# Molecular, Cytogenetic, and Hematologic Effects of Ethylene Oxide on Female Hospital Workers

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*Women comprise the majority of workers exposed to ethylene oxide during sterilization of medical instruments and supplies. This article evaluates molecular, cytogenetic, and hematologic effects of ethylene oxide on 68 women workers employed in nine hospitals in the United States and one hospital in Mexico. Workers were classified by three exposure categories: none (0), low (>0–32 ppm-hrs), and high (>32 ppm-hrs). Hematologic effects were evaluated using complete blood count with differential, which has been questioned as a test for screening ethylene oxide-exposed workers. A statistically significant decrease in hematocrit ($n = 0.02$) and hemoglobin ($P = 0.03$) levels, an increase in lymphocyte percentages ($P = 0.04$), and a relative decrease in neutrophil percentages ($P = 0.03$) with exposure were observed in US workers. The absolute number of lymphocytes, however, showed no relationship with exposure. No statistically significant results were seen for Mexican workers, although hematocrit decreased with exposure. An exposure-response relationship for the percentage for lymphocytes (positive) and neutrophils (negative) in US subjects and for neutrophils (positive) in Mexican subjects was seen. No overall relation with exposure was observed for total number of white cells. Molecular and cytogenetic results are also reported for the 68 women, who constitute a subgroup from a previous report. US women workers showed a statistically significant exposure-response relationship for ethylene oxide and hemoglobin adducts ($P = 0.0002$) and sister chromatid exchanges ($P = 0.001$). For micronuclei, the difference ($P = 0.02$) between low and high exposure was statistically significant. In Mexican workers, an exposure-response relationship was observed ($P = 0.002$) for hemoglobin adducts but not for sister chromatid exchanges or micronuclei.*

Ethylene oxide is an alkylating agent that has been linked with cancer and reproductive and hematologic effects.[1] Little is known about biological changes that occur at current levels of low exposure resulting from reducing the US permissible exposure limit to less than 1 ppm (8-hr time-weighted average (TWA)).[2] Our study reports on the relationship between ethylene oxide and a battery of biological markers: hemoglobin adducts, sister chromatid exchanges (SCEs), chromosomal micronuclei, and hematologic indicators. Some results were published earlier.[3] This article focuses on women workers and extends the analysis to include hematologic effects.

Investigating hematologic data is important because use of complete blood counts (CBCs) with differential leukocyte counts for routine surveillance has been called into question by LaMontagne et al.[4] Medical surveillance for workers exposed to ethylene oxide, stipulated in the 1984 Occupational Safety and Health Administration (OSHA) standard, must include CBCs with differential leukocyte counts. LaMontagne et al, using their own data and the literature, concluded that the association between ethylene oxide exposure and lymphocytosis would probably not be apparent until workers were also symptomatic. Thus, when obvious symptoms are present to indicate exposure, observation of lymphocytosis in such a worker would not serve the function of detecting otherwise unrecognized exposure, and therefore would not be a good med-

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**TABLE 1**
Demographic Characteristics of Study Participants

| Variables | United States | | | Mexico* | |
|---|---|---|---|---|---|
| | Cumulative Exposure Categories (ppm-hr) | | | Cumulative Exposure Categories (ppm-hr) | |
| | 0 | >0–32 | >32 | >0–32 | >32 |
| Number | 8 | 28 | 10 | 9 | 12 |
| Mean age (years) | 48.1 | 41.8 | 45.2 | 28.9 | 30.0 |
| Percentage white | 62.0 | 60.7 | 40.0 | 100† | 100† |
| Mean education (years) | 12.5 | 12.3 | 12.8 | 14.2 | 12.7 |
| Mean length of employment in present job | 7.4 | 5.5 | 10.0 | 5.9 | 4.2 |

* Only one subject was available for the 0 ppm-hr cumulative exposure category for Mexico, and she is not shown in this table.
† Mexican subjects were all hispanic.

