Cytogenetic, immunological, and haematological effects in workers in an ethylene oxide manufacturing plant

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ABSTRACT Samples of blood were collected from a group of plant workers engaged in the manufacture of ethylene oxide (EO) for periods of up to 14 years and also from a group of control personnel matched by age and smoking habits. Peripheral blood lymphocytes were cultured for cytogenetic analysis. Selected immune and haematological parameters were also investigated. The results of these studies showed no statistically significant difference between the group of plant workers and the control group in respect of any of the biological parameters investigated in this study. Nevertheless, duration of employment in EO manufacturing was positively correlated ($p < 0.05$) with the frequency of chromosome breaks and with the percentage of neutrophils in a differential white blood cell count and negatively correlated ($p < 0.05$) with the percentage of lymphocytes. As the values of these parameters remained within the normal limits of control populations, the correlations were considered to have no significance for health. Atmospheric concentrations of EO were determined using personnel air samplers and were generally below the detection limit (< 0.05 ppm) during stable plant operations, although transient concentrations of up to 8 ppm were occasionally recorded. The amount of alkylation (2-hydroxyethyl groups) of the N¹ atom of histidyl residues in haemoglobin was also measured in an attempt to gauge recent individual exposures to EO. Variable but, in most instances, readily measurable amounts of N¹-(2'-hydroxyethyl)-L-histidine (N¹ represents the N₁ atom of histidine) were found in the haemoglobin of plant workers and in the control group who had not knowingly been exposed to an exogenous source of EO. There was no statistically significant difference between the results obtained in the control group and in the group of plant workers.

Ethylene oxide (EO) has been shown to induce mutations in bacteria, plants, and animals.¹⁻⁴ The results of a recent series of publications provide clear evidence of a causal association between prolonged exposure to EO in excess of 1 ppm (8 h TWA) and an increased occurrence of chromosome aberrations in the lymphocytes of human peripheral blood.⁵⁻⁷ The exposure data lack precision, however, and are therefore not entirely satisfactory for application in quantitative risk assessment. It was thus considered important to determine

whether or not employment in a modern EO manufacturing plant was associated with an increased frequency of chromosome aberrations. Furthermore, because an increase in the incidence of chromosome aberrations is regarded as direct evidence of genotoxic action in vivo, it was considered pertinent to include an evaluation of certain parameters that might prove to be sensitive health indicators of exposure to genotoxic agents. For example, genotoxic agents, such as alkylating agents can depress immunological responsiveness in patients treated with cytostatic drugs.⁸ Accordingly, we included an assessment of the status of the cells of the immune system and related haematological parameters.

The measurement of exposure to EO was an essential component of this study. The accurate determination of exposure is a problem common to most industrial epidemiological studies. In an attempt to overcome this problem two independent methods, one direct the other indirect, were used to determine exposures to EO. In the first method atmospheric concentrations of EO were measured using personal air samplers whereas in the second a procedure developed by Ehrenberg and his colleagues⁹⁻¹³ was used to develop precise data regarding recent individual exposures to EO. This method is based on measurements of the amount of a stable product, N^t-(2'-hydroxyethyl)-L-histidine,* formed by reaction of EO with histidine residues in the haemoglobin of circulating erythrocytes. The method was applied to the plant workers and to a control group of personnel employed in administrative duties unconnected with EO manufacturing operations and remote from any possible exposure to EO.

