American Journal of Industrial Medicine 24:191–206 (1993)

Utility of the Complete Blood Count in Routine Medical Surveillance for Ethylene Oxide Exposure

Anthony D. LaMontagne, MA, MEd, David C. Christiani, MD, MPH, MS, and Karl T. Kelsey, MD, MOH

The 1984 OSHA Standard for ethylene oxide (EtO) mandates medical surveillance under various circumstances. When performed, medical surveillance for EtO must include a complete blood count (CBC) with differential leukocyte count. This requirement is based on reports of EtO-associated absolute lymphocytosis and other hematologic effects. This paper describes our experiences in providing EtO medical surveillance for a 300 bed hospital over a 6 year period. We observed an apparent relative lymphocytosis which persisted over 3–4 years in sterilization workers with documented TWA personal EtO exposures averaging 0.07 ppm. In addition, three workers had a history of acutely toxic overexposure to EtO as a result of a sterilizer malfunction. These workers became symptomatic following the high accidental overexposure, but did not show absolute lymphocytosis or altered patterns in the relative lymphocytosis. Finally, a cross-sectional comparison of the CBC data from the EtO-exposed workers to data from non-EtO-exposed hospital workers showed no significant differences, ruling out an association of the relative lymphocytosis with EtO exposure. These observations led us to review the basis for the inclusion of the CBC in routine EtO medical surveillance. Our experience, review of the literature on EtO-associated lymphocytosis and anemia, and review of the literature on the use of the CBC with differential as a screening test suggest that the leukocyte differential may not be useful in routine medical surveillance for EtO exposure. © 1993 Wiley-Liss, Inc.

Key words: health care workers, hospital hazards, screening tests, chemical hazards, hematology, OSHA, medical surveillance

INTRODUCTION

Ethylene oxide (EtO) is a widely used synthetic chemical precursor and biocide. It is used in most hospitals and in the medical products industry for the sterilization of heat- and moisture-sensitive medical equipment. The Occupational Safety and Health Administration (OSHA) estimated in 1984 that 140,000 workers were potentially exposed to EtO in all industries, including 80,000 hospital workers [OSHA,

Department of Environmental Health, Occupational Health Program, Harvard School of Public Health, Boston, MA (A.D.L., D.C.C., K.T.K.).
Center for Occupational and Environmental Medicine, Massachusetts Respiratory Hospital, Braintree, MA (A.D.L., D.C.C., K.T.K.).
Laboratory of Radiobiology, Harvard School of Public Health, Boston, MA (K.T.K.).
Address reprint requests to Anthony D. LaMontagne, Department of Environmental Health, Occupational Health Program, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.
Accepted for publication October 6, 1992.

© 1993 Wiley-Liss, Inc.

1984:25767]. Due to the current lack of adequate substitutes, EtO will remain a significant occupational hazard for the foreseeable future.

An unusually broad spectrum of serious health effects are associated with EtO. Overexposure to EtO has been clearly linked to genetic damage, neurologic effects, and tissue irritation in exposed workers [Landrigan et al., 1984; IARC, 1985]. Epidemiologic studies suggest that EtO exposure leads to increased risks of leukemia and other cancers [Hogstedt et al., 1986; Stayner et al., 1992; IARC, 1985], spontaneous abortions [Hemminki et al., 1982], and other reproductive problems [Yakubova et al., 1976]. These studies are supported by animal evidence of EtO carcinogenicity and reproductive toxicity [Landrigan et al., 1984; IARC, 1985; Glaser, 1979]. EtO exposure has also been associated with cataract formation [Jay et al., 1982].

OSHA passed a Final Standard on EtO in 1984 which was subsequently revised in 1988 to include a Short Term Exposure Limit (STEL) [OSHA, 1988]. Most provisions of the Standard, including medical surveillance, are triggered by the action level of 0.5 ppm time-weighted average (TWA). Employers are required to institute a medical surveillance program for all employees: (1) who **are or may be** (authors' emphasis) exposed to EtO at or above the action level for at least 30 days a year; (2) who have been exposed to EtO in an emergency situation; (3) who are showing EtO-associated signs and symptoms; or (4) who desire medical advice concerning the effects of EtO on their ability to produce a healthy child [OSHA 1984:25798]. Under normal circumstances, therefore, practitioners, employers, and others are allowed considerable latitude in deciding when EtO surveillance is required. Personal monitoring is usually performed far less frequently than 30 days per year, with samples taken being assumed to be representative of typical exposures. OSHA has stated that observation of one personal monitoring result at or above 0.5 ppm implies that such exposure is typical, unless shown to be otherwise [Siegel and Bunn, 1985]; this should trigger medical surveillance requirements. To be most protective of workers' health, anyone who works with EtO "may be exposed" beyond OSHA's specified action levels and hence should be offered medical surveillance. A recent examination of OSHA inspection records, however, suggests that EtO medical surveillance is under-implemented by employers and under-cited by OSHA inspectors [Schwartz et al., 1992].

When EtO medical surveillance is provided, OSHA mandates that surveillance includes, at a minimum, medical and work histories, physical examination, and complete blood count (CBC) that includes a differential white cell count, red cell count, hematocrit, and hemoglobin. Histories and physical exam are to focus on pulmonary, hematologic, neurologic, and reproductive systems, with attention paid to the eyes and skin, as indicated by EtO's multiple toxicities. OSHA justified the inclusion of histories, physical exam, and the complete blood count asserting that this would help to detect otherwise unrecognized overexposure [OSHA 1984:25784–25786].

In this paper, we describe our experience in providing EtO medical surveillance for sterilization workers at a 300 bed hospital over a 6 year period. We observed a persistent relative lymphocytosis in sterilization workers over a 3–4 year period. This finding led us to analyze our surveillance data and to review the literature supporting the use of the CBC in EtO medical surveillance. Our observations and a discussion of their implications are presented below.

