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8. TRIA has also been dispersed by immersion in water of a wettable substrate [cellulose (*6*, *9*) or clay (*2*)] on which a film of crystalline triacontanol has been deposited. Vesicles of polar lipids have been generated by a similar method (*10*), but the yield of dispersed particles is small and uncertain (0 to 20 percent) (*11*). This information suggests that the fraction of the TRIA applied by these methods that will actually be dispersed is also uncertain and likely to be small.
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Exposure to Ethylene Oxide at Work Increases Sister Chromatid Exchanges in Human Peripheral Lymphocytes

Abstract. *Sister chromatid exchange rates increased significantly in the peripheral lymphocytes of a small group of hospital workers exposed to ethylene oxide for as little as 3.6 minutes per day regularly over a period of months. Results based on breathing zone exposure and task frequency estimates over a 6-month period for 14 workers suggest that sister chromatid exchanges are a sensitive indicator of exposure and that cumulative dose and dose rate are important predictors of sister chromatid exchange response.*

A sister chromatid exchange (SCE) is the visual manifestation of a four-stranded exchange in the DNA (*1*). Determining the average number of SCE's per cell is a sensitive method for measuring the chromosomal effects of some mutagenic and carcinogenic agents and correlates with other short-term tests for DNA damage (*2*). The exact mechanism of SCE formation is unknown, but is thought to reflect changes in DNA resulting from adduct formation or changes in conformational structure after exposure to exogenous agents (*3*). Mutations may be a subset of lesions that elicit SCE's (*4*).

Although SCE rates in human lymphocytes have been investigated as a biological monitor for exposure to harmful agents in the workplace (*5*), exposure data have been lacking. In the few studies that included estimates of airborne exposure, values were reported as air concentrations averaged over an 8-hour workday [time-weighted average (TWA)] without regard to specific patterns of exposure or epidemiological factors that may impinge on the specificity of the SCE response. Results presented here suggest that, when a precise description of short-term exposure is obtained, an exposure-response relation in humans is shown even though the 8-hour TWA exposure is low.

Ethylene oxide (ETO), a known mutagen and a suspected carcinogen, has been shown to increase SCE's in the rabbit in vivo (*6*). The current Occupational Safety and Health Administration (OSHA) standard for workplace exposure is 50 ppm as an 8-hour TWA. Previous studies showed an increase in SCE's in persons occupationally exposed to ETO; however, no breathing zone information on the levels of exposure that elicit this response was reported (*7*).

Our study was conducted in two hospitals that use ETO to sterilize supplies. There appeared to be no significant exposure to other chemicals in these work areas. A single-beam infrared spectrophotometer (Miran 1A, Foxboro) connected to a strip-chart recorder was used to measure and record short-term exposures of operators as they performed their work with sterilizers containing ETO. We had already determined that there was no extraneous exposure to ETO, such as might occur from leaking gaskets on the sterilizer doors.

To express an estimated dose of ETO received by a worker during a brief exposure it can be assumed that alveolar uptake and absorption of ETO approach 100 percent (*8*). The expression for estimated dose per task is then $m = \bar{V}_m \bar{c} T$, where m is estimated dose per task in milligrams, $\bar{V}_m$ is mean respiratory minute volume (estimated to be about 21 liters per minute for humans performing light work) (*9*), $\bar{c}$ is mean concentration of ETO in milligrams per cubic meter, and T is elapsed time (minutes) during task performance.