Methods

STUDY POPULATION

Table 1 shows the characteristics of a group of EO plant workers and the control group. The plant workers were 36 men aged 20-59 (average age 32) who at the time of the examination (1980) had been engaged in EO manufacturing for periods of between one to 14 years. In the control group were 35 men matched by age and smoking habits, who had no operational involvement in the manufacturing process and who were believed to have had no previous occupational exposure to EO or other chemicals. The plant workers were divided into two groups: in group 1 were 19 men with less

Table 1 *Characteristics of study groups*

	Plant workers	Controls
No of individuals	36	35
Mean age (years)	32	34
Smokers	22	26
Non-smokers	14	9
Shift workers	32	14
Non-shift workers	4	21
Exposure to radiographs (past 5 years)	15	15
Age group 20-29	15	13
Age group 30-39	14	14
Age group 40-59	7	8
>5 years' exposure to EO (6-14 years)	17	—
<5 years' exposure to EO (1-5 years)	19	—
Previous employment in other chemical plants	17	0

*According to IUPAC nomenclature the N^t atom represents the N₃ atom of histidine.

than five years' employment in the EO plant and in group 2 17 men with more than five years' employment. In addition to EO exposure the influence of other variables such as smoking, age, shift work, recent exposure to radiography, and previous employment in other chemical plants was also examined.

The plant workers have been employed in a modern, open air, EO manufacturing plant. Apart from EO, various other chemicals are used in the plant. None of these chemicals was believed to be associated with the biological effects investigated in this study. All individuals participated in the cytogenetic study but because of technical difficulties not all the plant workers and control subjects were examined by immunological and haematological analysis.

The study took place over a period of four weeks in January and February 1980.

EO EXPOSURE MONITORING

EO air monitoring surveys using personal samplers were carried out on occasions in 1974, 1978, 1980, and 1981, using the sampling method and the analytical technique recommended by NIOSH.¹⁴ The limit of detection of EO in air with this method was 0.05 ppm for an eight hour sampling period. Exposure to EO was not measured during 1966-73.

BLOOD SAMPLING PROCEDURE AND DESPATCH OF SAMPLES

Approximately 20 ml of blood was collected by venepuncture in heparinised polystyrene tubes (25 IU preservative free heparin per ml blood). Syringes and tubes used in this study were not sterilised with EO.

Equal numbers of blood samples from the plant workers and control groups were despatched by air and road to the Shell Toxicology Laboratory (Tunstall), Sittingbourne, United Kingdom, for chromosome analysis, assessment of immunological parameters, and determination of the degree of alkylation of histidine residues in haemoglobin. Samples reached their destination within 24 hours of venepuncture. Haematological analyses were carried out in the medical department of the manufacturing site using standard haematological methods.

CHROMOSOME ANALYSIS

Cultures were begun on receipt of the samples, generally about 24 hours after the blood had been collected. The detailed method for the culture, harvesting, and staining of lymphocytes has been described by Evans and O'Riordan.¹⁵ An aliquot sample of 0.8 ml blood was incubated at 37°C for either 48 h or 72 h in 10 ml Hams F10 culture

medium, supplemented with fetal calf serum, NaHCO_3 solution, benzyl penicillin, streptomycin sulphate, and phytohaemagglutinin. Three hours before the end of the incubation period, colcemid was added to a final concentration of $0.2 \mu\text{g/ml}$. After centrifugation, cells were incubated in $10 \text{ ml } 0.075 \text{ M KCl}$ at 37°C for eight minutes and after another centrifugation the cells were fixed in a 3:1 mixture of methanol and acetic acid. For each culture two slides were prepared and then randomised and coded. One hundred metaphases were analysed from cultures incubated for 48 hours. If insufficient metaphases were present after 48 hours cultures incubated for 72 hours were examined. The cells were analysed for gaps, chromatid and chromosome aberrations, and for polyploidy, provided that they contained at least 44 chromosomes. Chromatid aberrations included breaks, single fragments, and exchanges, and chromosome aberrations included breaks, rings, dicentrics, and translocations.

IMMUNOLOGICAL ANALYSIS

T cells were identified using the E rosette technique and B cells by direct immunofluorescence after tagging of phagocytic cells with latex particles. T lymphocyte function was assessed by a transformation test in which radiolabelled thymidine incorporation was measured in control cultures and in cultures stimulated by a range of concentrations of the T cell mitogen, phytohaemagglutinin (PHA). The serum concentrations of immunoglobulins G, M, and A were determined by the Laurell rocket immunoelectrophoretic technique, giving an indirect estimate of B cell function. Haematological analysis included white blood cell counts and differential white blood cell counts.