METHODS

Study Populations

Periodic medical surveillance has been provided since 1985 for the sterilization department of a 300 bed eastern Massachusetts community hospital. Generally, 14 people were employed in the sterilization department at any one time between 1985 and 1991; each of these workers rotated through EtO sterilization duties. Maintenance workers, who change EtO cylinders and work on or near the sterilization equipment, were also included in surveillance. Persons who have retired or changed jobs and could be contacted were also offered surveillance. A total of 36 people have participated in surveillance at least once. Over the course of surveillance (offered 5 times in 6 years), the number of participants has ranged from 11 to 22, with an average of 17 workers per screening. Eleven workers have participated in surveillance 3 or more times, with the remainder participating twice or less.

Complete blood counts with differentials were performed once on each of two control groups. One control group consisted of 15 volunteers from the same hospital as the sterilization workers (on-site control). These volunteers were primarily from clerical jobs; none were potentially exposed to EtO or other known toxic chemicals. The second control group consisted of 12 medical and clerical staff volunteers from the Massachusetts Respiratory Hospital (off-site control).

Exposure Assessment

Continuous area monitoring for EtO has been performed by a multi-point gas chromatograph (AMSCO Enviroguard III) since 1985. Frequent personal monitoring has been performed since 1987 using AMSCO's "EO Self Scan" system. "Self Scan" monitoring results, which are generated by workers using an on-site processor, are periodically checked against monitoring badges from other manufacturers that are sent out for processing. The results are reportedly always in close agreement. All exposure data were collected by the employer in fulfillment of monitoring requirements of the EtO Standard.

Medical Surveillance Procedure

Medical surveillance for EtO consisted of thorough occupational and medical histories, physical exam, and CBC with differential. A several page, EtO-specific, medical and occupational history questionnaire developed by the authors was mailed to participants 1–2 weeks before the session. Questionnaires were reviewed with participants and checked for completeness by an assistant before the physical exam and blood drawing. Physical exams emphasized pulmonary, hematologic, neurologic, and reproductive systems, and included exams of the eyes and skin. Approximately 5 ml of blood was drawn by venipuncture. All blood samples throughout the 6 year surveillance period were processed at the same clinical laboratory. One hundred microliter samples were analyzed on a Coulter T660 for the CBC, and 100 cell leukocyte differential counts were performed manually. Standard quality control, using the Coulter Quality Assurance Program, was performed daily for automated counts. Additionally, the lab participates in a quarterly proficiency testing program through the College of American Pathologists. Over the 6 year surveillance period, the same three technicians performed all manual differential counts. Abnormal differential counts (e.g., lymphocyte counts above 45%) were recounted once or twice

TABLE I. Exposure of Surveillance Participants to Ethylene Oxide, 1987–1991: 8 Hour TWA Personal Monitoring Results by Individual

Participant number	Months (n)	Samples (n)	Mean (ppm)	Range (ppm)
1	34	70	0.07	(0.0, 0.3)
3	26	63	0.07	(0.0, 0.85)
7	5	18	0.05	(0.0, 0.20)
8	30	67	0.08	(0.0, 0.55)
10	31	73	0.09	(0.0, 0.45)
12	31	82	0.07	(0.0, 0.60)
14	32	84	0.07	(0.0, 0.90)
16	29	97	0.07	(0.0, 0.45)
17	24	47	0.09	(0.0, 0.70)
19	30	71	0.07	(0.0, 0.55)
23	1	2	0.0	(0.0, 0.0)
30	9	20	0.05	(0.0, 0.20)
31	46	149	0.08	(0.0, 1.05)
32	32	114	0.09	(0.0, 0.75)
34	36	110	0.08	(0.0, 0.45)
35	28	75	0.14	(0.0, 2.00)

by the same lab supervisor during the entire 6 year period described, with reported variability of approximately 5–10%. This laboratory used a ''normal'' percent lymphocyte range of 20–35% [Seiverd, 1972].

Statistical Analysis

Complete blood count values, dates of surveillance, exposure status (yes/no), date of birth, sex, and smoking status (current/ non) were coded for each subject and analyzed using SAS [SAS Institute, 1987]. Race was not included in analysis because all participants were Caucasian. CBC and differential count comparisons between sample groups were analyzed using the Wilcoxon rank sum test. Sample means were compared to normal ranges by taking the implied mean of the normal range as the ''true'' mean, and performing one-sample two-sided t tests.

RESULTS

EtO Exposure

Area EtO concentrations over 6 years averaged below 0.1 ppm, with occasional higher excursions of variable magnitude. Personal monitoring data were available for 16 of the 36 workers who participated one or more times in surveillance (Table I). The mean 8 hour TWA was 0.07 ppm. All of the 16 workers with personal monitoring data available were sterilizer operators. Of the 20 without data, approximately half were maintenance workers (none were monitored) and half were sterilizer operators employed before personal monitoring began in 1987 or employed for a short time after 1987. Since maintenance workers typically experience less exposure than sterilizer operators, we believe these data to fairly represent the highest average exposure which would be detected by this procedure for all participants in this surveillance program. Personal monitoring, however, has been performed only since 1987, and exposures may have been higher before then.

TABLE II. Lymphocyte and White Blood Cell Counts in Workers Acutely Overexposed to EtO in an Accident

Subject #	Lymphocyte count (% of WBC)	White blood cell count (WBC) (× 1,000/ml)	Acute symptoms reported
22	28%	6.8	Metallic taste in mouth and headache persisting several days after incident; respiratory irritation.
31	39%	11.5	Sore throat persisting several days after incident; muscle aches in upper arm and upper back; "nerves."
36	31%	5.9	Metallic taste persisting 3–4 days after incident; dry mouth and burning sensation in throat; slight nausea; shaking and tremors.

All sterilization department workers perform EtO sterilization duties on a rotating basis. Combining this knowledge with area and personal monitoring results provides an exposure profile which suggests fairly uniform and low individual exposures. However, temporary excursions to levels well in excess of the PEL of 1.0 ppm were also reported. These were usually caused by equipment malfunctions. The best documented incident involved high level, temporary exposure of three sterilization workers as a result of rupture of the sterilizer door gasket and discharge of the pressurized contents of the sterilizer into the work area. Neither personal nor area monitoring devices were in place during these excursions. Therefore, exposure levels were not documented.

