AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 50:293–302 (2007)

DNA Adducts in Granulocytes of Hospital Workers Exposed to Ethylene Oxide

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Background *Ethylene oxide (EtO), an important industrial chemical intermediate and sterilant, is classified as a human carcinogen. Occupational EtO exposure in many countries is regulated at 1 ppm (8-hr TWA), but levels of EtO-DNA adducts in humans with low occupational EtO exposures have not been reported.*

Methods *We examined the formation of N7-(2′-hydroxyethyl)guanine (N7-HEG), a major DNA adduct of EtO, in 58 EtO-exposed sterilizer operators and six nonexposed workers from ten hospitals. N7-HEG was quantified in granulocyte DNA (0.1–11.5 μg) by a highly sensitive and specific gas chromatography-electron capture-mass spectrometry method. Cumulative exposure to EtO (ppm-hour) was estimated during the 4-month period before the collection of blood samples.*

Results *There was considerable inter-individual variability in the levels of N7-HEG with a range of 1.6–241.3 adducts/10^7 nucleotides. The mean levels in the nonexposed, low (≤32 ppm-hour), and high (>32 ppm-hour) EtO-exposure groups were 3.8, 16.3, and 20.3 adducts/10^7 nucleotides, respectively, after the adjustment for cigarette smoking and other potential confounders, but the differences were not statistically significant.*

Conclusions *This study has demonstrated for the first time, detectable levels of N7-HEG adducts in granulocytes of hospital workers with EtO exposures at levels less than the current U.S. standard of 1 ppm (8-hr TWA). A nonsignificant increase in adduct levels with increasing EtO exposure indicates that further studies of EtO-exposed workers are needed to clarify the relationship between EtO exposure and N7-HEG adduct formation.* Am. J. Ind. Med. 50:293–302, 2007. © *2007 Wiley-Liss, Inc.*

KEY WORDS: ethylene oxide; DNA adducts; granulocytes; hospital workers; occupational exposure; N7-(2′-hydroxyethyl)guanine; gas chromatography-electron capture-mass spectrometry

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The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Accepted 14 January 2007
DOI 10.1002/ajim.20443. Published online in Wiley InterScience www.interscience.wiley.com

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INTRODUCTION

Ethylene oxide (EtO) is an important industrial chemical used primarily as an intermediate for the manufacture of ethylene glycol, glycol ethers, and other products that have many applications [IARC, 1994]. EtO is also used directly in the gaseous form as a sterilizing agent for a variety of heat-sensitive materials, such as agricultural and medical products and hospital equipment [IARC, 1994]. It has been estimated that approximately 80,000 U.S. health care workers, 144,000 medical device and related industry workers, and as many as 100,000 health care technicians are exposed directly or incidentally to EtO in the workplace [10th Report on Carcinogens, NTP 2002]. In addition to occupational sources, EtO is formed metabolically from ethylene, a constituent of cigarette smoke and polluted urban air [Tornqvist et al., 1986, 1988; Granath et al., 1996] as well as a product of endogenous metabolic processes [Tornqvist et al., 1989; Filser et al., 1992].

EtO has been shown to be mutagenic and genotoxic in a variety of biological systems, including the induction of gene mutations, sister chromatid exchanges (SCE), chromosomal aberrations, and micronuclei in both experimental animals and occupationally exposed workers [Dellarco et al., 1990; IARC, 1994; Kolman et al., 2002]. The International Agency for Research on Cancer has classified EtO as a human carcinogen based largely on animal and mechanistic data from cytogenetic studies [IARC, 1994]. The findings on EtO exposure and cancer risk from the epidemiologic studies conducted thus far [IARC, 1994], including the recent updates of a large U.S. [Steenland et al., 2004] and a British [Coggon et al., 2004] cohort of EtO-exposed workers, are conflicting. The carcinogenic risk of EtO in humans, particularly in relation to low levels of occupational exposure, is therefore uncertain and requires further investigation. Occupational exposure to EtO in many countries is regulated at 1 ppm (8-hr TWA) [IARC, 1994].

Many carcinogenic chemicals (either directly or after metabolic activation) cause DNA damage by reacting covalently with nucleophilic sites in DNA to form adducts [Miller and Miller, 1981]. The formation of DNA adducts is believed to be an important early event in carcinogenesis because it may give rise to mutations that can trigger the carcinogenesis process [Hemminki et al., 2000]. In addition, DNA adducts are considered as a measure of DNA damage which reflects not only exposure to a genotoxic agent, but also individual differences in absorption, metabolism, and DNA repair capacity [Nestmann et al., 1996; Vineis and Perera, 2000]. The quantification of EtO-induced DNA adducts in biological samples is therefore of interest since this can provide individual exposure and mechanistic data for the evaluation of the carcinogenic effect of EtO.

