race[c] | | | | |
| White | 2 (20)[d] | 20 (41.7) | 12 (52.2) | 10 (29.4) |
| Black | 4 (40) | 15 (31.3) | 2 (8.7) | 17 (50) |
| Hispanic | 4 (40) | 13 (27.1) | 9 (39.1) | 7 (20.6) |
| Exposure status | | | | |
| Nonexposed | 0 | 5 (10.4) | 2 (8.7) | 3 (8.8) |
| ≤32 ppm-h | 5 (50) | 31 (64.6) | 15 (65.2) | 20 (58.8) |
| >32 ppm-h | 5 (50) | 12 (25.0) | 6 (26.1) | 11 (32.4) |
| Smoking status | | | | |
| Never | 3 (30) | 29 (60.4) | 11 (47.8) | 20 (58.9) |
| Former | 2 (20) | 5 (10.4) | 3 (13.0) | 4 (11.8) |
| Current | 5 (50) | 14 (29.2) | 9 (39.1) | 10 (29.4) |

[a] *GSTM1* genotype status is unknown for one subject.
[b] Mean ± SE.
[c] Significant difference by *GSTM1* genotype (Fisher's exact test, $P$ = 0.004).
[d] Number (percentage is in parentheses).

none were in the nonexposed group. In contrast to the *GSTT1* genotype, a higher prevalence of the *GSTM1*-null genotype was observed (40%), particularly among the white (52%) and Hispanic (39%) subjects as compared with the black (9%) subjects. The *GSTM1*-null and -positive subjects are comparable in the distributions of the smoking and exposure status as well as the other sociodemographic characteristics (except for race) presented in Table 2.

The mean levels of HEV adducts and SCE differed by *GSTT1* genotype status (Table 3). The mean HEV adduct level was significantly higher in the *GSTT1*-null subjects than in the *GSTT1*-positive subjects. For SCE, a reverse pattern was observed, with the mean frequency significantly lower in the *GSTT1*-null subjects than in the *GSTT1*-positive subjects. The *GSTM1*-null and -positive subjects had a similar mean HEV adduct level. Although the *GSTM1*-null subjects had a higher mean SCE frequency than the *GSTM1*-positive subjects, the difference was not statistically significant. When the subjects were further examined with respect to the various combinations of the *GSTT1* and *GSTM1* genotypes, the HEV adduct level or SCE frequency did not differ by *GSTM1* genotype in either the *GSTT1*-null or -positive group of subjects.

The mean HEV adduct level was significantly higher in smokers (either former or current) than in never smokers but was not significantly different between former and current smokers (Table 3). In addition, former smokers did not differ significantly from current smokers with respect to the number of cigarettes smoked/day ($\bar{x}$ = 12.0 *versus* 11.6), years smoked ($\bar{x}$ = 10.0 *versus* 16.2), and cumulative dose of cigarette exposure, as indicated by pack-years ($\bar{x}$ = 8.4 *versus* 9.7). When all smokers (both former and current) were further categorized by the intensity of smoking, the mean HEV adduct level was found to be significantly higher in those with ≥10 ($\bar{x}$ = 0.18 pmol/mg) than in those with <10 cigarettes/day ($\bar{x}$ = 0.09 pmol/mg). The SCE frequency did not differ significantly by smoking status, intensity, or cumulative dose of cigarette exposure.

The raw HEV adduct and SCE data of the individual *GSTT1*-null and -positive subjects in relation to cumulative EtO exposure are displayed in Figs. 1 and 2, respectively. The HEV adduct level was found to reflect the extent of EtO exposure: the mean level in the high exposure group was twice that of the low exposure group and four times that of the nonexposed group; and these differences were statistically significant (Table 3). No significant difference in HEV adduct level was observed between the low exposure group and the nonexposed group. The mean SCE frequency was significantly higher in the high exposure group and in the low exposure group as compared with the nonexposed group, but these differ-

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*Table 3* Mean levels of HEV adducts and SCEs by EtO exposure, *GSTT1*/*GSTM1* genotype, and smoking status

| Variable | HEV adducts (pmol/mg HB) ($n$ = 58) | | | SCEs (freq/cell) ($n$ = 56)[a] | | |
|---|---|---|---|---|---|---|
| | $n$ | Mean ± SE | $P^{b,c}$ | $n$ | Mean ± SE | $P^{b}$ |
| *GSTT1* genotype | | | | | | |
| Null | 10 | 0.17 ± 0.03 | 0.02 | 9 | 5.31 ± 0.39 | 0.04 |
| Positive | 48 | 0.08 ± 0.01 | | 47 | 6.21 ± 0.17 | |
| *GSTM1* genotype[d] | | | | | | |
| Null | 23 | 0.10 ± 0.02 | 0.95 | 23 | 6.26 ± 0.25 | 0.35 |
| Positive | 34 | 0.10 ± 0.02 | | 32 | 5.96 ± 0.21 | |
| Combined genotype[d] | | | | | | |
| *GSTT1*-null genotype | | | | | | |
| *GSTM1*-null genotype | 3 | 0.17 ± 0.07 | 0.79 | 3 | 5.23 ± 0.44 | 0.82 |
| *GSTM1*-positive genotype | 7 | 0.17 ± 0.04 | | 6 | 5.36 ± 0.31 | |
| *GSTT1*-positive genotype | | | | | | |
| *GSTM1*-null genotype | 20 | 0.08 ± 0.02 | 0.85 | 20 | 6.42 ± 0.27 | 0.38 |
| *GSTM1*-positive genotype | 27 | 0.09 ± 0.01 | | 26 | 6.10 ± 0.24 | |
| Smoking status | | | | | | |
| Never (1) | 32 | 0.06 ± 0.03 | 1 *versus* 2 = 0.01 | 32 | 5.91 ± 0.21 | 0.47 |
| Former (2) | 7 | 0.16 ± 0.03 | 1 *versus* 3 = 0.0004 | 7 | 6.06 ± 0.45 | |
| Current (3) | 19 | 0.14 ± 0.02 | | 17 | 6.36 ± 0.29 | |
| Smokers (no. of cigarettes/day) | | | | | | |
| <10 | 10 | 0.09 ± 0.03 | 0.04 | 10 | 5.78 ± 0.39 | 0.12 |
| ≥10 | 16 | 0.18 ± 0.02 | | 14 | 6.62 ± 0.33 | |
| Exposure status | | | | | | |
| Nonexposed (1) | 5 | 0.04 ± 0.04 | 1 *versus* 3 = 0.001 | 5 | 5.00 ± 0.52 | 1 *versus* 2 = 0.049 |
| ≤32 ppm-h (2) | 36 | 0.08 ± 0.01 | 2 *versus* 3 = 0.0001 | 35 | 6.11 ± 0.20 | 1 *versus* 3 = 0.03 |
| >32 ppm-h (3) | 17 | 0.16 ± 0.02 | | 16 | 6.31 ± 0.29 | |
| Adjusted for smoking[e] | | | | | | |
| Exposure Status | | | | | | |
| Nonexposed (1) | 5 | 0.05 ± 0.03 | 1 *versus* 3 = 0.0001 | 5 | 5.01 ± 0.51 | 1 *versus* 2 = 0.05 |
| ≤32 ppm-h (2) | 36 | 0.08 ± 0.01 | 2 *versus* 3 = 0.0001 | 35 | 6.09 ± 0.19 | 1 *versus* 3 = 0.03 |
| >32 ppm-h (3) | 17 | 0.16 ± 0.02 | | 16 | 6.34 ± 0.28 | |