**TABLE 2**
Exposure to Ethylene Oxide of Hospital Workers in the United States and Mexico

| | Cumulative Exposure Category (ppm-hr) | No. | Mean 4-month Cumulative Exposure (ppm-hr) | Mean and Range of 8-hour Time-Weighted Averages (ppm)* |
|---|---|---|---|---|
| United States† | | | | |
| | 0 | 8 | 0.0 (0)‡ | 0 (0) |
| | >0–32 | 28 | 11.7 (10.5) | 0.08 (0.0–0.30) |
| | >32 | 10 | 106.4 (48.0) | 0.17 (0.13–0.30) |
| Mexico§ | | | | |
| | 0 | 1 | 0 (—) | 0 (—) |
| | >0–32 | 9 | 10.5 (0) | 0.02 (0.02–0.02) |
| | >32 | 12 | 349.1 (215.8) | 0.54 (0.27–1.36) |

* The mean shown is the average of the 8-hour time-weighted averages for the job-tasks of subjects in designated exposure category; the range is for the time-weighted averages in the category.
† Based on from 3 to 13 long-term personal and from 3 to 12 long-term area air samples in each of nine US hospitals.
‡ Numbers in parentheses are standard deviations.
§ Based on 20 long-term personal and eight long-term area air samples in one Mexican hospital.

ical surveillance test. However, the body of data on which this assessment was made is small and inconclusive; further investigations are necessary to resolve the debate.

## Methods

Methods and materials for molecular and cytogenetic components of the study have been described in detail in a previous paper,[3] which presented results for 73 male and female workers. Previously unreported data are also described for hematologic analyses in this current evaluation for the subset of 68 female workers from nine hospitals in the United States and one hospital in Mexico. The workers were generally employed in the central stores or central services departments of hospitals. They were exposed to ethylene oxide during the unloading of sterilizers or when working adjacent to sterilizers. In each hospital, we selected workers with the highest and lowest potential cumulative exposure to ethylene oxide, based on hospital records of the number of times they had unloaded sterilizers during the previous 4 months. Eight nonexposed female US workers were also included.

Exposure to ethylene oxide was measured by personal breathing zone samples, station and area air samples, and grab samples collected in each of the 10 hospitals over a 2- to 4-day period.[3] Each participant's 4-month cumulative exposure was estimated by an algorithm that is the product of the mean exposure per job task in her hospital and the number of days worked in that task. Each participant was put into the one of the following exposure categories: none (0), low (>0–32 ppm-hrs), or high (>32 ppm-hrs). These latter categories were selected upon inspection of the data, which revealed a break in the distribution near 32 ppm-hrs. The 4-month cumulative exposure for each individual was selected to correspond with the life span of the erythrocyte for the adduct analysis and was computed based on measured mean exposure concentration for each task, individual usage of the gas sterilizer equipment based on hospital records, personal interviews, questionnaires, and the professional judgment of an industrial hygienist.[3]

Other covariates were obtained by a health and occupational history questionnaire. Questionnaire data included demographic characteristics; occupational history (including sterilizer unloadings and health history); information on cigarette smoking; intake of coffee, tea, alcohol, and other drugs; immunizations; history of cancer and viral infections; and exposure to x-rays. Laboratory assays were conducted on aliquots of 40 ml of venous blood obtained from each subject drawn during the 2- to 4-day exposure monitoring periods. Details of the assays have been described previously.[3] Hydroxyl ethyl hemoglobin adducts were assessed using a radioimmunoassay method