EtO is a direct-acting alkylating agent which forms adducts with cellular macromolecules, including proteins and DNA, without requiring metabolic activation [Dellarco et al., 1990; Segerback, 1990; IARC, 1994]. N7-(2′-hydroxyethyl)guanine (N7-HEG), which represents about 90% of the alkylated sites, is the major DNA adduct induced in vitro [Segerback, 1990; Li et al., 1992] and in experimental animals [Segerback, 1983; Walker et al., 1993; Wu et al., 1999; van Sittert et al., 2000]. Although N7-HEG adducts are not considered directly promutagenic, they are measurable, and thus could serve as a surrogate biomarker for other minor and more promutagenic DNA adducts of EtO [van Sittert et al., 2000]. Several analytical methods have been used for DNA adduct determination [Phillips et al., 2000]; however, they are limited in sensitivity or specificity for quantifying the low endogenous or occupationally induced levels of N7-HEG adducts in a small quantity of DNA.

In a group of sterilizer operators occupationally exposed to less than 1 ppm of EtO (8-hr TWA) and nonexposed controls from ten hospitals, we have previously shown that the level of the hemoglobin adducts, N-(2′-hydroxyethyl)valine (HEV) was significantly associated with EtO exposure [Schulte et al., 1992]. Subjects with homozygous deletion of the *glutathione S-transferase T1* (*GSTT1*) gene (null genotype) were also found to have a significantly higher mean level of HEV as compared to subjects with at least one copy of the gene (positive genotype) [Yong et al., 2001]. Recently, an improved chromatography-electron capture-mass spectrometry (GC-EC-MS) method has been developed for the analysis of N7-HEG adducts in human tissues [Kao and Giese, 2005]. Here this technique is used to quantify N7-HEG adducts in the small quantity of granulocyte DNA remaining from earlier studies of this group of subjects. The granulocytes, which have a unique short life span [Godschalk et al., 1998], are of interest because they have not been examined for the formation of N7-HEG adducts in relation to occupational EtO exposure.

In this report, we present the levels of N7-HEG adducts detected in this group of hospital workers and relate them to two measures of EtO exposure: (i) the estimated 4-month cumulative occupational EtO exposure, and (ii) the HEV adduct level as an indicator of individual internal EtO dose. Additionally, we examine the possible confounding effects of age, gender, race, education, cigarette smoking, and polymorphism in the *GSTT1* gene on the N7-HEG adduct-EtO exposure relationship.

MATERIALS AND METHODS

Subjects

The subjects of this study were a subset of a group of 73 workers from nine hospitals in the U.S. and one hospital in Mexico City previously studied in 1987 [Schulte et al., 1992]. The workers were generally employed in the central sterile

supply departments and were exposed to EtO during the unloading of sterilizers or when working adjacent to sterilizers. In each hospital, workers with the lowest and highest potential for cumulative exposure to EtO were selected based on hospital records of the number of times they had unloaded sterilizers during the previous four months. A group of controls consisting of nonexposed workers who handled no unloadings or who worked in departments where EtO was not used, was also included in the study. For each subject, a blood sample was collected during the 2- to 4-day EtO-exposure monitoring period. Data on demographic information, health, medical and occupational history, and cigarette smoking history were obtained from each subject using an interviewer-administered questionnaire. All subjects provided written informed consent, and the study was approved by the National Institute for Occupational Safety and Health's Human Subjects Review Board.

Exposure Assessment

Exposure to EtO was measured based on the personal breathing zone, stationary area, and grab air samples collected in all work areas of each of the ten hospitals during the 2- to 4-day monitoring period, as described in detail previously [Schulte et al., 1992]. For each exposed subject, a 4-month cumulative exposure to EtO was estimated 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 judgment of an industrial hygienist. Each task was considered a discrete activity, such as the unloading of sterilizers, taking inventory or wrapping instrument packages. Using an algorithm, each subject's 4-month cumulative EtO exposure (in ppm-hour) was computed as 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 subject's estimated cumulative exposure largely reflects the duration of time spent in the performance of the various tasks over a 4-month period which was selected to correspond to the life span of the erythrocytes. The mean 8-hr TWA was also computed for each subject based on his/her job tasks, weighted by the duration of each task at his/her hospital [Schulte et al., 1992]. For the nonexposed subjects, the EtO-exposure concentrations of the personal breathing zone and stationary area air samples in their work areas were below the limit of detection. Consequently, the estimated 4-month cumulative exposure and 8-hr TWA of the nonexposed subjects were arbitrarily assigned a value of zero.