[a] Two subjects were excluded due to laboratory technical problems in SCE values.
[b] $P$ for difference between groups by ANOVA/analysis of covariance and pairwise comparisons by Student's $t$ test.
[c] Statistical test based on $\log_e$-transformed data.
[d] One subject with unknown *GSTM1* genotype status.
[e] Adjusted for no. of cigarettes/day for smokers.

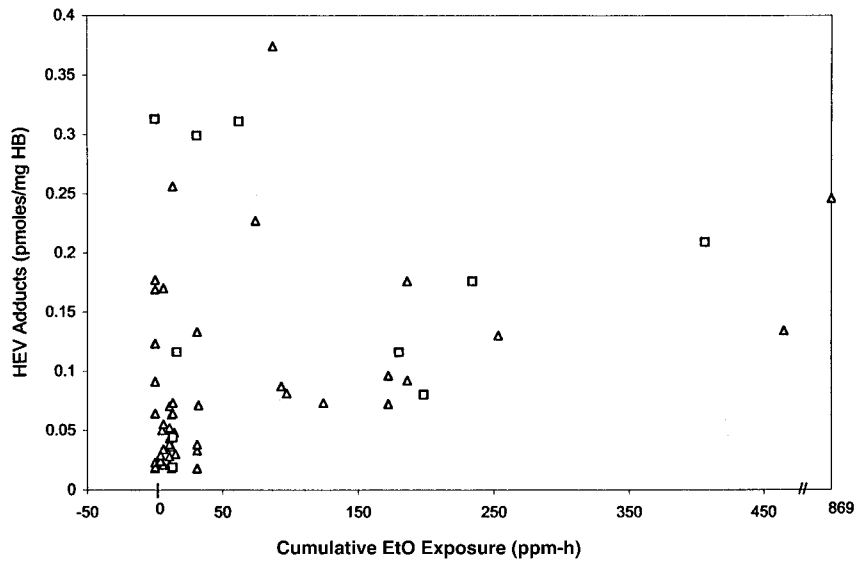


*Fig. 1.* HEV adduct level *versus* cumulative EtO exposure in 58 subjects; □, individuals with *GSTT1*-null genotype; △, individuals with *GSTT1*-positive genotype.

ences were of lower magnitudes. Because cigarette smoke and occupational exposure are both important sources of EtO, the levels of both biomarkers among the EtO exposure groups were further compared after adjusting for cigarette smoking. The mean levels of HEV adducts and SCE observed among the EtO exposure groups remained essentially unchanged after adjusting for the number of cigarettes smoked/day (Table 3) or pack-years (data not known).

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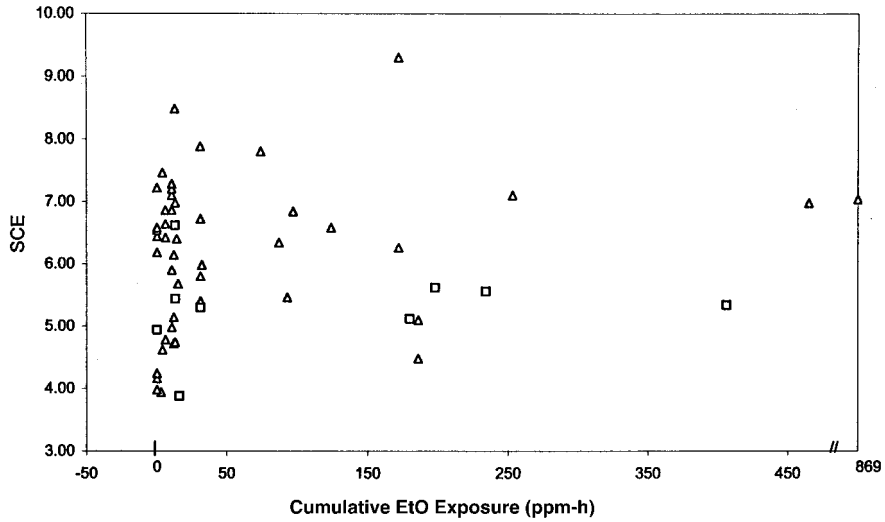


*Fig. 2.* SCE frequency *versus* cumulative EtO exposure in 56 subjects; □, individuals with *GSTT1*-null genotype; △, individuals with *GSTT1*-positive genotype.

*Table 4* Simple and multiple linear regression results for HEV adducts ($n$ = 58)

| Variable | Regression model | | | | | | |
|---|---|---|---|---|---|---|---|
| | Simple[a] | | Multiple[b] | | | | |
| | | | Model 1 ($R^2$ = 0.67) | | Model 2 ($R^2$ = 0.63) | | |
| | Coefficient[c] | *P* | Coefficient[c] | *P* | Coefficient[c] | *P* | Partial $R^2$ (× 100)[d] |
| Intercept | | | 0.01 | 0.0001 | 0.03 | 0.0001 | |
| EtO exposure (reference group, nonexposed) | | | | | | | |
| ≤32 ppm-h | 1.62 | 0.18 | 1.49 | 0.15 | 1.43 | 0.18 | 1.3 (4) |
| >32 ppm-h | 4.06 | 0.001 | 3.25 | 0.0002 | 3.53 | 0.0001 | 29.5 (2) |
| Cigarette smoking | | | | | | | |
| Cigarettes/day | 1.06 | 0.0001 | 1.06 | 0.0001 | 1.06 | 0.0001 | 27.5 (1) |
| *GSTT1* (null *vs.* positive) | 2.05 | 0.02 | 1.62 | 0.02 | 1.58 | 0.02 | 4.4 (3) |
| Race (reference group, white) | | | | | | | |
| Black | 1.27 | 0.39 | 1.17 | 0.42 | | | |
| Hispanic | 1.34 | 0.31 | 1.38 | 0.17 | | | |
| Gender (female *vs.* male) | 0.75 | 0.52 | 0.65 | 0.16 | | | |
| Age (yrs) | 1.00 | 0.90 | 1.01 | 0.35 | | | |
| Education (yrs) | 1.00 | 0.98 | 1.05 | 0.26 | | | |