**TABLE 3**
Mean Levels of Molecular and Cytogenetic Biological Markers Adjusted for Confounding Factors, in Hospital Workers Exposed to Ethylene Oxide

| Ethylene Oxide Exposure (ppm-hr) | No. | Hemoglobin adducts (pmole/mgHb) | Sister Chromatid Exchanges | Micronuclei (96 hr) (frequency/cell) |
|---|---|---|---|---|
| United States: Means* | | | | |
| 0 | 8 | 0.06 (0.02)† | 4.64 (0.40) | 0.50 (0.28) |
| >0–32 | 28 | 0.08 (0.01) | 5.89 (0.20) | 0.43 (0.14) |
| >32 | 10 | 0.17 (0.02) | 6.91 (0.36) | 1.11 (0.25) |
| Comparisons: | | | *P* values | |
| 0 vs >0–32 | | 0.30 | 0.01 | 0.83 |
| 0 vs >32 | | 0.001 | 0.0002 | 0.12 |
| >0–32 vs >32 | | 0.002 | 0.02 | 0.02 |
| Mexico: Means* | | | | |
| 0 | 1 | — | — | — |
| >0–32 | 9 | 0.07 (0.03) | 6.44 (0.33) | 3.12 (1.19) |
| >32 | 12 | 0.16 (0.03) | 6.48 (0.28) | 1.91 (1.02) |
| Comparison: | | | *P* values | |
| >0–32 vs >32 | | 0.04 | 0.94 | 0.45 |

* Means adjusted for regression covariates.
† Numbers in parentheses represent standard error of the mean.

developed by Wraith et al.[5] SCEs and micronuclei were assessed in cultured lymphocytes. Assay of SCEs was performed by the method of Carrano and Moore,[6] and micronuclei were assayed using the method of Hogstedt et al.[7]

The hematologic analysis (CBC with differential) was conducted on 40-μl aliquots of blood. All blood samples were analyzed at the same clinical laboratory. Blood samples were analyzed using a Baker 150 Cell Counter (Baker Instruments Corporation, Bethlehem, PA). Standard quality control was performed daily using Equinox 8 (tri-level) hematology controls (Hematronic, Inc, Benica, CA). Additionally, the laboratory participates three times yearly in comparison analyses of samples with reference laboratories. Lymphocyte and neutrophil percentages were determined by manual differential counts performed on 100 cells. All analyses and counts were performed by the same analyst.

Statistical analyses were performed on relative and absolute counts. Statistical analysis involved assessing the relationship between the biomarker (molecular, cytogenetic, and hematologic) and cumulative ethylene oxide exposure by analysis of covariance and multiple linear regression. Mean biomarker values for each cumulative exposure category were adjusted for covariates by analysis of covariance. A stepwise regression procedure was performed in selecting the strongest predictors for the final regression models. To create best-fitting models that were also similar for different biomarkers, some variables were selected based on theoretical reasons or because they were significant predictors for related biomarkers. This method has been described in detail.[3] Residuals and several diagnostic statistics were examined to evaluate the regression models.

## Results

Demographic characteristics of the study population are shown in Table 1. All subjects are female and the mean age was 43.6 years (±12.9 years) for the United States and 29.4 years (±3.6 years) for Mexican subjects. Mean duration of employment in their present job was 6.8 years (±5.5 years) for US subjects and 5.0 years (±2.7 years) for Mexican subjects.

Mean cumulative exposure for a 4-month period for three exposure categories is shown in Table 2. Adjusted mean levels of molecular and cytogenetic biomarkers for subjects in each exposure category are shown in Table 3. A statistically significant exposure-response relationship in US workers was observed for hemoglobin adducts when means were adjusted for age, race, cigarette smoking, tea consumption, and education. A similar finding was observed for SCEs when adjusting for age, race, cigarette smoking, and tea consumption. For micronuclei, there was a statistically significant difference between low exposure and high exposure when adjusting for age, race, cigarette smoking, and coffee consumption. A similar relationship with exposure for hemoglobin adducts was observed for Mexican subjects when the no-exposure category ($n = 1$) was excluded. No relationship was observed for SCEs and micronuclei in the Mexican subjects.