Histories and Physical Exams

Most occupational and medical histories and physicals were normal in each surveillance session. Medical surveillance in 1985 and 1987, however, revealed a history of apparently exposure-related eye, nose, and throat irritation in several workers. These complaints were no longer present in 1989. Additionally, in the first year of surveillance, peripheral neuropathies were observed in two workers. These patients' histories suggest that EtO overexposures of the magnitude necessary to induce these neuropathies may have taken place before exposure monitoring and medical surveillance were implemented.

Three workers were seen five days after the above described accidental release and overexposure. While they all suffered symptoms of acute overexposure, ranging from irritation to central nervous system effects, only one showed a relative lymphocyte count above 35% (Table II). One of the three workers showed a high absolute white blood cell count of 11,500/ml (normal range 5,000–10,000/ml). Examination of this worker's white blood cell counts over time, however, showed alternately high and within normal range values over several surveillance sessions in the absence of known or suspected EtO overexposures.

Significant anxiety about EtO's potential reproductive and carcinogenic effects was also noted among surveillance participants. This excessive anxiety can also be regarded as an undesirable health effect. Consultation about potential chronic effects seemed to reduce anxiety. Finally, cataracts were also detected in a woman with a long occupational history of EtO exposure as a sterilizer operator.

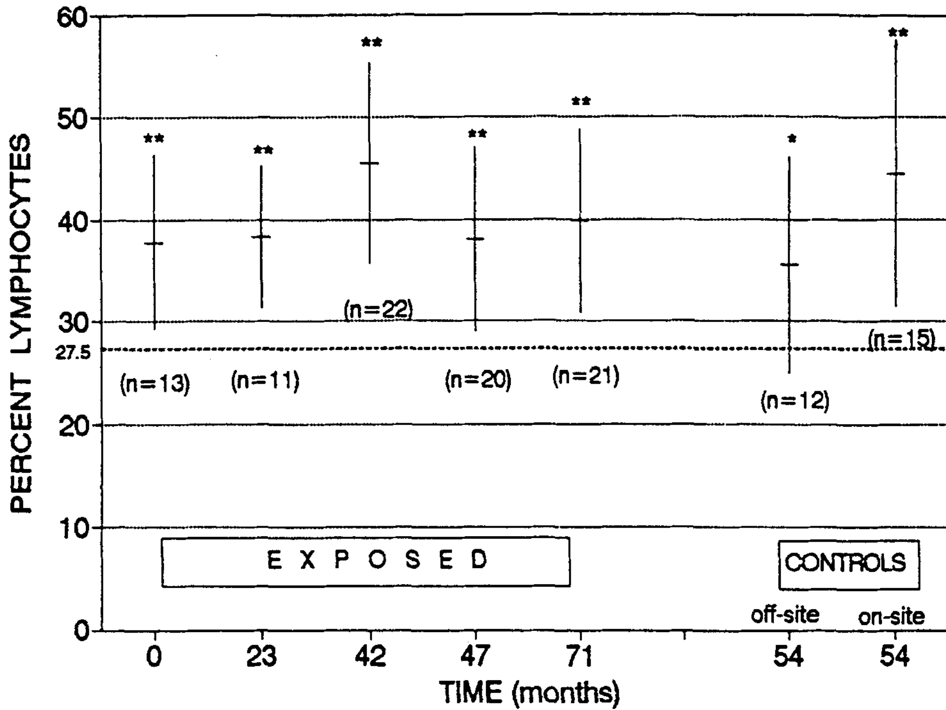

Fig. 1. Group average percent lymphocyte counts (mean and standard deviation) at various times over a six year period. Dashed line represents the population true mean implied by "normal" range of 20–35%. Results of one-sample two-sided t tests comparing sample means to "normal" population mean of 27.5% indicated as follows: * = $p < 0.05$, ** = $p < 0.002$.

Complete Blood Counts and Leukocyte Differentials: Initial Findings

A large proportion of patients participating in surveillance had high relative lymphocyte differential counts. For example, in the first year of surveillance, 9/13 patients were at or above the upper "normal" reference limit of 35% (Fig. 1, "0" month time point). When compared to the implied "true" mean of 27.5% from the reference range of 20–35%, the exposed group sample mean was significantly different from the reference mean (Fig. 1, "0" month time point). This pattern of *relative* lymphocytosis occurred in the absence of *absolute* lymphocytosis. Absolute lymphocytosis has been reported to be associated with EtO overexposure, whereas relatively lymphocytosis has not. Hence, this observation was initially believed to be insignificant. Personal monitoring data that later became available from these workers indicated minimal EtO exposures (Table I), also suggesting nonassociation with relative lymphocytosis.

All other potentially EtO-exposure-related hematologic parameters were unremarkable at the first and all subsequent surveillance sessions: total leukocyte counts were normal (5,000–10,000/ml) in the EtO-exposed group over various surveillance sessions. Red blood cell counts, hemoglobin levels, and hematocrits, likewise, were normal in the exposed group over various surveillance sessions with no suggestion of exposure-associated anemia.

TABLE III. Inferring Association Between EtO Exposure and Relative Lymphocyte Counts Using Various "Normal" Reference Values

Potentially exposed sample group lymphocyte count (% of WBC)		Reference "normal" lymphocyte count (% of WBC)	p value
EtO sterilization workers (n = 22, 42 month time point), mean = 45.5%	vs.	*Off-site* hospital "normal range": 20–35%, mean = 27.5%	<0.002[a]
EtO sterilization workers (n = 22, 42 month time point), mean = 45.5%	vs.	*Off-site* hospital employee controls, mean = 35.5% (n = 12)	0.016[b]
EtO sterilization workers (n = 22, 42 month time point), mean = 45.5%	vs.	*On-site* hospital employee controls, mean = 44.5% (n = 15)	0.721[b]
EtO sterilization workers (n = 22, 42 month time point), mean = 45.5%	vs.	*On-site* hospital "normal range": 20–45%, mean = 32.5%	<0.005[a]

[a]One-sample two-sided t test.
[b]Wilcoxon Rank Sum test.