[a] Not adjusted.
[b] Adjusted for all variables in the model.
[c] Regression coefficients derived from a model based on $\log_e$-transformed HEV adduct values that have been exponentiated back to the ordinary scale.
[d] Percentage of variance explained in HEV adducts by each independent variable in the model using a forward stepwise procedure. Numbers in parentheses indicate the order in which the variables entered the model.

The results of the regression analysis for the $\log_e$-transformed HEV adducts that have been exponentiated back to the original scale are presented for all 58 subjects (Table 4). Two subjects with technical laboratory problems in their SCE values were not excluded from this analysis because the results remained unchanged except for a reduction in sample size. In univariate analysis, EtO exposure, cigarette smoking (number of cigarettes smoked/day), and the *GSTT1* genotype were each significantly associated with an increase in HEV adduct level. We next examined the independent effects of these variables in a multiple regression model in which adjustment was made for all variables included in the model. As shown in model 1, the positive association between EtO exposure and HEV adducts remained unchanged after adjusting for cigarette smoking, age, race, gender, education, and the *GSTT1* genotype. The *GSTT1*-null genotype was found to be significantly associated with an increase in HEV adduct level ($\beta$ = 1.62, $P$ = 0.02). The regression coefficient gives an estimate of the change in the HEV adduct level in relation to each variable after adjusting for all other variables in the model. Therefore, in the case of the *GSTT1* genotype, the HEV adduct level was estimated to be significantly increased by a mean of 1.62 pmol/mg HB in subjects with the null genotype *versus* those with the positive genotype (reference group). The *GSTT1* genotype and HEV adduct relationship remained unchanged after further adjustment for the *GSTM1* genotype. There was also no indication of a modification of this relationship by the *GSTM1* genotype, EtO exposure status, or cigarette smoking. To further illustrate the effect of the *GSTT1* genotype on the HEV adduct-EtO exposure relationship, a plot of the adjusted HEV adduct level (from model 1) *versus* cumulative EtO exposure in individual *GSTT1*-null and -positive subjects, as distinguished by different symbols, is shown in Fig. 3.

A multiple regression model that includes only the vari-

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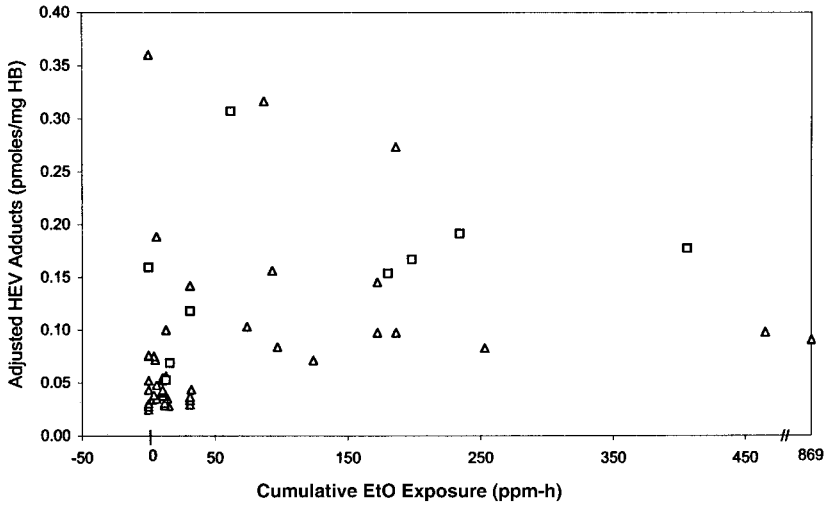


*Fig. 3.* HEV adduct level in relation to cumulative EtO exposure in 58 subjects, adjusted for age, race, gender, education, cigarette smoking, and *GSTT1* (see Table 4, model 1); □, individuals with *GSTT1*-null genotype; △, individuals with *GSTT1*-positive genotype.

*Table 5* Simple and multiple linear regression results for SCE in relation to EtO exposure ($n = 56$)

| Variable | Regression model | | | | | | |
|---|---|---|---|---|---|---|---|
| | Simple[a] | | Multiple[b] | | | | |
| | | | Model 1 ($R^2 = 0.38$) | | Model 2 ($R^2 = 0.21$) | | |
| | Coefficient | *P* | Coefficient | *P* | Coefficient | *P* | Partial $R^2$ ($\times$ 100)[c] |
| Intercept | | | 6.61 | 0.0001 | 4.94 | 0.0001 | |
| EtO exposure (reference group, nonexposed) | | | | | | | |
| ≤32 ppm-h | 1.11 | 0.049 | 1.24 | 0.02 | 1.26 | 0.02 | 8.9 (3) |
| >32 ppm-h | 1.31 | 0.03 | 1.37 | 0.02 | 1.59 | 0.008 | 3.1 (2) |
| Cigarette smoking | | | | | | | |
| Pack-years | 0.01 | 0.44 | 0.03 | 0.12 | 0.02 | 0.35 | 1.4 (4) |
| *GSTT1* (null *vs.* positive) | −0.90 | 0.04 | −1.25 | 0.003 | −1.11 | 0.009 | 7.9 (1) |
| Race (reference group, white) | | | | | | | |
| Black | −0.34 | 0.36 | 0.05 | 0.89 | | | |
| Hispanic | 0.50 | 0.20 | 0.48 | 0.26 | | | |
| Gender (female *vs.* male) | 0.45 | 0.47 | 0.56 | 0.33 | | | |
| Age (yrs) | −0.03 | 0.02 | −0.03 | 0.054 | | | |
| Education (yrs) | −0.05 | 0.56 | −0.10 | 0.26 | | | |

[a] Not adjusted.
[b] Adjusted for all variables in the model.
[c] Percentage of variance explained in SCE by each independent variable in the model using a forward stepwise procedure. Numbers in parentheses indicate the order in which the variables entered the model.

ables that were significantly associated with HEV adducts, *i.e.*, EtO exposure, cigarette smoking, and the *GSTT1* genotype, was also presented in Table 4 (model 2). The results of this reduced model were similar to those of fully-adjusted model 1. Using a forward stepwise procedure, we next assessed the relative importance of each of these variables in explaining the variability in HEV adducts after adjusting for all other variables in the model. Based on their contributions to the percentage of total variability and the order in which each variable entered the regression model, cigarette smoking (28%) and EtO exposure (30% for those in the high exposure group *versus* nonexposed group) have stronger effects on the HEV adduct level than the *GSTT1* genotype (4%).