Multivariate analyses (Table 4) of US subjects showed an association between cumulative ethylene oxide exposure and hemoglobin adducts ($\beta = 0.19$; $P = 0.0002$) when controlling for age, race, number of cigarettes smoked per day, tea consumption, and years of education. Ethylene oxide exposure was also associated with SCE formation ($\beta = 0.05$; $n = 0.001$) when controlling for age, race, cigarette smoking, and tea consumption. In the Mexican subjects (Table 5), an exposure-response relationship was observed for hemoglobin adducts ($P = 0.002$) when controlling for age, cigarette smoking, and liquor consumption.

Hematologic markers (CBC with leukocyte differential) were assessed to determine if there was a relationship with ethylene oxide exposure (Table 6). For US subjects, hematocrit and hemoglobin levels were re-

**TABLE 4**
Regression (β) Coefficients from Multiple Regression Models of Biomarkers (Natural Log Transformation) Among Female Hospital Workers, United States

| Variable | Hemoglobin Adducts | Sister Chromatid Exchanges* | Micronuclei* (96 hr) |
|---|---|---|---|
| In cumulative exposure | 0.19 ($P$ = 0.0002) | 0.05 ($P$ = 0.001) | 0.05 ($P$ = 0.34) |
| Age (years) | 0.008 ($P$ = 0.29) | −0.002 ($P$ = 0.30) | −0.001 ($P$ = 0.91) |
| Race (1 = white, 0 = black) | −0.52 ($P$ = 0.008) | 0.05 ($P$ = 0.38) | 0.15 ($P$ = 0.47) |
| Cigarettes/day | 0.08 ($P$ = 0.0001) | 0.006 ($P$ = 0.11) | −0.006 ($P$ = 0.58) |
| Tea/coffee | −0.20† ($P$ = 0.29) | −0.12† ($P$ = 0.05) | 0.11‡ ($P$ = 0.07) |
| Education (years) | 0.11 ($P$ = 0.14) | — | — |
| $R^2$ | 0.66 ($P$ = 0.0001) | 0.35 ($P$ = 0.006) | 0.14 ($P$ = 0.31) |

* Three sister chromatid exchange and micronuclei assays excluded due to laboratory technical problems.
† Coefficient for tea (1 = yes, 0 = no).
‡ Coefficient for cups of coffee/day.

**TABLE 5**
Regression (β) Coefficients from Multiple Regression Models of Biomarkers (Natural Log Transformation) Among Female Hospital Workers, Mexico

| Variable* | Hemoglobin Adducts | Sister Chromatid Exchanges | Micronuclei (96 hr) |
|---|---|---|---|
| In cumulative exposure | 0.24 ($P$ = 0.002) | 0.01 ($P$ = 0.65) | 0.04 ($P$ = 0.75) |
| Age (years) | 0.04 ($P$ = 0.41) | 0.001 ($P$ = 0.91) | −0.10 ($P$ = 0.14) |
| Cigarettes/day | 0.05 ($P$ = 0.21) | 0.02 ($P$ = 0.16) | −0.18 ($P$ = 0.04) |
| Liquor (1 = yes, 0 = no) | 0.51 ($P$ = 0.32) | — | — |
| Immunizations (1 = yes, 0 = no) | — | 0.11 ($P$ = 0.11) | — |
| $R^2$ | 0.66 ($P$ = 0.0008) | 0.25 ($P$ = 0.27) | 0.27 ($P$ = 0.12) |

* Sex and race not used, since all Mexican subjects were Hispanic females.