Over the second and third surveillance sessions (23 and 42 months), percent lymphocyte counts remained elevated, with 8/11 and 19/22 subjects, respectively, showing percent counts at or above 35%. As above, both exposed group surveillance sample means were significantly different from the reference mean ($p < 0.002$ at 23 and at 42 months) (Fig. 1, Table III, first line). Observing the persistence of the relative lymphocytosis, as well as a marked increase at 42 months (Fig. 1), we felt compelled to investigate further and recruited two control groups.

Exposed Versus Control Group Comparisons

Two volunteer control groups were studied, one from the same hospital as the sterilization workers (on-site), and one from the site of the authors' occupational medicine clinic (off-site). The on-site controls allowed us to evaluate the association between high relative lymphocyte counts and EtO exposure controlling for other potential influences within the hospital or local population. The off-site control allowed us to check our own laboratory's "normal" range, as well as to test for variability between different work sites.

To evaluate the potential effect of EtO exposure on percent lymphocyte counts, we compared control groups to the surveillance session with most pronounced lymphocytosis (42 months, mean = 45.5%). We observed that the exposed group was significantly different from the off-site control group, but not significantly different from the on-site control group (Table III, second and third comparisons). Hence, the use of either our hospital lab's "normal ranges" or the off-site control group suggested a potential association between EtO exposure and relative lymphocytosis, but comparison to on-site controls ruled out the association. We later learned that the surveillance hospital (on-site) used a "normal range" of 20–45%; the percent lymphocytes in the EtO-exposed group was also significantly different from their own hospital's "normal range" (Table III, last comparison).

For all of the above comparisons, relative or percent counts were used because it is the standard form of reporting. When percent counts were transformed to abso-

TABLE IV. Cross-Comparisons of Relative Lymphocyte Count Reference Values

Reference value lymphocyte count (% of WBC)		Reference value lymphocyte count (% of WBC)	p value
Off-site hospital employee controls, mean = 35.5% (n = 12)	vs.	*Off-site* hospital "normal range': 20–35%, mean = 27.5%	<0.05[a]
On-site hospital employee controls, mean = 44.5% (n = 15)	vs.	*Off-site* hospital "normal range": 20–35%, mean = 27.5%	<0.001[a]
On-site hospital employee controls, mean = 44.5% (n = 15)	vs.	*On-site* hospital "normal range": 20–45%, mean = 32.5%	<0.005[a]
Off-site hospital employee controls, mean = 35.5% (n = 12)	vs.	*On-site* hospital "normal range": 20–45%, mean = 32.5%	>0.20[a]
Off-site hospital employee controls, mean = 35.5% (n = 12)	vs.	*On-site* hospital employee controls, mean = 44.5% (n = 15)	0.10[b]

[a]One-sample two-sided t test.
[b]Wilcoxon Rank Sum test.

lute counts (multiply percent count by total white blood cell count), the difference in lymphocyte count between the exposed group at 42 months and the off-site control became nonsignificant ($p = 0.101$), suggesting less potential for misinterpretation when absolute rather than relative counts are used.

Age distributions of the groups were not significantly different; all subjects in both groups were Caucasian; and gender compositions were similar. The same comparisons as above were made between groups stratified by smoking status (current or non). Results were qualitatively similar to the crude analysis described above.

Comparing Controls and "Normal" Ranges

The two control groups and the two "normal ranges" were cross-compared in order to assess their consistency. The off-site control group sample mean (35.5%) significantly differed from the off-site hospital's reference mean (27.5%) ($p < 0.05$, Table IV, first comparison). Similarly, the on-site control group sample mean (44.5%) was compared to the off-site hospital's normal range (mean = 27.5%) and also was found to be significantly different (Table IV, second comparison).

After observing the high on-site control group sample mean (44.5%), we inquired and learned that the on-site "normal" reference range was 20–45% (implied reference mean of 32.5%). In contrast to the off-site hospital laboratory, this range was generated by sampling 20–30 hospital laboratory employees several years previously. However, the on-site control group sample mean was still significantly different from its own "normal" reference range (Table IV, third comparison). Only the off-site control group fell within the on-site reference range (Table IV, fourth comparison). Comparison of the off-site (mean = 35.5%) and on-site (mean = 44.5%) control groups, which were approximately 70 miles apart, showed that percent lymphocyte counts were not significantly different (Table IV, final comparison).

As described above, potential confounders of age, race, and sex are not likely to have had a significant effect on these comparisons. To examine confounding by smoking, comparisons were made between groups stratified by smoking status (current or non). Results were qualitatively equivalent to the non-stratified analysis above.

DISCUSSION

Findings From Single Hospital Observations

We have presented the results of 6 years of medical surveillance for EtO-exposed sterilization and maintenance workers in a medium-sized hospital. The data used in this analysis were generated by employers in compliance with OSHA's EtO Standard. Interpretation of these observations is somewhat limited by incomplete data and the lack of an experimental design. Importantly, however, our findings provide an evaluation of the medical surveillance requirements of the EtO Standard as practiced in the hospital work environment, where surveillance populations are typically small and monitoring, surveillance, and other data are often fragmentary. Despite these challenges to performing effective surveillance, hospitals account for greater than half of all potentially EtO-exposed workers in various industries. Hence, it is important to examine OSHA medical surveillance requirements as they function in this context.

These workers were exposed to low levels of EtO with occasional high excursions. Based on our observations at many different hospitals, as well as published accounts [Elliott et al., 1988; Currier et al., 1984; Van Sittert et al., 1985], we would describe this exposure profile as typical of many hospitals as well as industrial sterilization plants.

Medical complaints potentially related to EtO exposure were diagnosed in several workers. Occupational and medical histories and physical exams were crucial for detecting EtO-associated illness. The personal and area monitoring programs in place were not adequate to prevent these outcomes, highlighting the utility of medical surveillance in detecting otherwise unrecognized overexposure and illness. Appropriate measures were initiated in response to these findings, including upgrades of equipment and engineering controls, initiation of annual worker training, and revisions of policies and procedures.