The *GSTM1* genotype was not associated with HEV adduct level, either univariately or multivariately, after the adjustment for age, race, gender, education, EtO exposure, and cigarette smoking (data not shown). A similar result was observed after further adjustment for the *GSTT1* genotype. There was an indication of a modification in the effect of the *GSTM1* genotype by cigarette smoking. In a multiple regression model, the interaction term between the *GSTM1* genotype and cigarette smoking (number of cigarettes smoked/day) was marginally significant ($\beta = 1.04$; $P = 0.045$; data not shown). This effect of the *GSTM1* genotype, however, was no longer significant after further adjustment for the *GSTT1* genotype.

The results of the regression analysis for SCE are shown in Table 5. Due to the exclusion of two subjects whose SCE values were found to have technical laboratory problems, only 56 subjects were available for this analysis. EtO exposure and the *GSTT1* genotype but not cigarette smoking (pack-years) were significantly associated with SCE frequency, both univariately and multivariately (model 1). EtO exposure (for those in either the high or low exposure group *versus* the nonexposed group) was significantly associated with an increase in SCE frequency. However, in contrast to HEV adducts, the *GSTT1*-null genotype was significantly associated with a decrease in

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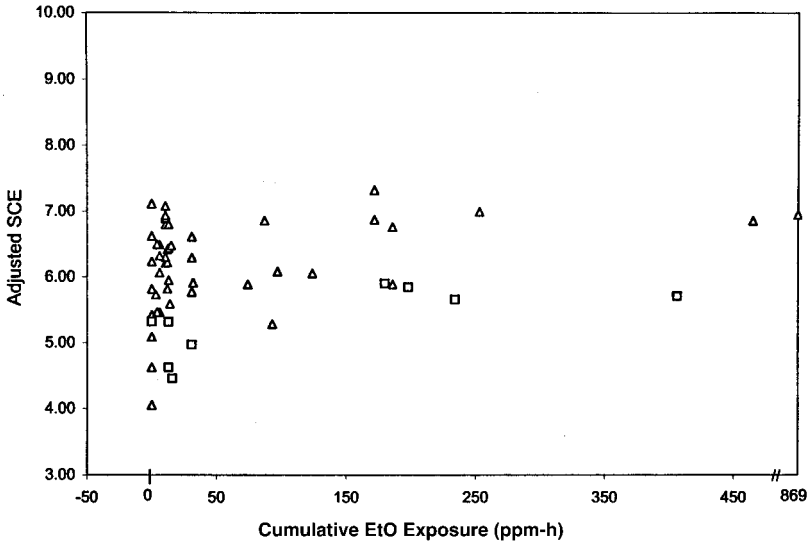


*Fig. 4.* SCE frequency in relation to cumulative EtO exposure in 56 subjects, adjusted for age, race, gender, education, cigarette smoking, and *GSTT1* genotype (see Table 5, model 1); □, individuals with *GSTT1*-null genotype; △, individuals with *GSTT1*-positive genotype.

*Table 6* Multiple linear regression results for SCE in relation to HEV adducts ($n = 56$)

| Variable | Regression model | | | | |
|---|---|---|---|---|---|
| | Model 1 ($R^2 = 0.36$) | | Model 2 ($R^2 = 0.19$) | | |
| | Coefficient | *P* | Coefficient | *P* | Partial $R^2$ (×100)[c] |
| Intercept | 9.35 | 0.0001 | 7.78 | 0.0001 | |
| HEV adducts ($log_e$-transformed) (pmol/mg HB) | 0.50 | 0.03 | 0.54 | 0.01 | 10.7 (2) |
| Cigarette smoking | | | | | |
| Pack-years | −0.001 | 0.96 | −0.01 | 0.54 | 0.6 (3) |
| *GSTT1* (null *vs.* positive) | −1.35 | 0.002 | −1.22 | 0.005 | 7.9 (1) |
| Race (reference group, white) | | | | | |
| Black | −1.00 | 0.78 | | | |
| Hispanic | 0.28 | 0.49 | | | |
| Gender (female *vs.* male) | 0.76 | 0.21 | | | |
| Age (yrs) | −0.03 | 0.07 | | | |
| Education (yrs) | −0.11 | 0.19 | | | |

[a] Not adjusted.
[b] Adjusted for all variables in the model.
[c] Percentage of variance explained in SCE by each independent variable in the model using a forward stepwise procedure. Numbers in parentheses indicate the order in which the variables entered the model.

SCE frequency, either univariately ($\beta = -0.90$; $P = 0.04$) or multivariately ($\beta = -1.25$; $P = 0.003$). That is, the SCE frequency was estimated to be significantly decreased by a mean of 1.25 in subjects with the null genotype *versus* those with the positive genotype (reference group). A plot of the adjusted SCE frequency (from model 1) *versus* cumulative EtO exposure in individual *GSTT1*-null and -positive subjects, as distinguished by different symbols, is presented in Fig. 4 to illustrate the effect of the *GSTT1* genotype on the SCE-EtO exposure relationship. The SCE results of the multiple regression model that includes only the variables of EtO exposure, cigarette smoking, and the *GSTT1* genotype (model 2) are similar to those of fully adjusted model 1. The *GSTT1* genotype and EtO exposure (low and high exposure groups *versus* nonexposed group) each contributed 8% and 12% to the variability in SCE, respectively.

When external EtO exposure was replaced by $log_e$-transformed HEV adducts as a measure of internal EtO dose in the multiple regression model, the SCE frequency was similarly observed to be significantly associated with the HEV adduct level ($\beta = 0.50$; $P = 0.03$; Table 6, model 1). In addition, subjects with the *GSTT1*-null genotype showed a significant decrease in SCE frequency as compared with the *GSTT1*-positive subjects ($\beta = -1.35$; $P = 0.002$). The *GSTT1* genotype and HEV adducts each contributed 8% and 11% to the variability in SCE, respectively (model 2).