DETERMINATION OF THE AMOUNT OF N^L -(2'-HYDROXYETHYL)-L-HISTIDINE IN HAEMOGLOBIN

Red blood cells were sedimented at 400 g for 10 minutes, washed by gently suspending in isotonic saline (10 ml), and recentrifuged at 400 g . The washed red cells were frozen and stored at -40°C before analysis. The isolation of haemoglobin from red blood cells and the hydrolysis of protein was performed using a modification of the method of Calleman *et al.*¹¹ Globin (100 mg) was dissolved in 6 M HCl (10 ml). An aliquot of $15\,000\text{--}20\,000 \text{ dpm}$ of N^L -(2'-hydroxyethyl)-L-(ring-2- ^{14}C) histidine (synthesised in Sittingbourne Research Centre; specific activity 55 mCi/mmol) was added to each sample before hydrolysis to calculate losses during the experiment. Mercaptoethanol (0.1 ml) and 1-octanol (0.1 ml) were added, and hydrolysis was

conducted at 120°C for 16 hours in vacuo. N^L -(2'-hydroxyethyl)-L-histidine was isolated from the hydrolysate by preparative HPLC on Partisil 10 SCX. This was done by washing the column first with $0.025 \text{ M NH}_4\text{H}_2\text{PO}_4$ buffer, pH 4.6 for 10 minutes at a flow rate of 8 ml/min , at which conditions the neutral and acidic amino acids were eluted. Then the column was washed with $0.075 \text{ M NH}_4\text{H}_2\text{PO}_4$ buffer, pH 4.6 at the same flow rate, at which conditions the basic amino acids were eluted. The retention time of N^L -(2'-hydroxyethyl)-L-histidine was 18 minutes. The solution containing the adduct was desalted on Dowex 50W-X8 by elution with $400 \text{ ml } 0.5 \text{ M HCl}$ and the adduct was eluted with $2 \times 100 \text{ ml } 4 \text{ M HCl}$. After evaporation of the solution at low temperature the adduct was derivatised after addition of 75 ng of deuterated N^L -(2'-hydroxyethyl)-L-histidine to serve as internal standard, by reaction with methanolic HCl and heptafluorobutyric anhydride. Quantitation was carried out by gas chromatography on Ultrabond II coupled with chemical ionisation mass spectrometry. Isobutane was employed as the ionising gas, and the $\text{M}+1$ ions of both N^L -(2'-hydroxyethyl)-L-histidine and deuterated internal standard were monitored using the mass spectrometer in the multiple ion detection mode. The amount of alkylation was calculated as nanomoles N^L -(2'-hydroxyethyl)-L-histidine per gram of globin. The average recovery value was 70%. The limit of detection was 0.02 nmol/g of globin.

STATISTICAL ANALYSIS

The percentage of cells showing chromosome damage were calculated with their standard errors assuming a Poisson distribution of aberrations. This analysis was also done on the different types of cells in a differential white blood cell count. Normality of distribution of immunological and haematological parameters and of haemoglobin alkylation was tested by drawing normal probability plots. Parameters that were not normally distributed were logarithmically transformed. Differences between two variables at the 5% significance level were calculated using Student's *t* test. The relation between variables was investigated using linear regression analysis.

Results

EXPOSURE MEASUREMENTS

EO air concentrations

Table 2 shows EO exposures of plant workers during 1974–81; most were below the limit of detection (0.05 ppm). In surveys carried out in 1974

and 1980, however, about 10% of the measurements yielded EO exposures higher than 0.1 ppm, with a maximum of 8 ppm in 1980. During 1981, 50% of the measurements exceeded 0.1 ppm. The geometric mean EO exposure, calculated by linear extrapolation, was below 0.01 ppm in surveys carried out in 1974, 1978, and 1980. The corresponding value in 1981 was 0.12 ppm.