Laboratory Assays

During the 2- to 4-day exposure monitoring periods in 1987, when the study was conducted, blood specimens from each subject were collected in heparinized tubes, coded, and immediately transported on ice to a central laboratory for processing, as described previously in detail [Schulte et al., 1992]. Within 12 hr, the mononuclear (lymphocytes and monocytes) and polymorphonuclear cells (granulocytes) along with erythrocytes were isolated by Ficoll–Hypaque density gradient separation. The HEV adducts in erythrocytes were determined by the radioimmunoassay method developed by Wraith et al. [1988]. The assay of SCE was performed using the method of Carrano and Moore [1982] on lymphocytes. The remaining granulocytes were treated with lysis buffer to eliminate the contaminating erythrocytes, washed with media, frozen at a feedback-controlled rate, and stored in liquid nitrogen (-120°C) for the conduct of future assays.

In 1998, DNA was extracted from the stored granulocytes using a QIAamp Kit (QIAGEN, Inc., Valencia, CA) in accordance to instructions except that the digestion was conducted for 2 hr at room temperature, and the elution was performed in 400 µl of water. DNA concentration was measured with a Hoechst dye-binding assay. The DNA solutions in water were stored in plastic vials at -20°C until the N7-HEG adduct analysis in 2003.

The N7-HEG adducts were analyzed in 0.1–11.5 µg of DNA by a GC-EC-MS method, as described in detail elsewhere by Kao and Giese [2005]. With the exception of one sample, all coded samples were analyzed only once due to the small amount of available DNA. In this method, the DNA samples were spiked with a stable isotope internal standard (N7-HEG-d_4), functioning as a mimic of the analyte, at the onset of the analysis to provide accurate and precise results. This was followed by neutral thermal hydrolysis where the samples were heated in water to release the adducts in a nucleobase form. After the adducts have been extracted into 1-butanol, they were purified by reverse phase HPLC and derivatized with HONO, pentafluorobenzyl bromide, and pivalic anhydride. Further purification by silica solid phase extraction and reverse phase HPLC was done prior to injection into a GC-EC-MS. This has contributed to the high sensitivity observed as shown by the relatively clean GC-EC-MS chromatograms [Kao and Giese, 2005]. The N7-HEG adducts were detected in all samples with levels of 11.1 and 15.2 adducts/10^7 nucleotides in the duplicate sample.

GSTT1 Genotyping

The *GSTT1* genotype was determined previously on the stored DNA using a PCR method as described by Wiencke et al. [1995]. For each gene, the subjects were categorized as

either null (homozygous gene deletion) or positive (at least one copy of the gene present).

Statistical Analysis

Linear regression analysis was used to examine the relationship between the level of N7-HEG adducts and the 4-month cumulative EtO exposure and HEV adduct level (as continuous variables) using separate models. Adjustment for cigarette smoking and other potential confounders was performed by including these variables in the separate multiple regression models. The relationships between the level of N7-HEG adducts and either measure of EtO exposure were not statistically significant, univariately or after adjusting for potential confounders. Therefore, to ease interpretation, we categorized the EtO exposure as described below. We used the analysis of variance (ANOVA) to compare the mean levels of N7-HEG adducts among the categories of EtO exposure, HEV adducts, cigarette smoking exposure, the *GSTT1* genotype, and other variables of interest. This was followed by the analysis of covariance to assess the difference in the mean levels of N7-HEG adducts among the EtO-exposure and HEV adduct groups after adjusting for potential confounders which included age, gender, race, years of education, and cigarette smoking. Effect modifications by cigarette smoking and the *GSTT1* genotype were examined by introducing cross-product terms in the ANOVA models. The analyses were also performed stratified by EtO and cigarette smoking exposure status.

Subjects were categorized as nonexposed or were in the low ($\leq$32 ppm-hour) or high ($>$32 ppm-hour) EtO-exposure group based on an observable break in the data (a range of 3–32 and 62–869 ppm-hour). The choice of 32 ppm-hour as the cut point between low and high EtO exposure is supported by a priori selection of the subjects with potential for low and high exposure and by the results of other previously studied cytogenetic markers based on this cut point [Schulte et al., 1992; Yong et al., 2001]. In the case of the HEV adducts, exposed subjects were dichotomized at the median level (0.07 pmol/mg HB).