The *GSTM1* genotype was not significantly associated with SCE frequency in the regression model with either the EtO exposure or $log_e$-transformed HEV adduct variable (data not shown). The *GSTT1* genotype-SCE relationship remained unchanged after further adjustment for the *GSTM1* genotype. There was also no indication of a modification of this relationship by the *GSTM1* genotype, EtO exposure, HEV adduct level, or cigarette smoking.

## Discussion

In this study of a group of hospital workers exposed to low levels of EtO (less than the United States 8-h TWA permissible level of 1 ppm) and unexposed controls, the *GSTT1*-null gen-

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otype was found to be significantly associated with an increase in the HEV adduct level and a decrease in the SCE frequency. There was no association between the *GSTM1* genotype and the level of either biomarker, and an indication of a modification of its effect by cigarette smoking on HEV adducts was no longer apparent after adjustment for the *GSTT1* genotype. These results persisted after the adjustment for potential confounders. To our knowledge, there have been no previous reports in the literature that relate the *GSTT1* genotype to the formation of HEV adducts and the frequency of SCE in a group of workers occupationally exposed to EtO.

EtO reacts covalently with the amino acids in hemoglobin (cysteine, histidine, and N-terminal valine), and the adducts formed (7) have been widely used for monitoring of occupational and environmental exposure to EtO (24). Because the extent of hemoglobin binding gives an estimate of the DNA dose, the HEV adducts may be used as a surrogate measurement for the corresponding adducts in DNA (25, 26). Due to the stability and lack of repair of hemoglobin adducts, the level measured reflects the integrated exposure over a period of 4 months (the life span of the erythrocytes) and thus gives a measure of the cumulative dose. In addition, the adducts provide a measure of the biologically effective dose of EtO that takes into account all routes of exposure and is the net result of the processes of absorption, distribution, and detoxification (27). Consistent with the observations from *in vitro* study (18, 28), our results have shown that the GSTT1 enzyme modified the formation of HEV adducts in subjects with *in vivo* exposure to EtO. This provides evidence that the GSTT1 enzyme is involved in the detoxification of EtO, and that the *GSTT1*-null subjects, due to reduced detoxification capacity, may have received a higher internal dose of EtO with a consequent increase in its binding to hemoglobin in the erythrocytes. In linear regression analysis, the HEV adduct level was estimated to be significantly increased by a mean of 1.62 pmol/mg HB in subjects with the null genotype *versus* those with the positive genotype after adjusting for cigarette smoking and other potential confounders.

In previous studies of smokers (29) and nonsmokers (29, 30) with nonoccupational exposure to EtO, the *GSTT1*-null genotype was found to be associated with an increase in the level of HEV adducts. This background level of HEV adducts from nonoccupational sources may be explained in terms of the exposure to ethylene (and a smaller amount of EtO) present in urban air and cigarette smoke (4). In a group of nonoccupationally exposed subjects, there was a direct relationship between the HEV adduct level and the number of cigarettes smoked (31, 32). Thus, an adequate control for cigarette smoking is important in assessing the contribution of occupational exposure to EtO. Our data indicate that cigarette smoking accounted for 28% of the variability in HEV adducts, and a comparable 30% was contributed by occupational EtO exposure after adjusting for cigarette smoking. Furthermore, the *GSTT1*-HEV adduct relationship in this group of subjects persisted after adjusting for cigarette smoking and several known potential confounders. However, in nonsmokers with no occupational EtO exposure, Filser *et al.* (5) have demonstrated that HEV adducts can also be formed from the EtO derived from the ethylene produced endogenously. This endogenous source of ethylene, which has been shown to originate from the lipid peroxidation of unsaturated fats and the metabolic processes of intestinal bacteria in animal studies (4), could be an important contributing factor that may not have been adequately accounted for in this study.

In contrast to what was observed with the HEV adducts, our results indicate that the *GSTT1*-null subjects have significantly lower SCE frequency than the *GSTT1*-positive subjects and therefore suggest that they were less susceptible rather than more susceptible to the genotoxic effects of EtO than were the *GSTT1*-positive subjects. A possible explanation for the differential results of the two end points based on the same group of subjects could be the use of a 4-month period (the life span of erythrocytes) before blood sampling for cumulative EtO exposure estimates. The choice of this reference period of exposure may be preferable to a one-time measurement, but it could not account for the occurrence of peak exposures or other variations in exposure pattern that could contribute to an individual's past exposure.

It is difficult to compare the actual levels of the HEV adducts and SCE of our subjects with those of other published studies due to differences in EtO exposure levels and conditions and study design. As has been reviewed by the IARC (1), there have been few studies that have examined these biomarkers in workers exposed to levels of EtO that are comparable to our study. Furthermore, studies varied in such subject characteristics as smoking distributions and intensity, and the control for cigarette smoking and other confounders was either not performed or differed across studies. In addition to low level EtO exposure, our subjects were predominantly female nonsmokers. For the 26 subjects who smoked, the average number of cigarettes smoked/day was $12 \pm 1.7$. Nevertheless, our study has demonstrated that both end points, although to a greater extent by the HEV adducts than SCE, reflected the estimated 4-month cumulative EtO exposure after adjusting for cigarette smoking and potential confounders. This suggests that SCE, as compared with HEV adducts, may reflect exposure during longer periods of time when EtO or other environmental mutagen/carcinogen exposures could have been different. However, the unexpected finding of lower SCE frequency among the *GSTT1*-null subjects as compared with the positive subjects remained unchanged when SCE was further examined in relation to HEV adducts as an indicator of the internal EtO dose. Thus, another possible explanation could be that the GSTT1 enzyme is not expressed in lymphocytes, although it is expressed in various tissues including erythrocytes (13, 33, 34). Because the presence of an enzyme in the target cell is necessary for the enhanced detoxification of its substrates, the lack of GSTT1 enzyme activity in lymphocytes may have accounted for the differential results observed between the *GSTT1*-null and -positive subjects with respect to the SCE frequency in lymphocytes *versus* the HEV adduct level in erythrocytes.