duced (compared to the no-exposure group) for the high-exposure group. However, only the difference between the high-exposure and low-exposure groups was statistically significant. In Mexican subjects, there were no statistically significant relationships between exposure and hemoglobin or hematocrit levels. Mean corpuscular volume was also assessed in evaluating red cell indices to control for confounding due to mild iron deficiency anemia in menstruating women. This did not appear to be a confounding factor. For US subjects only, lymphocyte percentages and relative neutrophil percentages (segmented) showed a relationship with exposure. Lymphocyte counts increased with exposure to ethylene oxide. The mean lymphocyte differential (percentage of total leukocytes) for the highest-exposure group (41.5%) was greater than for the no-exposure group (31.4%) ($P$ = 0.04) when adjusting for age, race, smoking, wine consumption, and history of mononucleosis and cancer. The low-exposure group was larger than the no-exposure group, but the difference was not statistically significant. The mean for neutrophils showed an inverse relationship with ethylene oxide exposure, ranging from a mean of 63.5% for the no-exposure group to 54.1% for the highest-exposure group ($n$ = 0.035). White cell differentials showed a reversed pattern in Mexican subjects, in contrast with US subjects. The highest exposure level had a lymphocyte count of 39.5%, compared with 42.2% for the low-exposure group ($P$ = 0.24). For the neutrophils, the highest exposure level had a neutrophil count of 57.3%, compared with 52.7% for the low-exposure groups ($P$ = 0.21). Since the differences are not statistically significant, any pattern may be an artifact.

Relationships between ethylene oxide and various hematologic markers for all subjects from each country were assessed using multiple linear regression (Tables 7 and 8). A positive exposure response was observed for relative lymphocyte counts in US subjects when controlling for age, race, cigarette and alcohol consumption, and past illnesses. An inverse relationship with exposure was observed for neutrophils, controlling for the same variables, except for alcohol. When absolute number of lymphocytes was used in the regression model, no exposure-response relationship existed (not shown). For neutrophils, an inverse relationship

was found using the absolute numbers, but it was not statistically significant (not shown). For Mexican subjects, a positive exposure-response relationship ($P = 0.03$) was observed for relative and absolute neutrophils when controlling for age, cigarette smoking, and tea and alcohol consumption (absolute results not shown).

## Discussion

This study restipulates for female subjects essentially the same results for a population already described[3] that levels of exposure of ethylene oxide below the OSHA threshold limit value (TLV) can result in biological changes (molecular and cytogenetic) potentially related to carcinogenesis. These include formation of hemoglobin adducts, SCEs, and chromosomal micronuclei. This study includes the largest group of women workers with ethylene oxide exposures of less than 1 ppm who have been shown to have simultaneous increased hydroxyethyl histidine adducts and SCEs. Mayer et al[8] conducted the only other study with similar molecular and cytogenetic results.

The ability to monitor chemical exposures via hemoglobin adducts can be affected by the presence of background adduct levels that tend to mask the effects of low levels.[9] Confounding exposures (ie, different chemicals or different sources of the same chemical) that can produce identical adducts from unknown and for endogenous sources contribute to these background levels.[10] In this study, cigarette smoking was one such factor that contributed to background levels. The pattern of adduct formation by smoking status for US and Mexican subjects is different. In US subjects, higher adduct levels for similar ethylene oxide exposures are found in smokers. This was not seen in Mexican subjects. The reason for this difference may be that the relatively higher level of ethylene oxide exposure in Mexican subjects masks the small contribution of cigarette smoke to the formation of adducts. In the less exposed US workers, smoking may contribute more to the adduct level.

This is the first study to observe hematologic effects at low levels of exposure to ethylene oxide in humans. Only three studies have evaluated hematologic effects at exposures less than 1 ppm,[4,11,12] and none of them found hematologic effects. Currier et al[11] studied a group of 84 workers involved in the manufacture of ethylene oxide, ethylene glycol, glycol ethers, or ethanolamine. Exposures to ethylene oxide were estimated and measured at generally less than 1 ppm. Despite the fact that exposed workers had more abnormalities of the hematologic, hepatic, or renal systems than matched controls (matched on age, date of hire, smoking, alcohol consumption, and date of examination), no differences were seen for hematologic parameters. Similarly, Van Sittert[12] found no hematologic effects in 36 workers with estimated 8-hr TWA exposures below 0.05 ppm in an ethylene oxide manufacturing plant, compared with matched controls. LaMontagne et al[4] observed lymphocytosis not associated with ethylene oxide exposure and no erythrocyte effects in hospital sterilizer operators exposed at levels averaging 0.07 ppm (8-hr TWA). Hematologic changes have been found in rats exposed to ethylene oxide. Snellings et al[13] reported elevated leukocyte counts in both sexes and depressed red cell counts and hemoglobin in female rats exposed to 10 ppm ethylene oxide for 2 years.