In this study, complete blood counts with differentials showed a relative lymphocytosis which, upon comparison to nonexposed groups, were found not to be associated with EtO exposure or work in the sterilization department. Confounding by age, race, sex, and smoking in our sample seems unlikely. Differential count variability by age, sex, and race is relatively small and only becomes apparent in large samples [Van Assendelft et al., 1977]. Smoking has been reported to have a significant effect on total white cell counts [Schwartz and Weiss, 1991], but not on differential counts [Lellouch and Schwartz, 1971; Malech and Gallin, 1987].

The lack of altered patterns of WBC or lymphocyte counts in three workers who were symptomatically overexposed to EtO suggests that lymphocytosis may occur only in very highly overexposed workers who simultaneously show severe acute neurotoxic and other symptoms. Additionally, although anemia has been reported to be associated with EtO exposures, there was no evidence of hemolytic effects in these three workers.

The described control group and reference range comparisons demonstrate that percent reference ranges are not reliable, and that there is a lack of standardization of clinical laboratory practice in deriving reference values. The use of absolute counts reduces misclassification of white cell differential counts as abnormal. Our results also suggest that relative lymphocyte counts can vary significantly between geograph-

ically close sites and that misinterpretations can arise in the absence of on-site controls.

Basis of the Requirement for the CBC With Differential in EtO Medical Surveillance

The above described findings suggest potential problems with the use of the CBC with differential in medical surveillance for EtO. Although the association between EtO overexposure and lymphocytosis seems to be widely accepted, the studies supporting this association are few and inconclusive. The CBC with differential requirement in the OSHA EtO Standard is based primarily on one report of an association of lymphocytosis with EtO overexposure [Ehrenberg and Hallstrom, 1967]. In this study of Swedish manufacturing workers, a group of 31 workers potentially exposed to "high" EtO levels was compared to a group of 26 unmatched workers exposed to "low" EtO levels. No exposure data were available, and no adjustment was made for smoking or other potential confounders. The high exposure group had significantly higher absolute lymphocyte counts (although within normal range), a significantly higher prevalence of relative anemia, a higher prevalence of chromosomal abnormalities, and one case of chronic lymphocytic leukemia. When the analysis was repeated one year later on expanded groups of workers, the difference in lymphocyte counts between exposed and control workers was no longer significant. The authors suggested that this may have been due to the improved ventilation and other control measures prompted by their initial observations. Although their findings were inconclusive, the authors recommended regular medical surveillance similar to that provided for radiological workers. This recommendation was based on the various hematologic changes in EtO-exposed workers and the expected radiomimetic effects of EtO as an alkylating agent. Such surveillance was to include blood counts and chromosome analysis.

There are additional case reports of lymphocytosis and leukocytosis associated with acute EtO exposure which were not cited by OSHA. Von Oettingen [1939] described three cases in which lymphocytosis was observed together with headache, vomiting, dyspnea, and diarrhea (EtO-associated flu-like syndrome); CBC data were not provided and exposure levels were not documented, but are likely to have been very high. Hess and Tilton [1950] described a private communication from Sexton of one case in which severe neurologic symptoms were observed together with a lymphocytosis of 17,000 with 60% lymphocytes. Finally, Joyner [1964] reported an elevated average WBC count in a group of 37 EtO-exposed chemical workers, noting the WBC in exposed workers to be 9,124/ml versus 7,553/ml in controls (normal range is 5,000–10,000/ml). The statistical significance of this apparent difference was not reported and differential counts were not performed. Workers in this study were reportedly exposed continuously to 5–10 ppm of EtO. Animal evidence of EtO-associated lymphocytosis is mixed, with some studies showing marked lymphopenia [Popp et al., 1986], some showing no effect [WHO, 1985], and some showing lymphocytosis [WHO, 1985].

The requirements for red cell count, hematocrit, and hemoglobin were included on the basis of limited evidence of EtO-associated anemia [Ehrenberg and Hallstrom, 1967; Glaser, 1979; and WHO, 1985]. Hemolysis has also been reported in patients exposed in residues in EtO-sterilized medical devices [Hirose et al., 1963; Stanley et al., 1971]. This exposure involves direct blood contact as opposed to inhalation

exposure, but nonetheless does establish hemolytic potential. Animal studies of EtO-associated hemolysis show conflicting results, with some studies showing hemolysis and some showing no effect [Glaser, 1979; WHO, 1985].

Since the passage of OSHA's 1984 EtO Standard, two more extensive studies including hematologic data have been published. Currier et al. [1984] examined hemoglobin, hematocrit, red blood cell count, white blood cell count, and percent lymphocytes as part of a cross-sectional study of 84 employees with an estimated 8 hour TWA EtO exposure "generally below 10 ppm with most below 1 ppm." These were compared to a control group individually matched on age, hire date, race, smoking habits, alcohol history, and date of examination. The differences between the hematologic values in exposed and control groups were small, and none approached statistical significance. Additionally, a series of blood chemistry analyses showed no statistically significant differences, with the exception of a significant excess of proteinuria in the potentially exposed group.

Van Sittert et al. [1985] examined cytogenetic, immunologic, and hematologic parameters in 36 EtO manufacturing plant workers with estimated 8 hour TWA EtO exposures below 0.05 ppm with occasional excursions to higher levels. These were compared to a group of 35 control personnel matched by age and smoking habits. There were no statistically significant differences between the two groups in white cell counts, percent lymphocytes, percent monocytes, and percent neutrophils.

OSHA, in promulgating the EtO Standard, asserted that the CBC was not useful in screening for EtO-associated leukemia [OSHA 1984:24784–25786]. This position is consistent with data from Cowles et al. [1991] and Tsai et al. [1983], who found CBC surveillance of petrochemical manufacturing workers to be an ineffective tool in identifying leukemia or pre-leukemic states (potentially associated with benzene exposures in this context). Chronic leukemias may have asymptomatic periods during which they could be detected by CBC; however, treatment during the asymptomatic period does not improve outcome over treatment after symptoms have developed [Rose, 1988]. Hence, detection of leukemia by CBC with differential would confer no therapeutic benefit to the affected individual and would not function well as a sentinel health event since the relevant exposure would have occurred years before.