Although the mechanism of formation and biological significance of SCEs are unclear, this end point is considered a sensitive indicator of cytogenetic damage in humans exposed to genotoxic carcinogens (35). HEV adducts have been shown to be a more sensitive end point for the detection of EtO exposure in humans than SCE (36). The end point of SCE, as compared with HEV adducts, is also less specific because it is induced by numerous mutagens/carcinogens and factors other than EtO (22, 35). Although cigarette smoking has been observed to be associated with the SCE frequency in most studies (37), it is not a significant contributor in this study. On the other hand, there have been reports of an association between the *GSTT1* genotype and the background SCE frequency, with higher values observed among the null subjects as compared with the positive

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subjects, independent of cigarette smoking (23, 38). It is therefore possible that the SCE frequency observed in this study may also reflect exposure to other endogenous or environmental mutagens and carcinogens that are substrates for the *GSTT1* and other GST enzymes. In particular, diet could be an important factor because it contributes to the endogenous production of potential GST substrates, which include reactive oxygen species (*e.g.*, lipid hydroperoxides), as well as being a source of some plant components (*e.g.*, phytochemicals) that can induce GST activity (11).

The GST-catalyzed conjugation reaction is generally assumed to be a detoxification process. However, some classes of chemicals such as methylene chloride, a widely used industrial solvent (39) and a substrate of *GSTT1* (19), and ethylene dibromide, a petrol additive and soil fumigant (40), are activated instead by GSH conjugation to form mutagenic intermediates. The mutagenicity of these and other chemicals has been demonstrated in the *Salmonella typhimurium* tester strain TA 1535 expressing the rat $\theta$ class enzyme GST5-5, which has a structural and enzymatic activity similar to that of the human GSTT1 enzyme (39, 40). Although it is unclear whether this high level of GSTT1 enzyme expression in a bacteria test system is relevant in humans, it is possible that *GSTT1* gene deletion could also be associated with protection against the cytotoxic effects of some ubiquitous exposures in the general environment.

The results of our study indicate that the *GSTT1* genotype may not play a major role in EtO metabolism because it only explained a relatively small percentage of the variability in HEV adducts. EtO is metabolized by hydrolysis and by enzyme-mediated conjugation with GSH (8). This may suggest that under conditions of low exposure to EtO, if an individual is deficient in the GSTT1 enzyme, detoxification may proceed mainly via the hydrolytic pathway. This interpretation of a less important effect of the *GSTT1* genotype at low level EtO exposure seems to agree with the observation based on a physiologically based pharmacokinetic model that predicts that a large percentage of the inhaled EtO in humans is metabolized by hydrolysis, and only approximately 10% is metabolized by GSH (41). Alternatively, the contribution of an individual GST enzyme to the metabolism of a particular chemical is small because its absence could be compensated for by the expression of other available polymorphic and nonpolymorphic enzymes. This is due to the existence of several multigene families of isoenzymes other than the GSTs such as the cytochrome P450s and the *N*-acetyltransferases, all of which have many broad and overlapping substrates (42). Consequently, it is not just the effect of an individual genotype but rather the interactive effects of combinations of genotypes that may be more important in influencing susceptibility to chemical exposure.

Our data do not provide support for a role of the GSTM1 enzyme in the detoxification of EtO. This is shown by the lack of an association between the *GSTM1* genotype and HEV adducts in the overall group of subjects in accordance with the findings observed in the combined group of nonoccupationally EtO exposed smokers and nonsmokers (29, 30, 43). Similarly, the *GSTM1* genotype was not associated with SCE frequency. An indication of a modification of its effect on the HEV adducts by cigarette smoking was observed, but this was no longer significant after adjustment for the *GSTT1* genotype. These observations may suggest the presence of epoxides of polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines, or other carcinogenic intermediates in cigarette smoke that are common substrates for the GSTM1, GSTT1, and other biotransformation enzymes (*e.g.*, cytochrome P-450 1A1; Ref. 42). Another explanation could be that smokers may be exposed to an additional dose of EtO arising from cigarette smoke and from the conversion of ethylene, but the small number of EtO-exposed smokers with absence of both the GSTT1 and GSTM1 enzymes limited further stratified analysis.

Our study results need to be interpreted with caution due to several methodological limitations. In addition to low statistical power, the small sample size may not have allowed for adequate control for the many potential confounders evaluated. Therefore, although we have controlled for known confounders such as age, gender, ethnicity, years of education, and cigarette smoking, and there are no apparent alterations of study results by several health and lifestyle factors and length of employment in current job, we could not rule out chance findings due to uncontrolled confounding. The study of the effect of a null genotype with low and varying ethnic prevalence, such as *GSTT1*, is also problematic. Other than the higher prevalence of the *GSTT1*-null genotype among the Hispanics in our study as compared with that reported by Nelson *et al.* (15), the prevalence estimates of the *GSTT1*-null and *GSTM1*-null genotypes among whites and blacks and the *GSTM1*-null genotype among Hispanics in our study are, in general, comparable with those of other studies (15, 44, 45). Instead, the major limitation is the small number of *GSTT1*-null genotype subjects who, although evenly distributed between the low and high EtO exposure groups, are absent in the nonexposed group. Such an uneven distribution would have resulted in more biological effects from EtO or other substrates of *GSTT1* in the exposed subjects and a tendency toward finding a *GSTT1*-null genotype effect association, but this relationship is not observed with respect to SCE in our study.

Another issue that needs to be addressed is the effect of factors that could influence the outcome of the HEV adduct and SCE assays. This would more likely affect SCE than HEV adducts because factors such as duration of sample storage before lymphocyte culture can lead to the loss or repair of cells with lesions that result in SCE formation. In this study, due to longer shipping time, the lymphocytes for the SCE assay of the Hispanic subjects from Mexico were cultured 12–20 h longer after the collection than those of United States white and black subjects. Although all culturing and assays were performed in the same laboratory, it is possible that this difference in lag time in culturing may have resulted in some reduction in the SCE frequency of the Mexican subjects. However, this is unlikely to have had a major effect on our study results because there was a positive exposure-response trend for SCE in both the Mexican and United States group of subjects. In addition, consistent with what is expected from the higher range of EtO exposures in the Mexican subjects as compared with the United States subjects, a higher SCE frequency (although it was nonsignificant due to small numbers) was observed in the low and high exposure groups of Mexican subjects as compared with those of the United States subjects, respectively.