In our study, ethylene oxide exposures were generally below the OSHA TLV of 1 ppm. At this level, we observed a decrease in hemoglo-

**TABLE 6**
Hematologic Effects of Ethylene Oxide Exposure

| Exposure-Category (ppm-hr) | Red Blood Cells ($10^6$/mm$^3$) | | Hemoglobin (gm/dl) | | Hematocrit (vol/dl) | | White Blood Cells ($10^3$/mm$^3$) | | Lymphocytes (% total leukocytes) | | Neutrophils (% total leukocytes) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | US | Mexico | US | Mexico | US | Mexico | US | Mexico | US | Mexico | US | Mexico |
| (1) 0† | 4.48* (0.15) | — | 13.34 (0.40) | — | 40.44 (1.27) | — | 6.88 (1.24) | — | 31.37 (3.64) | — | 63.82 (3.41) | — |
| (2) >0–32 | 4.56 (0.09) | 5.09 (0.14) | 13.7 (0.21) | 13.80 (0.45) | 41.98 (0.68) | 43.60 (1.41) | 7.84 (0.65) | 4.89 (0.58) | 37.23 (1.86) | 42.15 (1.64) | 57.81 (1.75) | 52.68 (1.84) |
| (3) >32 | 4.27 (0.15) | 4.99 (0.12) | 12.76 (0.36) | 14.46 (0.39) | 38.82 (1.15) | 44.24 (1.22) | 5.42 (1.09) | 6.37 (0.51) | 41.46 (3.08) | 39.51 (1.42) | 54.07 (2.90) | 57.29 (1.59) |
| | ‡ | ‡ | $P = 0.03$ (2) vs (3) | ‡ | $P = 0.02$ (2) vs (3) | ‡ | ‡ | ‡ | $P = 0.04$ (1) vs (3) | ‡ | $P = 0.03$ (1) vs (3) | ‡ |

* Top number is mean adjusted for age, race, smoking, and other covariates determined by regression models. Number in parentheses is standard error.

† Only one person represents Mexican values in this category.

‡ Not statistically significant ($P < 0.05$).

**TABLE 7**
Regression (β) Coefficients from Multiple Regression Models of Hematological Measures Among Female Hospital Workers, United States

| Independent Variable | Red Blood Cells ($10^6$/mm$^3$) | Hemoglobin (gm/dl) | Hematocrit (vol/dl) | White Blood Cells ($10^3$/mm$^3$) | Lymphocytes (% total leukocytes) | Neutrophils (% total leukocytes) |
|---|---|---|---|---|---|---|
| Number | 46 | 46 | 46 | 46 | 46 | 46 |
| Intercept | 4.49<br>P = 0.0001 | 13.50<br>P = 0.0001 | 40.63<br>P = 0.0001 | 7.46<br>P = 0.001 | 31.62<br>P = 0.0001 | 60.78<br>P = 0.0001 |
| ln cumulative exposure | −0.04<br>P = 0.25 | −0.11<br>P = 0.25 | −0.33<br>P = 0.27 | −0.38<br>P = 0.16 | 1.64<br>P = 0.03 | −1.46<br>P = 0.04 |
| Age (years) | 0.0002<br>P = 0.98 | −0.01<br>P = 0.47 | −0.02<br>P = 0.68 | −0.008<br>P = 0.85 | −0.01<br>P = 0.95 | 0.01<br>P = 0.93 |
| Race (1 = white or hispanic, 0 = black) | 0.09<br>P = 0.56 | 0.66<br>P = 0.07 | 2.01<br>P = 0.08 | 2.01<br>P = 0.06 | 1.16<br>P = 0.69 | 1.05<br>P = 0.70 |
| Cigarettes/day | −0.01<br>P = 0.24 | 0.02<br>P = 0.35 | 0.05<br>P = 0.41 | 0.03<br>P = 0.64 | 0.002<br>P = 0.99 | 0.06<br>P = 0.69 |
| Coffee (1 = yes) | — | — | — | −1.98<br>P = 0.10 | — | — |
| Alcohol | 0.28*<br>P = 0.14 | — | — | — | 7.98†<br>P = 0.12 | — |
| Past illness | — | 1.69‡<br>P = 0.01 | 5.12<br>P = 0.02 | — | 14.18§<br>P = 0.05 | −15.17§<br>P = 0.03 |
| Cancer | — | — | — | — | 22.82‖<br>P =0.03 | −21.52‖<br>P =0.03 |
| $R^2$ | 0.10<br>(P = 0.47 | 0.31<br>P = 0.01 | 0.28<br>P = 0.02 | 0.21<br>P = 0.08 | 0.29<br>P = 0.05 | 0.27<br>P = 0.04 |