Haemoglobin alkylation

Table 3 shows the mean values and the ranges of the amounts of N¹-(2'-hydroxyethyl)-L-histidine in the haemoglobin of plant workers and control subjects. The mean value was slightly higher in the plant workers than in the control group but the increase was not statistically significant. The equivalent tissue (in vivo) doses* of EO were calculated from histidine alkylation levels using the criteria developed by Ehrenberg and co-workers^{10,11} and are also shown in table 3.

CHROMOSOME ANALYSIS

Table 4 shows the percentages of aberrant cells in the plant workers and in the control group. There was no statistically significant difference between the frequency of any of the types of aberrations noted in the lymphocytes of the plant workers' group compared with the controls.

*The in vivo dose is a function of concentration and exposure time and is expressed in terms of a composite unit, concentration $\times$ time—for instance, $\mu\text{M} \times \text{h}$. The calculation of the in vivo dose assumes a repeated and regular pattern of exposure to EO during the 18 week period immediately before blood sampling.

Table 3 Degree of alkylation of histidine in haemoglobin and corresponding weekly in vivo EO doses in plant workers and the control group

Group	No of individuals	N ¹ -(2'-hydroxyethyl)-L-histidine (nmol/g globin)		In vivo dose; ($\mu\text{M} \times \text{h} \times \text{week}^{-1}$)	
		Mean $\pm$ SEM	Range	Mean $\pm$ SEM	Range
Plant workers	32	2.08 $\pm$ 0.24	<0.02–9.7*	<16.5 $\pm$ 1.9	<0.16–76.9
Controls	31	1.59 $\pm$ 0.18	<0.02–4.7	<12.6 $\pm$ 1.4	<0.16–37.3

*This high value is based on a poor chromatogram and is therefore considered unreliable. Nevertheless, the result has not been excluded from the statistical analyses. If this result is excluded the range would be <0.02–6.5.

Table 4 Chromosome analysis of plant workers and controls. Data show the percentage of aberrant cells $\pm$ 1SEM

	Plant workers	Controls	Statistical analysis
No of metaphases analysed	3600	3444	
Polyploidy	0.06 $\pm$ 0.04	0.20 $\pm$ 0.08	NS
Gaps	0.89 $\pm$ 0.16	0.80 $\pm$ 0.15	NS
Chromatid aberrations			
Chromatid breaks	0.50 $\pm$ 0.11	0.31 $\pm$ 0.09	NS
Chromatid exchanges	0.03 $\pm$ 0.03	0.00	NS
Chromosome aberrations			
Chromosome breaks	0.22 $\pm$ 0.08	0.26 $\pm$ 0.08	NS
Dicentric chromosomes	0.00	0.00	NS
Ring chromosomes	0.00	0.00	NS

NS = Not significant ($p > 0.05$).

Table 2 Exposure of plant workers to ethylene oxide 1974–81. (Number of samples taken are given in parentheses)

Year of monitoring	Sampling period (h)	EO air concentration (ppm)	Geometric mean* EO air concentration (ppm)
1974	0.5	<0.1 (152); 0.1–4.9 (18)	<0.01
1978	8	<0.05 (26); 0.05–0.1 (20)	<0.01
1980	8	<0.05 (40); 0.1–8.0 (5)	<0.01
1981	8	<0.05 (6); 0.1–1.3 (6)	0.12

*Calculated by linear extrapolation on log probability paper.

Table 5 summarises the data of individuals in the respective groups 1 and 2. There was no statistically significant difference in the frequency of aberrant cells in group 1 compared with group 2 or between these groups and the control group.