The tasks during which ETO exposure occurred were identified and defined. Each exposed the workers to a different dose (Table 1) (*10*). Estimates of the doses received by workers performing the same task at hospitals A and B differ considerably. This is attributable to differing ventilation systems, equipment,

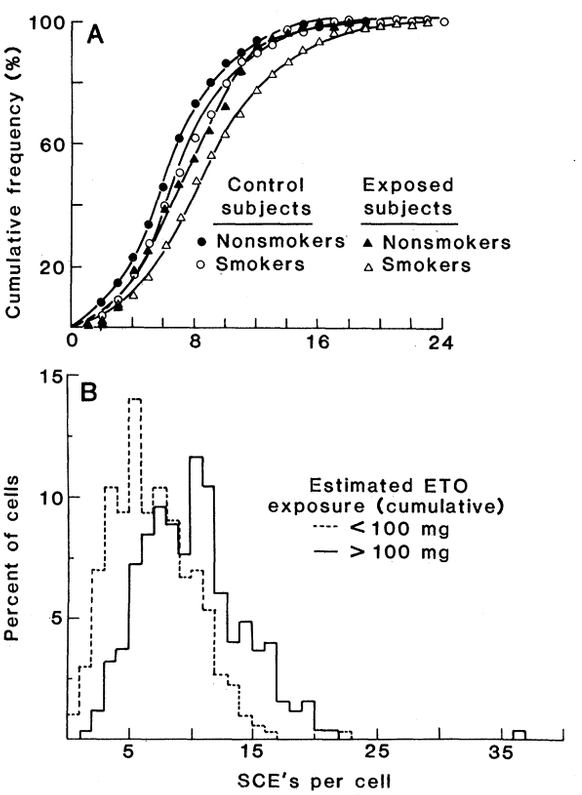

Fig. 1. (A) Cumulative frequency distribution of SCE's per cell. The mean number of SCE's per cell (± the standard deviation) was 7.04 ± 1.00 for control nonsmokers, 7.89 ± 0.92 for control smokers, 8.27 ± 2.06 for exposed nonsmokers, and 9.09 ± 2.00 for exposed smokers. (B) Frequency distribution of SCE's per cell in workers exposed to ETO at relatively low (D_w < 100 mg) and relatively high (D_w > 100 mg) cumulative doses.

and work practices, and illustrates the importance of carefully assessing actual work and exposure conditions.

When four different workers repeatedly performed task 1 at hospital A, there was no difference among them in estimated mean dose per task. Therefore, we assumed that exposures were homogeneous for all workers performing the same task. Even if this assumption is valid only for task 1 at hospital A, the conclusions would not be affected since that task was the major contributor to exposure (Table 1).

Table 1. Estimated ETO doses for defined tasks.

Task	Observations (No.)	Dose per task (mg)
Hospital A		
1. Open and unload sterilizer and load aerator	30	9.60 ± 3.60*
2. Open test package	5	5.90 ± 1.93*
3. Remove items from aerator before cycle is completed	2	0.26 ± 0.07*
Hospital B		
1. Open and unload sterilizer and load aerator	1	0.1

*Mean ± standard deviation.

Table 2. Estimated cumulative exposure of workers to ETO over the 6-month period (D_w) and SCE data. Blood samples (3 ml) were drawn by venipuncture into sterile heparinized tubes and coded. Samples were analyzed for SCE's as follows: replicate cultures containing 0.3 ml of whole blood in 5 ml of RPMI 1640 medium (Gibco) supplemented with fetal calf serum (15 percent by volume) (Gibco), 1 percent glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), 1 percent PHA-M (Difco), and 20 μ*M* 5-bromodeoxyuridine were grown at 37° ± 0.1°C for 72 hours in the dark. Four hours before the end of incubation, 0.1 ml of 10^{-5}*M* Colcemid was added. Cells were centrifuged, treated with 0.075*M* KCl to spread the chromosomes, and fixed and washed twice in methanol and acetic acid (3:1). Concentrated fixed cells were placed on slides, dried, and stained. For staining the slides were placed in Hoechst 33258 (5 μg/ml) in 0.07*M* Sorensen's buffer (*p*H 6.8) for 15 minutes, rinsed in distilled water, blotted, and mounted in Sorensen's buffer under cover slips. The slides were placed on a warming tray at 56°C and exposed to black light at a distance of 2.5 cm for 7 to 11 minutes so that differentiation would occur. The cover slips were then removed and the slides were stained in 3 percent Giemsa (Gurr's R66 in 0.07*M* phosphate buffer, *p*H 6.8) for 10 minutes. The slides were rinsed in distilled water, dried, passed through xylene, and mounted with Depex (Gurr). Metaphase cells containing 44 to 46 chromosomes were then examined to determine the number of SCE's. Fifty cells were scored per coded sample. Control and experimental samples were run concurrently and the same lot of fetal calf serum was used throughout.