* Coefficient for wine (1 = yes, 0 = no).
† Coefficient for glasses of wine/week.
‡ Coefficient for hepatitis (1 = yes, 0 = no).
§ Coefficient for mononucleosis (1 = yes, 0 = no).
‖ Coefficient for cancer (1 = yes, 0 = no).

bin and hematocrit with increased exposure between >0–32 ppm-hr and >32 ppm-hr (estimated in the 4 months prior to sampling), and a relative lymphocytosis and decrease in neutrophil (segmented) counts in US workers. These data are not conclusive for the following reasons: (a) they represent a one-time biological sampling of workers; (b) they may reflect physiologic variation and a rather high level of imprecision, since counts were performed on 100 cells, as is customary in the clinical laboratory; (c) many statistical comparisons could by chance alone account for a significant result; and (d) the failure to see the same pattern in Mexican workers does not support the validity of this effect. These factors, however, do not outweigh the supporting evidence.

The hematologic findings are supported by the fact that the exposure assessment was extensive and allowed for a representative characterization of 4 months of exposure. Hematologic effects were assessed for confounding factors such as smoking, drinking, education, and history of disease, in addition to age and race. In fact, 24 different confounding variables were considered in the analyses. This is generally a more extensive control for confounding than in any previous studies of hematologic effects of ethylene oxide. The finding of lymphocytosis has been observed in other studies at higher levels of exposure. Ehrenberg and Hallstrom[14] reported that manufacturing workers exposed to ethylene oxide intermittently over several years exhibited higher absolute lymphocyte counts than did controls. The level of ethylene oxide exposure was not specified, but it is likely that the exposure was greater than 10 ppm (half of the historical Swedish standard). Our study found a relative lymphocytosis related to exposure,

**TABLE 8**
Regression (β) Coefficients from Multiple Regression of Hematological Measures Among Female Hospital Workers, Mexico