Before the conduct of all statistical analyses, the distributions of the level of N7-HEG adducts and the continuous demographic and smoking variables (when necessary) were normalized, and their variances were stabilized by $\log_e$ transformation. The geometric means of the N7-HEG adduct levels within each group presented in the tables are the exponentiated means of the $\log_e$ transformed variable. However, for ease of interpretation and comparison with other studies, the arithmetic means, standard deviations, and standard errors of the mean adduct level are presented in the results and discussion. All analyses were performed using the SAS software version 9.0 (SAS Institute, Inc., Cary, NC), and a P-value <0.05 (two-sided) was considered statistically significant.

RESULTS

The subjects of this component of the study were restricted to those who had sufficient DNA for the N7-HEG adduct analysis. The ages of the 64 subjects (all females except for 4 males) ranged from 22 to 70 years, with a mean of 39 years (Table I). There were 26 white (41%) and 19 black (30%) subjects from the U.S., and 19 Hispanic (30%) subjects from Mexico. The majority of the subjects (53%) were never smokers, 31% were current smokers, and 16% were former smokers.

The characteristics of the subjects according to the EtO-exposure status are summarized in Table I. Of the total subjects, 6 (9%) were in the nonexposed group, 38 (59%) in the low ($\leq$32 ppm-hour), and 20 (31%) in the high ($>$32 ppm-hour) EtO-exposure group. The mean 4-month cumulative exposures for the low and high EtO-exposure groups were 12.3 and 234.7 ppm-hour, respectively. The corresponding mean 8-hr TWAs were 0.03 and 0.36 ppm. The EtO-exposure groups did not differ greatly with respect to age, years of education, length of employment in current job, and gender. There were moderate differences in terms of race and smoking status with the high EtO-exposure group being more likely to be Hispanic and to have smoked cigarettes. The prevalence of the *GSTT1* null genotype was 19% ($n = 12$) in the overall group of subjects, 18% ($n = 7$) in the low, and 26% ($n = 5$) in the high EtO-exposure group, but with none in the nonexposed group (Table I).

Table II presents the arithmetic mean $\pm$ SE, range, and geometric mean of the levels of N7-HEG adducts according to categories of age, gender, race, years of education, and the *GSTT1* genotype. The mean N7-HEG adduct level was higher in females versus males, black versus Hispanic or white, and in those with $>$40 versus $\leq$40 years of age as well as in those with $<$12 versus $\geq$12 years of education. The differences, however, were not statistically significant. The *GSTT1*-null genotype subjects had a significantly higher mean level for the HEV adducts (data not shown), but not for the N7-HEG adducts (Table II) as compared to the *GSTT1*-positive genotype subjects. Therefore, further results on the *GSTT1* genotype are not presented.

Because occupational EtO exposure and cigarette smoke are both important sources of EtO, we next examined the effect of EtO exposure on the N7-HEG adduct level stratified by smoking status (Table III). For the five never smokers not occupationally exposed to EtO, individual levels of 1.6, 2.7, 4.4, 7.0, and 38.5 adducts/10^7 nucleotides (mean = 10.8) were observed. Among the never smokers, there was an indication of a lower mean adduct level in the high versus the low EtO-exposure group, both before and after adjusting for age, gender, race, and education. The mean adjusted levels in the high (8.2 adducts/10^7 nucleotides) and low (21.4 adducts/10^7 nucleotides) EtO-exposure groups were higher than that of the nonexposed group (3.1 adducts/10^7

TABLE I. Selected Characteristics of Study Subjects (Overall and by EtO-Exposure Status)

		Exposure status		
Variable	**Total (n = 64)**	**Nonexposed (n = 6)[a]**	**≤32 ppm-hour (n = 38)**	**>32 ppm-hour (n = 20)**
Age (years)	38.7 ± 12.7[b]	48.0 ± 18.3	37.3 ± 11.6	38.7 ± 12.1
Education (years)	12.8 ± 1.8	12.7 ± 2.2	12.8 ± 1.6	12.8 ± 2.1
Employment (years)[c]	5.6 ± 4.6	8.0 ± 7.4	4.7 ± 3.6	6.5 ± 4.9
4-month cumulative exposure (ppm-hour)	80.6 ± 150.4	0	12.3 ± 9.6	234.7 ± 196.0
8-hr TWA (ppm)[d]	0.13 ± 0.23	0	0.03 ± 0.05	0.36 ± 0.31
Race				
White	26 (40.6)[e]	2 (33.3)	21 (55.3)	3 (15.0)
Black	19 (29.7)	3 (50.0)	9 (23.7)	7 (35.0)
Hispanic	19 (29.7)	1 (16.7)	8 (21.0)	10 (50.0)
Gender				
Male	4 (6.2)	0 (0)	3 (7.9)	1 (5.0)
Female	60 (93.8)	6 (100)	35 (92.1)	19 (95.0)
Smoking status				
Never	34 (53.1)	5 (83.3)	21 (55.3)	8 (40.0)
Former	10 (15.6)	0 (0)	3 (7.9)	7 (35.0)
Current	20 (31.3)	1 (16.7)	14 (36.8)	5 (25.0)
GSTT1 genotype[f]				
Null	12 (19.3)	0 (0)	7 (18.4)	5 (26.3)
Positive	50 (80.7)	5 (100)	31 (81.6)	14 (73.7)