The Use of the CBC With Differential as a Screening Tool

The CBC and leukocyte differential are two of the most commonly ordered clinical laboratory tests [Shapiro and Greenfield, 1987]. Despite its widespread use, however, the leukocyte differential also has been referred to as "one of the worst procedures offered by the hematology lab" [Dutcher, 1984; Wenz et al., 1986]. Some consider the leukocyte differential to be of little value in the ambulatory care setting [Rich et al., 1983; Shapiro and Greenfield, 1987; Ruttimann et al., 1992] or the hospital setting [Bull and Korpman, 1980]. A leading reference book in clinical preventive medicine describes the CBC as never having been shown to pick up any disease that is worth finding in the asymptomatic, nonpregnant adult [Rose, 1988].

The leukocyte differential count suffers from a lack of sensitivity and specificity, mechanical errors in slide preparation, physiologic variation by age, sex, ethnicity, and race, diurnal variation, subjective interpretation, and counting error due entirely to chance [Wenz et al., 1986; Shapiro and Greenfield, 1987]. As far back as 1933, Barnett recommended that differentials be based on at least 400 cells in order to be reliable. Currently, many labs perform manual counts on 100 cells. The in-

TABLE V. "Normal" Values for Lymphocyte Differential Counts

Reported as percentage of total leukocytes:	Reference:
20–35%	Seiverd [1972], range used by "off-site" control hospital
25–45%	Derived from 20–30 employees at eastern Mass. hospital ("on-site")
25–33%	Currier et al. [1984], range used in another EtO study
25–33%	Cecil and Loeb [1971]
22–40%	Sonnenwirth and Jarett [1980]
20–40%	Krupp [1991]
16–45%	Jordan et al., [1992]
Reported as percentage and absolute counts:	**Reference:**
19.6–52.7% 1,500–4,000 cells/ml	Orfanakis et al. [1970]
12.5–40.0% 832–3,140 cells/ml	Zacharski et al. [1971]
21–49% 1,490–3,930 cells/ml	Wintrobe. [1967]
15–60% 1,000–4,500 cells/ml	Whitby and Britton [1963]
20–45% 1,500–3,500 cells/ml	Dacie and Lewis [1963, 1970]
15–45% 600–4,500 cells/ml	Linman [1966]
34% 1,000–4,800 cells/ml	Albritton [1952]
Reported as mean percentage:	
36.3% (male, all races) 36.5% (female, all races)	Van Assendelft et al. [1977]
Reported as absolute counts:	
1,168–3,262 cells/ml (males) 1,149–3,664 cells/ml (females)	Bain and England [1975]
1,500–4,000 cells/ml	Wintrobe [1981]

creasing use of automated differential cell counters on larger numbers of cells will eliminate many of these sources of error. Where manual counts must be performed, they should be based on 400 or more cells.

In addition to the above limitations, a review of published reference values shows poor consensus on defining "normal" lymphocyte percentages. The "normal" values for lymphocyte differential counts at the hospital for which EtO surveillance was provided and the off-site control hospital are listed and compared to several other published values in Table V. A marked variability in "normal" values is observed.

Some investigators report differential lymphocyte and other white cell values in absolute numbers as 95% confidence limits (for examples, 1,500–4,000 or 832–3,140 lymphocytes per ml) (Table V). Wesson et al. [1980] showed this approach to be more reliable than reporting percentages in a study where differentials were performed on 119 healthy men with total leukocyte counts of 5,000–10,000/ml; it was

observed that 51% of the differentials were abnormal when reported as percentages, whereas only 10% were abnormal when absolute numbers were reported. The use of absolute lymphocyte counts in our study also reduced the misclassification of abnormal values. When the differential is performed, absolute reference ranges should be obtained (many lab slips do not provide them) and absolute counts should be used in preference to relative counts.

In the most thorough survey of "normal" leukocyte differentials that we were able to locate through literature review and direct communication with the College of American Pathologists, 5,800 single determination, 100 cell, manually performed leukocyte differentials were analyzed as part of the National Health and Nutrition Examination Survey [Van Assendelft et al., 1977] (Table V). Small but statistically significant differences in percent lymphocyte and segmented neutrophil counts were observed by sex, race, and age. Stratification by income group showed no statistically significant differences. The data were not analyzed for geographic variation. A more recent report by Saxena and Wong [1990] confirmed the finding of heterogeneity of CBC and leukocyte differential counts among racial, ethnic, and gender subgroups, and recommended the development of reference values for each subgroup. The biological basis of these variations is not understood.

It appears that clinical laboratories choose "normal" lymphocyte values in at least two different ways: by adopting from the literature or by sampling of employees or patient base. In the present study, the "off-site" hospital laboratory used a normal range from a 1972 textbook [Seiverd]. The "on-site" hospital lab used a range derived by sampling 20–30 lab employees several years before this study (lab employees could not recall the year). Neither of these reference ranges were representative of the surveillance population, as discovered through the use of control groups. Hence, before initiating surveillance, it is essential to ensure that the participating clinical laboratory uses the appropriate reference ranges.

The "normal" values used may not be of great consequence for routine clinical practice, since percent variations within even the widest of ranges are not associated with known disease processes. Clinical labs generate "normal" values primarily for the purpose of evaluating ill patients rather than detecting early illness in active workers. However, appropriate "normal" values are crucial to a medical surveillance program which is looking for abnormalities in lymphocyte counts in otherwise healthy subjects. In order to unambiguously interpret results when the CBC with differential is used for medical surveillance, clinicians should concurrently sample appropriate control groups. Concurrent controls are particularly important if one is to be able to detect potentially exposure-related changes in counts which occur within "normal ranges" [see citations of Joyner, 1964 and Ehrenberg and Hallstrom, 1967, discussed above]. In such a case, one could only detect and determine the need for further investigation and intervention through the use of nonexposed controls. This issue has been discussed previously with regard to the use of the CBC in benzene medical surveillance [Goldstein, 1988].