Certain other factors such as smoking, shift work, previous employment in other chemical plants, and recent exposure to radiography were found to have no statistically significant effect on the frequency of chromatid or chromosome aberrations in either the plant workers or the control group. Regression analysis showed that the frequency of chromosome breaks in cultures from plant workers was positively correlated ($p < 0.05$) with the duration of employment. This trend was not solely attributable to the age of the men, since there was no significant correlation ($p > 0.05$) between age and number of aberrations.

No significant correlations ($p > 0.05$) were found between either the frequency of gaps or the frequency of chromatid aberrations and duration of employment or age. Regression analysis showed no

Table 5 Duration of employment and chromosome damage. Data show the percentage of aberrant cells $\pm$ 1SEM

	Group 1 (less than 5 years)	Group 2 (more than 5 years)	Statistical analysis
No of metaphases analysed	1900	1700	
Polyploidy	0.11 $\pm$ 0.07	0.00	NS
Gaps	0.63 $\pm$ 0.18	1.18 $\pm$ 0.26	NS
Chromatid breaks	0.58 $\pm$ 0.17	0.47 $\pm$ 0.17	NS
Chromosome breaks	0.16 $\pm$ 0.09	0.29 $\pm$ 0.13	NS

NS = Not significant ($p > 0.05$).

significant correlation ($p > 0.05$) between the amount of N^t -(2'-hydroxyethyl)-L-histidine in haemoglobin and chromosome or chromatid aberrations.

IMMUNOLOGICAL AND HAEMATOLOGICAL ANALYSIS

Tables 6 and 7 summarise the haematological profiles and the data for B and T lymphocyte subpopulations in the plant workers and the control group. There was no statistically significant difference between the plant workers and the control group in any of the parameters examined. The lymphocyte count in split samples recorded in the Shell Toxicology Laboratory (Tunstall) (table 7) after a delay of 24 hours was greater than the number recorded with no intervening delay in the site medical department (table 6) in both the plant workers and the controls. This may have been due to a relative increase in lymphocyte count during storage because of a reduction in the numbers of the other cells of the differential count or to a difference

in methods between the two laboratories. A comparison of results in workers from groups 1 and 2 also showed no statistically significant difference in any of the parameters recorded (tables 6 and 7). With respect to the influence of other variables, smokers in both the control group and the plant workers showed higher lymphocyte count and B and T cell counts compared with non-smokers, but these differences were not statistically significant. A statistically significant difference was found, however, between the white cell count of smokers and non-smokers. Regression analysis showed that the duration of employment was positively correlated ($p < 0.05$) with the percentage of neutrophils in a differential white cell count and negatively correlated ($p < 0.05$) with the percentage of lymphocytes. This correlation did not hold when age was related with these parameters. Other parameters recorded in tables 6 and 7 showed no correlation with duration of employment or with age. Furthermore, no significant correlation was

Table 6 Haematological profile in plant workers and controls, smokers, and non-smokers. Data show the mean value $\pm$ SEM. (Number of individuals are given in parentheses)

	Plant workers (n = 35)	Controls (n = 33)	Statistical analysis	Smoking (n = 47)	Non-smoking (n = 21)	Statistical analysis
WCC ($10^9/l$ blood)*	6.10 $\pm$ 0.27	5.95 $\pm$ 0.27	NS	6.40 $\pm$ 0.15	5.05 $\pm$ 0.40	$p < 0.05$
Lymphocytes (%)†	38.3 $\pm$ 1.1	37.5 $\pm$ 1.0	NS	37.6 $\pm$ 0.9	38.4 $\pm$ 1.3	NS
Monocytes (%)†	4.6 $\pm$ 0.35	5.4 $\pm$ 0.35	NS	4.8 $\pm$ 0.3	5.5 $\pm$ 0.5	NS
Neutrophils (%)†	54.1 $\pm$ 1.3	53.6 $\pm$ 1.2	NS	54.1 $\pm$ 1.3	53.3 $\pm$ 1.5	NS

NS = Not significant ($p > 0.05$).