[a]Subjects with EtO-exposure levels below the lowest limit of detection were assigned a value of 0 ppm-hour for the 4-month cumulative exposure and the 8-hr TWA.
[b]Arithmetic mean ± SD.
[c]n = 63 in total and n = 37 for the ≤32 ppm-hour group.
[d]The mean shown is the average of the 8-hr TWAs for the job-tasks of subjects in designated exposure category.
[e]n (percentage in parentheses).
[f]Two subjects with unknown *GSTT1* genotype.

nucleotides), but the differences were not statistically significant.

The former smokers, as a group (n = 10) did not differ greatly from the current smokers (n = 20) with respect to several cigarette exposure indices: average number of cigarettes smoked per day (mean = 12.2 vs. 11.3); age first smoked (mean = 19.6 vs. 19.2); years of smoking (mean = 14.8 vs. 16.8); and pack-years (packs of cigarettes smoked per day multiplied by years of smoking) as an indicator of the cumulative dose of cigarette exposure (mean = 13.0 vs. 10.4) (data not shown). However, former smokers have a higher mean N7-HEG adduct level (30.8 adducts/10^7 nucleotides) than either the never (15.5 adducts/10^7 nucleotides) or current (9.7 adducts/10^7 nucleotides) smokers (data not shown). Additionally, because the majority of former smokers quit smoking within the 2-year period before blood sampling and cigarette smoking history was not verified but was based on self-reports, data from both former and current smokers were combined for the analysis (Table III). There was only one smoker not occupationally exposed to EtO: a current smoker with an average of eight cigarettes/day and a total of 3.5 pack-years, and an N7-HEG adduct level of 3.5 adducts/10^7 nucleotides. Therefore, this subject was excluded from the analyses that evaluated the separate effects of EtO and cigarette smoking exposure on the adduct levels. Among the smokers, the mean adduct level was found to be higher in the high than in the low EtO-exposure group, both before and after adjusting for age, gender, race, and years of education. However, the adjusted mean adduct levels for smokers in the high (37.3 adducts/10^7 nucleotides) and low (19.2 adducts/10^7 nucleotides) EtO-exposure groups were not statistically different.

There was also an indication of an increase in the mean adduct levels across the combined EtO-exposure (low and high) and intensity of cigarette exposure (≤10 and >10 cigarettes per day) groups (Table III). Among the smokers, the adjusted mean adduct level was highest in the high EtO-exposure and high cigarette smoking intensity group (63.6 adducts/10^7 nucleotides) and was lowest in the low EtO-exposure and low cigarette smoking intensity group (15.0 adducts/10^7 nucleotides), but the difference was not statistically significant.

Due to the lack of a significant interaction between EtO and cigarette exposure on the N7-HEG adduct levels and to

TABLE II. Levels of N7-HEG Adducts According to Age, Gender, Race, Education, and *GSTT1* Genotype*

	N7-HEG adducts/10^7 nucleotides			
Variable	**n**	**Arithmetic mean ± SE**	**Range**	**Geometric mean**
Age (years)				
≤40	40	11.4 ± 2.5	1.8–88.4	6.4
>40	24	23.8 ± 10.8	1.6–241.3	6.9
Gender				
Male	4	7.4 ± 2.1	1.9–11.8	6.1
Female	60	16.6 ± 4.6	1.6–241.3	6.6
Race				
White	26	10.0 ± 2.8	1.7–61.4	5.5
Black	19	26.6 ± 13.3	1.6–241.3	7.7
Hispanic	19	13.9 ± 4.6	1.9–88.4	7.1
Education (years)				
<12	7	46.2 ± 32.8	1.6–241.3	14.3
≥12	57	12.4 ± 2.7	1.6–109.8	6.0
GSTT1 genotype[a]				
Null	12	17.4 ± 9.0	1.6–109.8	6.8
Positive	50	15.2 ± 5.1	1.6–241.3	6.2

*All *P*-values for differences among groups on $\log_e$-transformed data using analysis of variance, are >0.05.
[a]Two subjects with unknown *GSTT1* genotype status.