Group	Sample number	Estimated dose per task (mg)*	Estimated number of times task performed in 6 months	D_w (mg)		SCE's per cell	
				Individual	Group mean	Individual mean	Group mean
Control	H15					6.80	7.56
	H16					8.22	
	H18					7.20	
	H20					5.50	
	H35					7.46	
	H07†					9.38	
	H19†					6.82	
	H22†					7.16	
	H27†					7.52	
	H32†					8.30	
	H33†					8.90	
	H34†					7.96	
	H36†					7.04	
Exposed							
Low exposure	H01†			0	13	7.50	7.76
	H03†			0		8.16	
	H08†			0		9.62	
	H30‡	0.1	96	10		7.24	
	H23†	0.26	48	12		7.76	
	H24†	0.26	48	12		6.60	
	H28†	0.26	48	12		8.54	
	H29‡	0.1	120	12		8.28	
	H05	9.6	6	58		6.16	
High exposure	H17†	9.6	18	173	501	9.64	10.69§
	H25	9.6	40	384		8.00	
	H11†	9.6	48	461		12.98	
	H04†	9.6 + 5.9	48	744		11.16	
	H12	9.6 + 5.9	48	744		11.66	

*From Table 1. †Smoker. ‡Worker in hospital B. §$P = .002$, analysis of variance.

To express exposure as an estimated cumulative dose (D_w) during the 6 months of observation preceding blood sampling for SCE analysis, we interviewed the workers to ascertain the number of times they performed specific tasks. The total of these estimates agreed with hospital A's daily logs on the number of sterilizer loads processed during the same period. [The decision to make observations during a 6-month period was based on an animal inhalation study (*6*)]. Each worker's estimated 6-month cumulative dose (Table 2) is then the product of the mean dose per task and the number of times that task was performed (Tables 1 and 2).

Control subjects were selected from the clerical and administrative staffs of the hospitals and from two nearby research institutions. Confidentiality of data, informed consent, and other ethical considerations in keeping with research involving human subjects were carefully observed. All the subjects were interviewed to obtain a health and occupational history including information on smoking, age, sex, recent immunizations and infections, and intake of caffeine, alcohol, and other drugs. Inspection of the data showed a similar distribution of all these factors in both populations. The populations were also well matched in terms of the number of smokers (there were five nonsmokers in each group) (Table 2). However, smokers among the controls smoked a mean of 21 cigarettes per day, whereas smokers among the exposed subjects smoked a mean of nine cigarettes per day. Smokers in both groups had cells containing the highest frequencies of SCE's (Fig. 1A) (*11*).

Mean frequency of SCE's per cell was significantly higher in the exposed subjects than in the controls ($P < .05$, Mann-Whitney U test). More important, when exposed subjects were divided into relatively low and high exposure groups, each containing a similar proportion of smokers (Table 2), the mean frequency of SCE's per cell in the high exposure group significantly exceeded that in both the low exposure group and the controls. An estimated cumulative dose of ETO of > 100 mg was chosen as the cutoff between groups because it is at this point in the continuum that there is no statistical overlap in exposure estimates. Figure 1B shows the frequency distribution of pooled cells from low and high exposure groups. The more highly exposed population shows a marked shift to the right (*6*, *11*).

These data suggest that ETO elicits an

increase in SCE frequency at average levels of exposure that are low in comparison with the current OSHA standard of 50 ppm. With the breathing zone data gathered in this study, it may be possible to determine whether the observed increase in SCE's arises exclusively from the cumulative effect of daily exposure or whether some component of the increase results from the rate at which that exposure occurs. Comparison of mean numbers of SCE's induced per cell per unit of cumulative exposure with those reported in a recent animal study (*6*) indicates that humans may be considerably more sensitive to SCE induction than animals.