In summary, despite the small sample size, our study has shown for the first time that the *GSTT1*-null genotype is associated with increased binding of EtO with N-terminal valine in the hemoglobin of humans with occupational exposure to EtO, independent of cigarette smoking and potential confounders. These results thus indicate that the GSTT1 enzyme is involved in the detoxification of EtO, and because the level of HEV adducts can potentially act as a surrogate measurement for the corresponding adducts in DNA, individuals deficient in the *GSTT1* gene may be more susceptible to the genotoxic effects of EtO exposure. However, the *in vivo* contribution of an individual GST enzyme to protection against the cytogenetic

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effects of EtO is more difficult to assess because it may reflect longer term exposures and is determined by the complex interplay of numerous polymorphic and nonpolymorphic metabolizing enzymes, multiple exposures present in the workplace and general environment as well as those derived from endogenous sources, and other contributing factors. The potential ability of the GSTT1 enzyme to activate some chemicals to reactive forms while detoxifying others such as EtO and the lack of its expression in target cells such as the lymphocytes other than erythrocytes also present additional difficulty in interpreting the findings. Consequently, no firm conclusions can be drawn from our finding of a protective effect of the *GSTT1*-null genotype against cytogenetic damage in relation to EtO exposure, based on the sensitive but nonspecific chemical end point of SCE. Thus, in view of the small contribution of the GSTT1 enzyme to the metabolism of EtO and the low prevalence of the null genotype that varies by ethnicity, additional studies with a larger sample size that take into consideration the varying background levels from exogenous and endogenous sources and numerous contributing factors will be needed to confirm our findings.

## References


1. IARC. Ethylene oxide. IARC Monogr. Eval. Carcinog. Risk Hum., *60:* 73–159, 1994.

2. United States Department of Labor. Occupational Exposure to Ethylene Oxide. United States Code of Federal Regulations, Title 29, Part 1910, Vol. 48, pp. 17289–17319, 1983.

3. Granath, F., Rohlen, O., Goransson, C., Hansson, L., Magnusson, A., and Tornqvist, M. Relationship between dose *in vivo* of ethylene oxide and exposure to ethene studied in exposed workers. Hum. Exp. Toxicol., *15:* 826–833, 1996.

4. Tornqvist, M., Gustaffson, B., Kautiainen, A., Harms-Ringdahl, M., Granath, F., and Ehrenberg, L. Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethene and ethylene oxide. Carcinogenesis (Lond.), *10:* 39–41, 1989.

5. Filser, J. G., Denk, B., Tornqvist, M., Kessler, W., and Ehrenberg, L. Pharmacokinetics of ethylene in man: body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene. Arch. Toxicol., *66:* 157–163, 1992.

6. Dellarco, V. L., Generoso, W. M., Sega, G. A., Fowle, J. R., III, and Jacobson-Kram, D. Review of the mutagenicity of ethylene oxide. Environ. Mol. Mutagen., *16:* 85–103, 1990.

7. Segerback, D. Reaction products in hemoglobin and DNA after *in vitro* treatment with ethylene oxide and *N*-(2-hydroxyethyl)nitrosurea. Carcinogenesis (Lond.), *11:* 307–312, 1990.

8. Krishnan, K., Gargas, M. L., Fennell, T. R., and Andersen, M. E. A physiologically based description of ethylene oxide dosimetry in the rat. Toxicol. Ind. Health, *8:* 121–140, 1992.

9. Brown, C. D., Wong, B. A., and Fennell, T. R. *In vivo* and *in vitro* kinetics of ethylene oxide metabolism in rats and mice. Toxicol. Appl. Pharmacol., *136:* 8–19, 1996.

10. Coles, B., and Ketterer, B. The role of glutathione and glutathione transferases in chemical carcinogenesis. Crit. Rev. Biochem. Mol. Biol., *25:* 47–70, 1990.

11. Hayes, J. D., and Pulford, D. J. The glutatione *S*-transferases supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol., *30:* 445–600, 1995.

12. Seidegard, J., Vorachek, W. R., Pero, R. W., and Pearson, W. R. Hereditary differences in the expression of the human glutathione *S*-transferase activity on *trans*-stilbene oxide are due to a gene deletion. Proc. Natl. Acad. Sci. USA, *85:* 7293–7297, 1988.

13. Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., and Taylor, J. B. Human glutathione *S*-transferase $\theta$ (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem. J., *300:* 271–276, 1994.

14. Schroder, K. R., Hallier, E., Peter, H., and Bolt, H. M. Dissociation of a new glutathione *S*-transferase activity in human erythrocytes. Biochem. Pharmacol., *43:* 1671–1674, 1992.

15. Nelson, H. H., Wiencke, J. K., Christiani, D. C., Cheng, T. J., Zuo, Z. F., Schwartz, B. S., Lee, B. K., Spitz, M. R., Wang, M., Xu, X. P., and Kelsey, K. T. Ethnic differences in the prevalence of the homozygous deleted genotypes of glutathione *S*-transferase $\theta$. Carcinogenesis (Lond.), *16:* 1243–1245, 1995.

16. Strange, R. C., and Fryer, A. A. The glutathione *S*-transferases: influence of polymorphism on susceptibility to cancer. *In:* P. Boffetta, N. Caproraso, J. Cuzick, M. Lang, and P. Vineis (eds.), Metabolic Polymorphisms and Cancer, pp. 231–249. Lyon, France: IARC, 1999.

17. van Poppel, G., de Vogel, N., van Balderen, P. J., and Kok, F. J. Increased cytogenetic damage in smokers deficient in glutathione *S*-transferase isoenzyme $\mu$. Carcinogenesis (Lond.), *13:* 303–305, 1992.

18. Fost, U., Hallier, E., Ottenwalder, H., Bolt, H. M., and Peter, H. Distribution of ethylene oxide in human blood and its implication for biomonitoring. Hum. Exp. Toxicol., *10:* 25–31, 1991.

19. Hallier, E., Langhof, T., Dannappel, D., Leubecher, M., Schroder, K., Goergens, H. W., Muller, A., and Bolt, H. M. Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. Arch. Toxicol., *67:* 173–178, 1993.

20. Schulte, P. A., Boeniger, M., Walker, J. T., Schober, S. E., Pereira, M. A., Gulati, D. K., Wojciechowski, J. P., Garza, A., Froelich, R., Strauss, G., Halperin, W. E., Herrick, R., and Griffith, J. Biologic markers in hospital workers exposed to low levels of ethylene oxide. Mutat. Res., *278:* 237–251, 1992.

21. Wraith, M. J., Watson, W. P., Eadsforth, C. V., van Sittert, N. J., Tornqvist, M., and Wright, A. S. An immunoassay for monitoring human exposure to ethylene oxide. *In:* H. Bartsch, K. Hemminki, and I. K. O'Neill (eds.), Methods for Detecting DNA Damaging Agents in Humans. Applications in Cancer Epidemiology and Prevention, pp. 271–274. Lyon, France: IARC, 1988.