increase the sample size, the results are presented for the overall group of subjects categorized according to the 4-month cumulative EtO exposure (Table IV). A large inter-individual variation in the adduct levels was observed among the groups: a range of 1.6–38.5, 1.6–109.8, and 1.9–241.3 adducts/10^7 nucleotides in the nonexposed, low, and high EtO-exposure groups, respectively. There was an increase in the mean levels of the adducts across the EtO-exposure groups, both before and after adjusting for age, gender, race, education, and cigarette smoking. A four- to five-fold difference in the adjusted mean levels in the low (16.3 adducts/10^7 nucleotides) and high (20.3 adducts/10^7 nucleotides) EtO-exposure groups as compared to the nonexposed group (3.8 adducts/10^7 nucleotides) was observed, but the differences were not statistically significant. The *GSTT1* genotype did not alter the results and therefore were not included as covariates in the analysis. No significant interaction was detected between the genotype and smoking status on the levels of the adducts (data not shown).

In this group of subjects, there was a significant relationship between the level of the HEV adducts and EtO exposure (data not shown). When the 4-month cumulative EtO exposure was replaced by the level of HEV adducts, a similar pattern of increasing mean levels of N7-HEG adducts was observed across the HEV adduct groups, both before and after adjusting for age, gender, race, education, and cigarette smoking (Table IV). The adjusted mean levels in the low (6.3 pmol/mg HB) and high (13.9 pmol/mg HB) HEV adduct group were two- to four-fold higher than that of the nonexposed group (3.6 pmol/mg HB), but the differences were not statistically different.

DISCUSSION

We utilized a GC-EC-MS method to evaluate the formation of N7-HEG, the major EtO-DNA adduct in a group of 58 hospital workers with low occupational EtO exposure (less than the U.S. 8-hr TWA permissible level of 1 ppm) and six nonexposed controls. Due to the high sensitivity of the method, the low μg amounts of granulocyte DNA were sufficient for the quantification of the low levels of N7-HEG adducts observed in this study. All subjects had detectable levels of N7-HEG adducts with a large range of 1.6–241.3 adducts/10^7 nucleotides. Although not statistically significant, the mean N7-HEG adduct levels in the low and high exposure groups were observed to be higher than in the nonexposed group when categorized according to the 4-month cumulative EtO exposure as well as the individual levels of the HEV adducts as an indicator of the internal EtO dose. This adduct pattern remained after adjusting for age, gender, race, years of education, and cigarette smoking. Cigarette smoking and the *GSTT1* genotype did not significantly modify the N7-HEG adduct-EtO exposure relationship at the level of EtO and cigarette smoking exposure examined in this study.

Our study findings may be explained by several factors, including the use of granulocytes with a life span of less than a day instead of lymphocytes with a life span of several years [Savela and Hemminki, 1991; Mustonen and Hemminki, 1992; Godschalk et al., 1998], and the timing of sample collection [Fang and Vaca, 1997]. EtO exposure in sterilization units is characterized by relatively low long-term TWA concentrations as background levels and short-term (2–30 min) peak levels which usually occur during the unloading of sterilizers [Elliott et al., 1988; Florack and Zielhuis, 1990]. In addition, the height of the background levels and the frequency, duration, and height of the peak levels which could be as high as 103 ppm, are dependent on the number of sterilization cycles, ventilation system, and work practices [Elliott et al., 1988]. Work routines for many individuals in this study typically appeared to be inconsistent. For example, the number of times they operated the sterilizer per day varied daily. Thus, in this study, the large inter-individual variation of the N7-HEG adduct levels quantified in granulocytes, may be predominantly a reflection of the recent individual variations in EtO exposure pattern. That is, they may reflect the transient peak exposures of individuals not captured in the low overall 4-month cumulative exposure estimates.

TABLE III. Levels of N7-HEG Adducts According to EtO-Exposure and Cigarette Smoking Status*