| Independent Variable | Red Blood Count ($10^6$/mm$^3$) | Hemoglobin (gm/dl) | Hematocrit (vol/dl) | White Blood Cells ($10^3$/mm$^3$) | Lymphocytes (% total leukocytes) | Neutrophils (% total leukocytes) |
|---|---|---|---|---|---|---|
| Number | 22 | 22 | 22 | 22 | 22 | 22 |
| Intercept | 4.2<br>$P = 0.0001$ | 14.8<br>$P = 0.0003$ | 61.3<br>$P = 0.0001$ | −2.65<br>$P = 0.44$ | 40.1<br>$P = 0.0006$ | 59.5<br>$P = 0.0001$ |
| ln cumulative exposure | −0.03<br>$P = 0.58$ | 0.14<br>$P = 0.36$ | 0.02<br>$P = 0.97$ | 0.39<br>$P = 0.08$ | −0.88<br>$P = 0.14$ | 1.45<br>$P = 0.03$ |
| Age (years) | 0.03<br>$P = 0.29$ | −0.03<br>$P = 0.76$ | −0.69<br>$P = 0.03$ | 0.22<br>$P = 0.06$ | 0.20<br>$P = 0.51$ | −0.41<br>$P = 0.24$ |
| Cigarettes/day | −0.01<br>$P = 0.77$ | 0.05<br>$P = 0.65$ | −0.22<br>$P = 0.56$ | 0.21<br>$P = 0.16$ | 1.30<br>$P = 0.005$ | −0.96<br>$P = 0.04$ |
| Tea/coffee | 0.12*<br>$P = 0.06$ | — | 1.90†<br>$P = 0.06$ | 0.58*<br>$P = 0.03$ | −7.26‡<br>$P = 0.004$ | 6.76‡<br>$P = 0.01$ |
| Alcohol | — | −2.46§<br>$P = 0.04$ | — | −3.17‖<br>$P = 0.01$ | 7.03‖<br>$P = 0.03$ | −7.84‖<br>$P = 0.03$ |
| $R^2$ | 0.23<br>$P = 0.31$ | 0.38<br>$P = 0.07$ | 0.31<br>$P = 0.16$ | 0.52<br>$P = 0.02$ | 0.74<br>$P = 0.0003$ | 0.68<br>$P = 0.001$ |

* Coefficient for cups of tea/day (1 = yes, 0 = no).
† Coefficient for cups of coffee/day.
‡ Coefficient for tea (1 = yes, 0 = no).
§ Coefficient for drinks of liquor/week.
‖ Coefficient for glasses of wine/week.

but absolute numbers of lymphocytes did not increase but, in fact, decreased from low-exposure to high-exposure categories. This parallels the total white blood cell counts by exposure category. The relative lymphocytosis is most likely caused by a decrease in total granulocytes rather than an increase in total lymphocytes.[15]

The finding of increased percentage lymphocytes and decreased percentage neutrophils in US workers is biologically plausible. It has been seen in situations where a chronic inflammatory response increases numbers of lymphocytes and decreases numbers of neutrophils.[16] Chronic exposure to ethylene oxide could provoke such a reaction. The failure to see the same pattern of increased lymphocytes and decreased neutrophils in Mexican subjects is partially explainable by the small number of subjects and that specimens from Mexico had a longer time (12–20 hr) from collection to counting. Since all specimens were analyzed at the same laboratory, Mexican specimens were subject to more extensive effects of travel, including vibration and temperature change. Shifts in differentials upon aging of blood have been observed following at least two different techniques.[17] Under such conditions, counts from automated devices show that the percentage of lymphocytes decreases and the percentage of granulocytes such as neutrophils increases. This pattern was observed for the Mexican subjects, but whether these time-related variances affect the manual differential counts used in this study is unknown. Additionally, the difference between Mexican and US white cell results may be accounted for by differences in environmental sources of pollution, allergy, or infection.[18,19]

Although CBC with leukocyte differential is known to be a variable test, in this study, the CBC was conducted using an automated device and counting was performed by the same laboratory. All laboratory tests were conducted blind, so it is not likely that the observed hematologic effects occurred by chance.

LaMontagne et al[4] have reviewed and critiqued the literature on this topic and pointed out the weaknesses of using the CBC with leukocyte differential as a screening test for ethylene oxide. Generally, few data have been available on the hematologic effects of ethylene oxide exposure. Our study provides further data to consider. In our study, substantial overlap in CBC existed between the groups that were most and least exposed, and most individual and all group results were within clinically acceptable levels. Results for an individual generally could not be

used to detect even the most heavily exposed workers. In contrast, this study does demonstrate the value of these same tests for comparing populations exposed to ethylene oxide.

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