22. Carrano, A. V., and Moore, D. H. The rationale and methodology for quantifying sister chromatid exchange in humans. *In:* J. A. Heddle (ed.), Mutagenicity: New Horizons in Genetic Toxicology, pp. 268–306. New York: Academic Press, 1982.

23. Wiencke, J. K., Pemble, S., Ketterer, B., and Kelsey, K. T. Gene deletion of glutathione *S*-transferase $\theta$: correlation with induced genetic damage and potential role in endogenous mutagenesis. Cancer Epidemiol. Biomark. Prev., *4:* 253–259, 1995.

24. Ehrenberg, L., and Tornqvist, M. The research background for risk assessment of ethylene oxide: aspects of dose. Mutat. Res., *330:* 41–54, 1995.

25. Segerback, D. Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. Chem. Biol. Interact., *45:* 139–151, 1983.

26. Farmer, P. B., Neumann, H-G., and Henschler, D. Estimation of exposure of man to substances reacting covalently with macromolecules. Arch. Toxicol., *60:* 251–260, 1987.

27. Fennell, T. R. Biological markers of exposure to chemical carcinogens. CIIT Activities, *10:* 1–7, 1990.

28. Fost, U., Tornqvist, M., Leutbecher, M., Granath, F., Hallier, E., and Ehrenberg, L. Effects of variation in detoxification rate on dose monitoring through adducts. Hum. Exp. Toxicol., *14:* 201–203, 1995.

29. Fennell, T. R., MacNeela, J. P., Morris, R. W., Watson, M., Thompson, C. L., and Bell, D. A. Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione transferase T1 and M1 genotypes. Cancer Epidemiol. Biomark. Prev., *9:* 705–712, 2000.

30. Muller, M., Kramer, A., Angerer, J., and Hallier, E. Ethylene oxide-protein adduct formation in humans: influence of glutathione *S*-transferase polymorphisms. Int. Arch. Occup. Environ. Health, *71:* 499–502, 1998.

31. Bailey, E., Brooks, A. G. F., Doller, C. T., Farmer, P. B., Passingham, B. J., Sleightholm, M. A., and Yates, D. W. Hydroxyethylvaline adduct formation in hemoglobin as a biological marker of cigarette smoke intake. Arch. Toxicol., *62:* 247–253, 1988.

32. Bono, R., Vinventi, M., Meineri, V., Pignata, C., Saglia, U., Giachino, O., and Scursatone, E. Formation of *N*-(2-hydroxyethyl)valine due to exposure to ethylene oxide via tobacco smoke: a risk factor for onset of cancer. Environ. Res., *81:* 62–71, 1999.

33. Juronen, E., Tasa, G., Uuskula, M., Pooga, M., and Mikelsaar, A-V. Purification, characterization and tissue distribution of human class $\theta$ glutathione *S*-transferase T1–1. Biochem. Mol. Biol. Int., *39:* 21–29, 1996.

34. Wang, L., Groves, M. J., Hepburn, M. D., and Bowen, D. T. Glutathione *S*-transferase enzyme expression in hematopoietic cell lines implies a differential protective role for T1 and A1 isoenzymes in erythroid and for M1 in lymphoid lineages. Haematologica, *85:* 573–579, 2000.

35. Tucker, J. D., Auletta, A., Cimino, M. C., Dearfield, K. L., Jacobson-Kram, D., Tice, R. R., and Carrano, A. Sister-chromatid exchange: second report of the Gene-Tox program. Mutat. Res., *297:* 101–180, 1993.

36. Tates, A. D., Grummt, T., Tornqvist, M., Farmer, P. B., van Dam, F. J., van Mossel, H., Schoemaker, H. M., Osterman-Golkar, S., Uebel, C., Tang, Y. S., Zwinderman, A. H., Natarajan, A. T., and Ehrenberg, L. Biological and chemical


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monitoring of occupational exposure to ethylene oxide. Mutat. Res., *250:* 483–497, 1991.

37. Sarto, F., Faccioli, M. C., Cominato, I., and Levis, A. G. Aging and smoking increase the frequency of sister-chromatid exchanges (SCE) in man. Mutat. Res., *144:* 183–187, 1985.

38. Schroder, K. R., Wiebel, F. A., Reich, S., Dannappel, D., Bolt, H. M., and Hallier, E. Glutathione *S*-transferase (GST) $\theta$ polymorphism influences background SCE rate. Arch. Toxicol., *69:* 505–507, 1995.

39. Thier, R., Taylor, J. B., Pemble, S. E., Humphreys, W. G., Persmark, M., Ketterer, B., and Guengerich, F. P. Expression of mammalian glutathione *S*-transferase 5-5 in *Salmonella typhimurium* TA1535 leads to base-pair mutations upon exposure to dihalomethanes. Proc. Natl. Acad. Sci. USA, *90:* 8576–8580, 1993.

40. Thier, R., Pemble, S. E., Kramer, H., Taylor, J. B., Guengerich, F. P., and Ketterer, B. Human glutathione *S*-transferase T1–1 enhances mutagenicity of 1,2-dibromoethane and 1,2,3,4-diepoxybutane in *Salmonella typhimurium.* Carcinogenesis (Lond.), *17:* 163–166, 1996.

41. Fennell, T. R. Biomarkers of exposure and susceptibility: application to ethylene oxide. CIIT Activities, *16:* 1–8, 1996.

42. Daly, A. K., Cholerton, S., Gregory, W., and Idle, J. R. Metabolic polymorphisms. Pharmacol. Ther., *57:* 129–160, 1993.

43. Farmer, P. B., Cordero, R., and Autrup, H. Monitoring human exposure to 2-hydroxyethylating carcinogens. Environ. Health Perspect., *104* (Suppl. 3)*:* 449–452, 1996.

44. Chen, C-L., Liu, Q., and Relling, M. V. Simultaneous characterization of glutathione *S*-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks. Pharmacogenetics, *6:* 187–191, 1996.

45. Lin, H. J., Han, C-Y., Bernstein, D. A., Hsiao, W., Lin, B. K., and Hardy, S. Ethnic distribution of the glutathione transferase $\mu$ 1-1 (GSTM1) null genotype in 1473 individuals and application to bladder cancer susceptibility. Carcinogenesis (Lond.), *15:* 1077–1081, 1994.

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# Hemoglobin Adducts and Sister Chromatid Exchanges in Hospital Workers Exposed to Ethylene Oxide: Effects of Glutathione S-Transferase T1 and M1 Genotypes

Lee C. Yong, Paul A. Schulte, John K. Wiencke, et al.

*Cancer Epidemiol Biomarkers Prev* 2001;10:539-550.

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