	N7-HEG adducts/10^7 nucleotides								
	Nonexposed			≤32 ppm-hour			>32 ppm-hour		
Variable	n	Arithmetic mean ± SE (range)	Geometric mean	n	Arithmetic mean ± SE (range)	Geometric mean	n	Arithmetic mean ± SE (range)	Geometric mean
Unadjusted									
Never smokers	5	10.8 ± 7.0 (1.6–38.5)	5.5	21	18.2 ± 6.6 (1.7–109.8)	7.3	8	11.1 ± 4.4 (1.9–39.2)	6.6
Smokers[a]									
All	1	—[b]	—	17	7.9 ± 2.8 (1.6–38.2)	4.6	12	30.4 ± 19.4 (2.0–241.3)	10.3
≤10 cigarettes/day	1	—	—	8	3.2 ± 0.5 (1.6–5.5)	2.9	7	15.2 ± 4.1 (3.6–33.2)	11.9
>10 cigarettes/day	0	NA[c]	NA	9	12.1 ± 4.9 (2.3–38.2)	6.9	5	51.6 ± 47.4 (2.0–241.3)	8.5
Adjusted[d]									
Never smokers[e]	5	3.1 ± 17.0	5.3	21	21.4 ± 11.8	10.2	8	8.2 ± 12.3	7.5
Smokers									
All[e]	1	—	—	17	19.2 ± 19.6	5.2	12	37.3 ± 21.4	10.8
≤10 cigarettes/day	1	—	—	8	15.0 ± 21.2	3.5	7	18.7 ± 26.4	10.9
>10 cigarettes/day	0	NA	NA	9	39.7 ± 27.3	12.8	5	63.6 ± 29.7	11.9

*All *P*-values for unadjusted and adjusted differences among groups on $\log_e$-transformed data using analysis of variance and covariance, respectively, are >0.05.
[a]Combined former and current smokers.
[b]One current smoker with 3.5 adducts/10^7 nucleotides excluded.
[c]Not applicable.
[d]Adjusted for age, gender, race, and years of education using analysis of covariance. SE of the adjusted mean involved pooling of unequal variances.
[e]Adjusted means are only comparable within the same row.

TABLE IV. Levels of N7-HEG Adducts According to EtO-Exposure Status and HEV Adduct Levels*

			N7-HEG adducts/10^7 nucleotides			
			Unadjusted		Adjusted[a]	
Variable	n	Range	Arithmetic mean ± SE	Geometric mean	Arithmetic mean ± SE	Geometric mean
Exposure status						
Nonexposed	6	1.6–38.5	9.6 ± 5.8	5.1	3.8 ± 17.9	4.7
≤32 ppm-hour	38	1.6–109.8	13.6 ± 3.9	5.9	16.3 ± 10.9	6.6
>32 ppm-hour	20	1.9–241.3	22.7 ± 11.7	8.6	20.3 ± 11.6	8.6
HEV adduct level[b]						
Nonexposed	6	1.6–38.5	9.6 ± 5.8	5.1	3.6 ± 10.5	4.5
≤0.07 pmol/mg HB	28	1.7–88.4	10.8 ± 3.6	5.3	6.3 ± 7.1	4.5
>0.07 pmol/mg HB	28	1.6–109.8	15.1 ±4.2	7.8	13.9 ± 6.4	8.5

*All *P*-values for unadjusted and adjusted differences among the EtO exposure and HEV adduct groups on $\log_e$-transformed data using analysis of variance and covariance, respectively, are >0.05.
[a]Adjusted for age, gender, race, years of education, and average number of cigarettes/day (0 for never smokers). SE of the adjusted mean involved pooling of unequal variances.
[b]HEV adduct level is unknown for two subjects. Exposed subjects are dichotomized at the median level of 0.07 pmol/mg HB.

Due to the variable nature of EtO exposures, our results suggest that granulocytes may allow a more sensitive detection of N7-HEG adducts in relation to short-term peak exposures than is possible using lymphocytes where all the exposures are integrated over a longer time period. Although exposure limits have been set for EtO in the workplace, an upward trend in exposures exceeding the short-term exposure limit has been observed in a recent survey of U.S. hospitals, suggesting that there may be a parallel increase in short-term peak exposures to workers not detected by personal monitoring [LaMontagne et al., 2004]. Since a dose-rate effect of EtO has been demonstrated in experimental mice under conditions of constant total dose [Generoso et al., 1986; Dellarco et al., 1990], our results may have important implications for workers exposed directly or incidentally to the short-term EtO peak exposures in the workplace in terms of adduct formation.

Other than exogenous sources, EtO is also formed endogenously from ethylene derived from polluted urban air and cigarette smoke as well as various metabolic processes [Tornqvist et al., 1986, 1988, 1989; Filser et al., 1992; Granath et al., 1996]. Therefore, there is a variable and measurable background level of N7-HEG adducts in addition to those derived from the occupational EtO to which the subjects of this study were exposed. One of the limitations of this study is the small sample size with only five never smokers and one current smoker in the control group. Based on quantification methods with varying degrees of sensitivity and specificity [Fost et al., 1989; Bolt et al., 1997; Wu et al., 1999; Zhao et al., 1999; Zhao and Hemminki, 2002], the background levels of N7-HEG adducts in various tissues of nonsmokers and smokers not occupationally exposed to EtO appear to be mostly less than 10 adducts/10^7 nucleotides. Thus, the N7-HEG adduct levels in our small group of control subjects (a range of 1.6–38.5 with 3.5 adducts/10^7 nucleotides detected in a smoker) are comparable to those of previous studies, and the mean level of 9.6 adducts/10^7 nucleotides falls within the expected range.