CONCLUSIONS

Our experience suggests that the use of the CBC with differential in routine medical surveillance for EtO exposure needs to be reexamined. Our findings and a review of the literature suggest problems with both the association between CBC

outcomes and EtO exposure, and pitfalls in the use of the leukocyte differential as a screening tool. Alterations in the CBC seem to occur only after EtO exposure at levels that also produce acutely toxic symptoms. Hence, the detection of CBC abnormalities during medical surveillance will likely not aid in the early detection of EtO health effects or in the evaluation of the adequacy of primary preventive measures. Furthermore, pitfalls regarding the use of absolute versus relative counts, proper reference ranges, and appropriate control groups can lead to misinterpretations of results, and follow-up that is costly both economically and in terms of worker anxiety and discomfort. Although initially justified on the basis of expected hematologic effects of high EtO exposure levels, experience since the passage of the 1984 EtO Standard suggests that the CBC with differential may not currently meet the criteria for an appropriate component of medical surveillance for EtO exposure [Halperin et al., 1986].

It is important to acknowledge that the CBC, excluding the differential, is very useful as a screening tool for certain outcomes and exposures, such as to detect pancytopenia in benzene-exposed workers or anemia in arsine-exposed workers. Each of these provide both potential therapeutic benefits to the affected individuals as well as indications for intervention to protect similarly exposed workers. Further study of the utility of the CBC with leucocyte differential in medical surveillance is warranted due to its current use, as well as its potential to be considered by OSHA in future standard-setting activities.

ACKNOWLEDGMENTS

The authors thank participating workers, supervisors, volunteer blood donors, and others. We thank hospital administration and the employees' union for their cooperation and support. We thank Dianne Plantamura and Margaret Bates from the Center for Occupational and Environmental Medicine, Massachusetts Respiratory Hospital for their support and help in obtaining medical records, and Patricia Quinn from the clinical laboratory of the same institution for her help in obtaining and interpreting hematology records. We thank Janna Frelich and Lucille Pothier from the Occupational Health Program, Harvard School of Public Health for their help in data analysis. Finally, we thank Joan Levine and Paul Schulte from NIOSH for their review of the manuscript. Funded in part by NIEHS Center Grant ES00002, NRSA T32 ES 07069, and K01-0H00110 and CCU 107404-1 from the CDC.

REFERENCES

Albritton EC (1952): "Standard Values in Blood." Philadelphia: WB Saunders Company, p 51.

Bain BJ, England JM (1975): Normal hematologic values: Sex differences in neutrophil counts. Br Med J 1:306–309.

Barnett CW (1933): The unavoidable error in the differential count of the leukocytes of the blood. J Clin Invest 12:77–85.

Bull B, Korpman RA (1980): Characterization of the WBC DIfferential Count. Blood Cells 6:411–419.

Cecil RL, Loeb RF (1971): "Textbook of Medicine, 13th Edition." Philadelphia: Saunders.

Cowles SL, Bennett JM, Ross CE (1991): Medical surveillance for leukemia at a petrochemical manufacturing complex: Four year summary. J Occup Med 33(7):808–812.

Currier MF, Carlo GL, Poston PL, Ledford W (1984): A cross-sectional study of employees with potential occupational exposure to ethylene oxide. Br J Ind Med 41:492–498.

Dacie JV, Lewis SM (1963): "Practical Hematology, Third Edition." New York: Grune and Stratton, Inc., pp 51–56.

Dacie JV, Lewis SM (1970): "Practical Hematology, Fourth Edition." London: Churchill.

Dutcher TF (1984): Leukocyte differentials: Are they worth the effort? Clin Lab Med 4:71–87.

Ehrenberg L, Hallstrom T (1967): Hematologic studies on persons occupationally exposed to ethylene oxide. In Kallings LO (ed): "Radiosterilization of Medical Products." Vienna: International Atomic Energy Agency, pp 327–334 (Report No. SM-92/96).

Elliott LJ, Ringenburg VL, Morelli-Schroth P, Halperin WE, Herrick RF (1988): Ethylene oxide exposures in hospitals. Appl Ind Hyg 3:141–145.

Glaser Z (1979): Ethylene oxide: Toxicology review and field study results of hospital use. J Environ Pathol Toxicol 2:173–208.

Goldstein BD (1988): Benzene toxicity. Occup Med 3:541–554.

Halperin WE, Ratcliffe J, Frazier TM, Wilson L, Becker SP, and Schulte PA (1986): Medical screening in the workplace: Proposed principles. J Occup Med 28:547–552.

Hemminki R, Mutanen P, Soloniemi I, Niemi ML, Vainio H (1982): Spontaneous abortion in hospital staff engaged in sterilizing instruments with chemical agents. Br Med J 285:1461–1463.

Hess LG, Tilton VV (1950): Ethylene oxide: Hazards and methods of handling. Ind Eng Chem 42:1251–1258.

Hirose T, Goldstein R, Bailey CP (1963): Hemolysis of blood due to exposure to different types of plastic tubing and the influence of ethylene oxide sterilization. J Thorac Cardiovasc Surg 45:245.

Hogstedt C, Aringer L, Gustavsson A (1986): Epidemiologic support for ethylene oxide as a cancer-causing agent. JAMA 255:1575–1578.

IARC (1985): Ethylene Oxide. In: "Alkyl Compounds, Aldehydes, Epoxides, and Peroxides; IARC Monographs, Volume 36." Lyons, France: International Agency for Research on Cancer, pp 189–226.

Jay WM, Swift TR, Hull DS (1982): Possible relationship of ethylene oxide exposure to cataract formation. Am J Ophthalmol 93:727–732.

Jordan CD, Flood JG, Laposata M, Lewandrowski KB (1992): Case records of the Massachusetts General Hospital: Normal reference laboratory values. NEJM 327(10):718–724.

Joyner RE (1964): Chronic toxicity of ethylene oxide. Arch Environ Health 8:700–710.