This difference would be much less, however, if SCE induction were also a function of dose rate, since the workers were exposed to ETO for short periods at five times the dose rate to which the animals were exposed. An effect of dose rate has been shown for ETO in other animal studies (*12*). If a dose rate effect is found for humans as well, then the evidence of ETO-induced SCE's may suggest that occupational exposure to ETO and other alkylating agents be controlled in terms of both cumulative dose and dose rate.

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10. The Miran 1A infrared spectrophotometer has a rapid response time and is sensitive to 1 ppm ETO. It was linked to a strip-chart recorder with integral output to continuously monitor air concentrations during the defined tasks. Five-foot-long tubing with a sampling probe was attached to the instrument and hand-held in the breathing zone of the worker while the specified tasks were performed. The instrument was calibrated at the recommended wavelength of 3.3 μm for the mixture used at hospital A (12 percent ETO and 88 percent Freon 12) and at 11.8 μm for the 100 percent ETO at hospital B. Thirty measurements were obtained for the task that contributed most to exposure (task 1 at hospital A) (Table 1). Statistical testing showed no detectable difference between the assumptions of a normal or a log-normal distribution for the samples. The 8-hour TWA was calculated to be 0.92 ppm (range, 0.51 to 1.26 ppm).
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In vivo Phosphorus-31 Nuclear Magnetic Resonance Reveals Lowered ATP During Heat Shock of *Tetrahymena*

Abstract. *Cells synthesize a characteristic set of proteins—heat shock proteins—in response to a rapid temperature jump or certain other stress treatments. The technique of phosphorus-31 nuclear magnetic resonance spectroscopy was used to examine in vivo the effects of temperature jump on two species of* Tetrahymena *that initiate the heat shock response at different temperatures. An immediate 50 percent decrease in cellular adenosine triphosphate was observed when either species was jumped to a temperature that strongly induces synthesis of heat shock proteins. This new adenosine triphosphate concentration was maintained at the heat shock temperature.*

Cells respond in a common manner to a rapid temperature jump. This heat shock response involves the immediate transcriptional activation of a small set of previously quiescent genes, the transcripts of which are then preferentially translated into a characteristic set of proteins, the heat shock proteins. Conversely, both transcription and translation of most non–heat shock genes and transcripts are greatly reduced at the elevated temperature (*1*). The synthesis of these heat shock proteins, especially a class with a molecular weight of approximately 70,000, has been strongly conserved during evolution, and their presence is diagnostic of the response. In addition to temperature jump, these proteins are synthesized by cells in response to a variety of other stresses, including treatment with respiratory inhibitors, amino acid analogs, release from anoxia, and, in the case of *Tetrahymena*, deciliation (*1, 2*). The functions of this general cellular response to stress and of the induced proteins are not known, but their uniform occurrence in diverse organisms suggests that they play a fundamental role in cellular homeostasis.

The physiological signals mediating the heat shock response are unknown. However, because many inhibitors of oxidative phosphorylation or electron transport also induce synthesis of heat shock proteins, it has been suggested that cellular adenosine triphosphate (ATP) may be involved in the response (*3*). We used ^{31}P nuclear magnetic resonance (NMR) spectroscopy to examine in vivo the effects of temperature jump on the ciliated protozoan *Tetrahymena*. We compared two species of *Tetrahymena* in which the response is induced by different temperatures.

Cells in logarithmic growth were concentrated by centrifugation, washed, and resuspended at high density. The ^{31}P NMR spectra of the cells maintained at a standard temperature were collected, and the cells were then subjected to a temperature jump and further spectra were obtained. Portions were removed at both temperatures, and protein synthesis was monitored in vivo by incubating the cells in the presence of tritiated amino acids at either temperature. The ^{3}H-labeled proteins were then identified by gel electrophoresis and fluorography (*4*).

Upon subjecting either species to the higher temperature, where heat shock