*Normal distribution.

†Poisson distribution.

Table 7 Percentage and absolute number of B and T cells in whole blood. Data show the mean value $\pm$ SEM. (Number of individuals are given in parentheses)

	Plant workers (n = 25)	Controls (n = 25)	Statistical analysis	Smoking (n = 34)	Non-smoking (n = 16)	Statistical analysis
Lymphocytes* ($10^9/l$)	3.11 $\pm$ 0.20	2.85 $\pm$ 0.20	NS	3.19 $\pm$ 0.16	2.54 $\pm$ 0.24	NS
T Lymphocytes* ($10^9/l$)	2.24 $\pm$ 0.14	2.01 $\pm$ 0.15	NS	2.29 $\pm$ 1.23	1.78 $\pm$ 0.16	NS
T Lymphocytes (%)	72.4 $\pm$ 1.0	70.5 $\pm$ 1.2	NS	71.8 $\pm$ 1.0	70.8 $\pm$ 1.4	NS
B Lymphocytes† ($10^9/l$)	0.212 $\pm$ 0.020	0.186 $\pm$ 0.023	NS	0.22 $\pm$ 0.021	0.15 $\pm$ 0.018	NS
B Lymphocytes† ($10^9/l$)	7.1 $\pm$ 0.6	7.0 $\pm$ 0.7	NS	7.3 $\pm$ 0.7	6.4 $\pm$ 0.7	NS

NS = Not significant.

*Normal distribution.

†Log normal distribution.

Table 8 *Lymphocyte activation by maximum stimulation of PHA in vitro. Data show the mean value $\pm$ SEM. (Number of individuals are given in parentheses)*

	Plant workers (n = 25)	Controls (n = 25)	Statistical analysis
Maximum stimulation* (cpm)	51864 $\pm$ 4133	45362 $\pm$ 3711	NS
Background† (cpm)	598 $\pm$ 104	428 $\pm$ 77	NS
Stimulation index†	80 $\pm$ 14	98 $\pm$ 17	NS

NS = Not significant ($p > 0.05$).

*Normal distribution.

†Log normal distribution.

Stimulation index = $\frac{\text{cpm Stimulated culture}}{\text{cpm Unstimulated culture}}$

found ($p > 0.05$) between the amount of N¹ - (2'-hydroxyethyl) - L - histidine in haemoglobin and haematological or immunological parameters (tables 6 and 7). Tables 8–10 summarise the effects of variables on T and B cell function. Table 8 shows the data for the in vitro lymphoproliferative response to the mitogen PHA of cultures from plant workers and the control group; table 10 shows the data for humoral immunity. There was no statistically significant difference between plant workers and the control group in respect of any of the parameters recorded in these tables. Furthermore, no statistically significant difference was found between groups 1 and 2 or between these groups and the control group in any of the parameters recorded in tables 8 and 10. Of the other variables, smoking was associated with a statistically significant decrease on serum IgG and IgM levels (table 10). The response of cultures stimulated by PHA was increased in smokers compared with non-smokers, but the difference was not statistically significant. Furthermore, a statistically significant

decrease in the PHA response was observed in the group aged 40–59 compared with groups aged 30–39 and 20–29 (table 9). Regression analysis showed that the PHA response of cultures from plant workers was negatively correlated ($p < 0.01$) with duration of employment. This effect was undoubtedly influenced by age: the relation between age and PHA response was highly significant ($p < 0.01$). Other parameters recorded (tables 8 and 10) showed no correlation with either duration of employment or with age.

No significant correlation was found between the amount of N¹-(2'-hydroxyethyl)-L-histidine in haemoglobin and immunological parameters (tables 8 and 10).