At the time of the original study, cumulative exposure to EtO was estimated during the 4-month period before the blood sampling to reflect the life span of the erythrocytes. The N7-HEG adduct levels would only be expected to reflect the exposures during at most, a few days before blood sampling that correspond to the life span of granulocytes, and a strong association with the 4-month exposure classification period was not likely. On the other hand, if exposures during this short period were representative of what occurred during the previous four months for most individuals, one would expect an increasing gradient in the level of the adducts from the nonexposed to the low and high exposure groups, as was found in this study. The mean levels of N7-HEG adducts in the nonexposed, low, and high exposure groups were 3.8, 16.3, and 20.3 adducts/10^7 nucleotides, respectively, after the adjustment for cigarette smoking and other potential confounders.

As has been previously reported [Schulte et al., 1992; Yong et al., 2001], the 4-month cumulative EtO exposure was significantly associated with the formation of HEV adducts in erythrocytes in this group of subjects. However, when we categorized the exposed subjects by HEV adducts (based on the median cut point) instead of external exposure, we did not find a significant relationship between the HEV and N7-HEG adducts, as one would expect (see below). Instead, an increasing gradient in the mean level of N7-HEG adducts of a lesser degree was observed in the nonexposed, low, and high HEV adduct groups (3.6, 6.3, and 13.9 adducts/10^7

nucleotides, respectively) after adjusting for cigarette smoking and other potential confounders. This may be partly explained by the stability of HEV adducts in hemoglobin as their removal is determined by the life span of the erythrocytes [Boogaard, 2002]. In contrast, the N7-HEG adducts with a half-life varying from 1.0 to 6.9 days in different rat and mouse tissues, are unstable as they are subjected to losses by spontaneous depurination and repair [Walker et al., 1993; van Sittert et al., 2000]. This complexity of correlating the N7-HEG and HEV adducts was similarly reported in a time-course study of rats and mice with repeated exposures to EtO by inhalation [Walker et al., 1993]. Thus, although the HEV adduct level is a better exposure metric for individuals than air monitoring and gives a measure of the individual internal EtO dose [Boogaard, 2002], it may not provide a good assessment of the formation of N7-HEG adducts in humans when the actual exposures are intermittent and vary on a day-to-day basis.

The granulocyte samples were stored together for 11 years at −120°C before DNA extraction, and the resulting DNA samples in water were subsequently stored in water at −20°C for another 5 years before the N7-HEG adduct analysis. During storage in water, there could have been some depurination of the adducts prior to complete depurination by neutral thermal hydrolysis. However, this would not affect the levels of adducts quantified, since the stored DNA samples were directly subjected to neutral thermal hydrolysis. It is also unlikely that artifacts could arise from the analytical process due to the use of an internal standard and an analyte with a unique structure, and with extra precautions taken to control contamination. Furthermore, although not all samples were analyzed in duplicate, little variation in the adduct level was seen in a duplicate sample. In prior work on method development [Kao and Giese, 2005], it has been demonstrated that the agreement (coefficient of variation) between triplicate 10 pg standards subjected to the method was 3%, and values for duplicate DNA samples spiked with 11 pg of N7-HEG differed by only 2%.

In summary, using a highly sensitive and specific GC-EC-MS method, this study has demonstrated for the first time, detectable levels of N7-HEG adducts in granulocytes of hospital workers with EtO exposures at levels less than the current U.S. standard of 1 ppm (8-hr TWA). Our data indicate that individuals varied considerably in the levels of N7-HEG adducts. The individual level of N7-HEG adducts was not significantly associated with either the average 4-month cumulative EtO exposure or the individual HEV adduct level. However, a nonsignificant increasing gradient in the mean N7-HEG adduct level was observed in the nonexposed, low, and high EtO-exposure group of workers when categorized by either exposure metric. Although this could be due to chance, it is also possible that the trend exists, but that this study had insufficient power to detect it statistically. Additionally, it could be speculated that granulocytes may allow a sensitive detection of N7-HEG adducts in relation to short-term peak EtO exposures. EtO is still being used extensively in hospitals and industries due to the lack of a satisfactory substitute for EtO as a sterilizing agent. Therefore, further studies of EtO-exposed workers are needed to clarify the relationship between EtO exposure and N7-HEG adduct formation.

ACKNOWLEDGMENTS

The authors thank Dr. James T. Walker, NIOSH, for his help with data management during the conduct of the original study.

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