Krupp MA (1991): Normal Laboratory Values. In Schroeder SA, Krupp MA, Tierney LM Jr., and McPhee SJ (eds): "Current Medical Diagnosis and Treatment 1991." Norwalk, CT: Appleton and Lange, pp 1202–1203.

Landrigan PJ, Meinhardt TJ, Gordon J, Lipscomb JA, Burg JR, Mazzuchelli LF, Lewis TR, Lemen RA (1984): Ethylene oxide: An overview of toxicologic and epidemiologic research. Am J Ind Med 6:103–115.

Lellouch J, Schwartz D (1971): Smoking and leukocyte counts. Lancet 18:632–635.

Linman JW (1966): "Principles of Hematology." New York: The Macmillan Company, pp 308–309.

Malech HL, Gallin JI (1987): Neutrophils in human diseases. New Engl J Med 317:687–702.

Orfanakis NG, Ostlund RE, Bishop CR, Athens JW (1970): Normal blood leukocyte concentration. Am J Clin Pathol 53:647–651.

OSHA (1984): Occupational Exposure to Ethylene Oxide: Final Standard. Federal Register 49:25734–25809.

OSHA (1988): Occupational Exposure to Ethylene Oxide: Final Standard. Federal Register 53:11414–11438.

Popp DM, Popp RA, Lock S, Mann RC, Hand REJr (1986): Use of multiparamater analysis to quantitate hematological damage from exposure to a chemical (ethylene oxide). J Tox Environ Hlth 18:543–565.

Rich EC, Crowson TW, Conelly DP (1983): Effectiveness of differential leukocyte count in case finding in the ambulatory care setting. JAMA 249:633–636.

Rose SD (1988): Screening tests of uncertain value. In Hudson TW, Reinhart MA, Rose SD, Stewart GK (eds): "Clinical Preventive Medicine: Health Promotion and Disease Prevention." Boston/Toronto: Little, Brown, and Company, p 481.

Ruttimann S, Clemencon D, Dubach UC (1992): Usefulness of complete blood counts as a case-finding tool in medical outpatients. Ann Intern Med 116:44–50.

SAS Institute (1987): "SAS/STAT Guide for Personal Computers, Version 6 Edition." Cary, North Carolina: SAS Institute Inc.

Saxena S, Wong ET (1990): Heterogeneity of common hematologic parameters among racial, ethnic, and gender sub-groups. Arch Pathol Lab Med 114:715–719.

Schwartz BS, Ford DP, Yodaiken R (1992): Analysis of OSHA inspection data with exposure monitoring and medical surveillance violations. J Occup Med 34(3):272–278.

Schwartz J, Weiss ST (1991): Host and environmental factors influencing the peripheral blood leukocyte count. Am J Epidemiol 134:1402–1409.

Seiverd CE (1972): "Hematology for Medical Technologists, Fourth Edition." Philadelphia, PA: Lea and Febiger, p 215.

Shapiro MF, Greenfield S (1987): The complete blood count and leukocyte differential count. Ann Intern Med 106:65–74.

Siegel WC, Bunn W (1985): The ethylene oxide standard: Medical and legal aspects of compliance. J Hosp Supp Process Distrib May/June:40–46.

Sonnenwirth AC, Jarett L (eds) (1980): "Gradwohl's Clinical Laboratory Methods and Diagnosis, Eighth Edition." Saint Louis: CV Mosby Company, p 798.

Stanley P, Bertranou E, Forest F, Langevin L (1971): Toxicity of ethylene oxide sterilization of polyvinyl chloride in open-heart surgery. J Thorac Cardiovasc Surg 61:309–314.

Stayner L, Steenland K, Greife A, Hornung R, Hyaes R, Nowlin S, Morawetz J, Ringenburg V, Elliot L, Halperin W (1992): An exposure-response analysis of cancer mortality among a cohort of workers exposed to ethylene oxide. Abstract from Ninth International Symposium on Epidemiology in Occupational Health, Cincinnati, Ohio, Sept 23–25, 1992, p 179.

Tsai SP, Wen CP, Weiss NS, Wong O, McLellan WA, Gibson RL (1983): Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J Occup Med 25:685–692.

Van Assendelft OW, McGrath C, Murphy RS, Schmidt RM (1977): The differential distribution of leukocytes. In Koepke JA (ed): "Differential Leukocyte Counting." Northfield, Illinois: College of American Pathologists, pp 11–22.

Van Sittert NJ, De Jong G, Clare MG, Davies R, Dean BJ, Wren LJ, Wright AS (1985): Cytogenetic, immunological, and hematological effects in workers in an ethylene oxide manufacturing plant. Br J Ind Med 42:19–26.

Von Oettingen WF (1939): Supplement to occupation and health. In: "Encyclopedia of Hygiene, Pathology, and Social Welfare." Geneva: International Labour Office.

Wenz B, Gennis P, Canova C, Burns ER (1986): The clinical utility of the leukocyte differential in emergency medicine. Am J Clin Pathol 86:298–303.

Wesson SK, Mercado T, Austin M, Schumacher HR (1980): Percentage and absolute differential counts on 119 healthy US Navy mid-shipmen (letter). Lancet 1:552.

Whitby LEH, Britton CJC (1963): "Disorders of the Blood: Diagnosis, Pathology, Treatment, Technique, Ninth Edition." New York: Grune and Stratton, Inc., p 15.

WHO (1985): "Environmental Health Criteria 55: Ethylene oxide." Geneva: World Health Organization.

Wintrobe MM (1967): "Clinical Hematology, Sixth Edition." Philadelphia: Lea and Febiger, p 260.

Wintrobe MM (1981): "Clinical Hematology, Eighth Edition." Philadelphia: Lea and Febiger, p 208.

Yakubova AN, Samova NA, Miftakhova FA, Shilova LF (1976): Gynecological disorders in workers engaged in ethylene oxide production. Kazanskii Meditsinskii Zhuranl 57:558–560.

Zacharski LR, Elveback LR, Linman JW (1971): Leukocyte counts in healthy adults. Am J Clin Pathol 56:148–150.