Discussion

Exposure levels to EO in modern manufacturing plants are usually low, generally below 1 ppm (M Kokeitsu, unpublished data). In the current study occupational EO exposure (8 h TWA) measured under normal plant operating conditions during 1974–82 was generally below the detection limit of 0.05 ppm. Nevertheless, occasional transient concentrations of up to 8 ppm were recorded. It is also possible that higher exposures than 0.05 ppm may have occurred before 1974 and during planned closures for maintenance purposes—that is, during the shut down and start up procedures.

No statistically significant increase in the frequency of chromatid or chromosome aberrations was observed in the current study. In the group of plant workers the positive correlation ($p < 0.05$) found between the frequency of chromosome breaks and duration of employment was independent of

Table 9 *Effect of other variables on lymphocyte activation. Data show the mean value $\pm$ SEM*

	No of individuals	Maximum stimulation (cpm)	Background (cpm)	Stimulation Index
Smoking	34	53594 $\pm$ 3460	487 $\pm$ 72	100 $\pm$ 14
Non-smoking	16	40152 $\pm$ 3932	547 $\pm$ 128	69 $\pm$ 16
Age group 20–29	22	58139 $\pm$ 3947	645 $\pm$ 136	86 $\pm$ 19
Age group 30–39	17	47472 $\pm$ 4555	521 $\pm$ 82	86 $\pm$ 14
Age group 40–59	11	31325 $\pm$ 3239*	297 $\pm$ 71	100 $\pm$ 22

*Statistical significant difference compared with age groups 20–29 and 30–39 years ($p < 0.05$).

Table 10 *Serum immunoglobulin concentrations. Data show the mean value $\pm$ SEM. (Number of individuals are given in parentheses)*

	Plant workers (n = 24)	Controls (n = 27)	Statistical analysis	Smoking (n = 35)	Non-smoking (n = 16)	Statistical analysis
IgG* (mg%)	1565 $\pm$ 58	1500 $\pm$ 41	NS	1467 $\pm$ 39	1679 $\pm$ 57	$p < 0.05$
IgM† (mg%)	152 $\pm$ 11	127 $\pm$ 9	NS	127 $\pm$ 9	164 $\pm$ 8	$p < 0.05$
IgA* (mg%)	159 $\pm$ 13	160 $\pm$ 10	NS	150 $\pm$ 9	181 $\pm$ 17	NS

NS = Not significant ($p > 0.05$).

*Log normal distribution.

†Normal distribution.

age, suggesting an occupational effect. This statistically significant correlation was based on a single finding of 2% chromosome breaks in a plant worker with nine years of employment in the EO plant. In six other plant workers and seven control subjects this type of aberration occurred at a rate of 1%. No chromosome breaks were recorded in 29 plant workers and 26 controls. When coupled with the absence of a statistically significant difference in the frequency of chromosome breaks in group 1 compared with group 2 or between these groups and the control group and the consistency with published values for control populations,^{16,17} it is unlikely that the statistically significant correlation between duration of employment and frequency of chromosome breaks is of biological significance. These essentially negative findings are in close agreement with the reported absence of chromosome damage in the lowest exposure group in the study conducted by Johnson and Johnson.⁷ It should also be noted that the EO exposures measured in the current study were similar to those determined for the lowest exposure group in the Johnson and Johnson study.

Various aspects of lifestyle may influence the degree of chromosome damage in peripheral blood lymphocytes. For example, several reports indicate increased frequencies of chromatid and chromosome aberrations in smokers compared with non-smokers.^{18,19} These increases appeared to be dose related—that is, dependent on the frequency and the total duration of smoking. The results obtained in the current study provide no evidence to support an association between smoking and chromosome aberrations. This failure to detect an effect may have been due to the relatively low average age of the subjects and, possibly, to a low frequency of smoking, particularly among plant workers who are not allowed to smoke on the plant. Analysis of the results of haematological and immunological tests showed no statistically significant differences between plant workers and the control group which suggests that immune function was not altered under the conditions prevailing in the EO manufacturing plant. The finding that in the group of plant workers the percentage of neutrophils in a differential white cell count was increased and the percentage of lymphocytes decreased with duration of employment to EO is probably not of biological significance, because the absolute numbers remained within the normal limits and also because there was no statistically significant difference in the percentages of neutrophils and lymphocytes observed in the plant workers and in the control group.

Smoking had a more profound effect on haematological and immunological parameters as evidenced by a statistically significant increase in white cell count and a decreased level of serum immunoglobulins. These findings are in agreement with several published reports.²⁰ Nevertheless, the values for smokers were within the normal range. The fact that these values were significantly different from the control values (non-smokers) suggests that a change in immunological parameters may provide a more sensitive criterion for detecting an effect of smoking than does an increase in chromosome aberrations. Although not directly relevant to the main issue, it should be noted that the previously reported age dependent decrease in the responsiveness of lymphocytes to PHA *in vitro*²¹ was confirmed in the present study.

In the present study the degree of 2-hydroxyethylation of the N¹ atom of histidine residues in the haemoglobin of circulating erythrocytes was measured in the plant workers and in the control group in an attempt to develop precise data regarding recent individual exposures to EO—that is, during the 18 week period immediately before blood sampling.* A surprising feature of the results was the finding of variable but, in most instances, readily measurable quantities of N¹-(2'-hydroxyethyl)-L-histidine in the haemoglobin of the control group, who had not knowingly been exposed to an exogenous source of EO. Indeed, there was no statistically significant difference between the results obtained in the control group and the plant workers, although the latter displayed a tendency towards higher values. Calleman and co-workers calculated that an exposure dose† of 1 ppm × h would be equivalent to an *in vivo* EO dose of 0.106 μM × h.¹¹ Using this criterion and assuming a repeated and regular pattern of exposure, the average weekly *in vivo* doses of EO in plant workers (16.5 μM × h.; table 3) and in control personnel (12.6 μM × h.; table 3) would be equivalent to weekly EO exposure doses of 156 ppm × h and 119 ppm × h respectively. The observation that there was no statistically significant difference between the *in vivo* doses in the plant workers and in the controls indicates minimal occupational exposures to EO during the present study. The variable and relatively high background levels of N¹-(2'-hydroxyethyl)-L-histidine in the control group prevented the calculation of the contribution of the *in vivo* dose, made by occupational exposure to EO, on an individual basis.

*The life duration of the erythrocyte is 18 weeks in man.

†The exposure dose is expressed in terms of a composite unit, concentration × time—for instance, ppm × h.

The origin of the variable background of 2-hydroxyethyl groups in the control group is unknown. Several possible sources exist including EO formed during the oxidative metabolism of endogenously synthesised ethylene or an exposure of both groups to ethylene used on site. Smoking and age were not contributing factors of these background alkylations. No statistically significant differences in N¹-(2'-hydroxyethyl)-L-histidine levels were found between smokers and non-smokers, and regression analysis showed that there was no significant correlation ($p > 0.05$) between the amount of adduct and age.

The detection of a variable background of 2-hydroxyethyl groups in the histidine residues of human haemoglobin has clear toxicological and methodological implications. It would be premature, however, to comment further on the possible significance of this finding until the origin of these 2-hydroxyethyl groups has been established and studies have been conducted to determine whether or not a corresponding background of 2-hydroxyethyl groups occurs in DNA. Studies are in progress to investigate these problems.

In summary, no statistically significant difference was found in any of the biological parameters investigated in this study between the group of plant workers, employed in EO manufacture for periods of up to 14 years, and the control group. The association between duration of employment in EO manufacture and changes in the frequency of chromosome breaks and in the percentage of neutrophils and lymphocytes in a differential white cell count was not considered to be of biological significance. No statistically significant correlation was found between the amount of N¹-(2'-hydroxyethyl)-L-histidine in the haemoglobin of a particular individual and the frequency of chromosome aberrations and immunological or haematological parameters.

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