Int Arch Occup Environ Health (1999) 72: 142–150

ORIGINAL ARTICLE

P. J. Boogaard · P. S. J. Rocchi · N. J. van Sittert

Biomonitoring of exposure to ethylene oxide and propylene oxide by determination of hemoglobin adducts: correlations between airborne exposure and adduct levels

Received: 28 July 1998 / Accepted: 28 November 1998

Abstract ***Objectives:*** Ethylene oxide (EO) and propylene oxide (PO) are important industrial chemicals. Exposure to these directly acting mutagens may be monitored by determination of their adducts to hemoglobin (Hb). This study establishes correlations between airborne concentrations of EO and PO and their Hb adducts in petrochemical workers. ***Methods:*** In three different studies conducted during maintenance shutdown of petrochemical plants the external occupational exposure to EO and PO was assessed by personal air monitoring (PAM). The internal exposure to EO and PO was concomitantly assessed by determination of *N*-(2-hydroxyethyl)valine (HOEtVal) and *N*-(3-hydroxypropyl)valine (HOPrVal) in blood samples of the operators using the *N*-alkyl-Edman degradation method. ***Results:*** In the first study, PAM was applied once a month at random over a period of 4 months. Blood samples for Hb-adduct determination were collected at the end of this period. No significant correlation was found between PAM and Hb-adduct data. In the next two studies, PAM was applied to the operators during the entire shift on every working day during the shutdown. Blood samples were collected before and immediately after the shutdown period. Highly significant correlations were found between the increment in the concentration of HOEtVal and HOPrVal over this period and the total exposure to EO and PO, respectively. ***Conclusions:*** Time-integrated exposure to EO or PO can be readily and reliably assessed by measurement of the concentration of HOEtVal or HOPrVal in a small blood sample. In workers occupationally exposed to low concentrations of EO or PO, good correlations were found between these Hb adducts and the airborne concentrations of EO and PO. These correlations allow the calculation of tentative biological exposure limits (BELs) for EO and PO. At the current Dutch occupational exposure limit (OEL) for EO (0.84 mg m^{-3}, 8-h TWA) the BEL is 3.2 nmol HOEtVal/g globin. At the value of 10 mg m^{-3} (8-h TWA), which is currently being investigated as the new Dutch OEL for PO, the corresponding BEL is 5.3 nmol HOPrVal/g globin.

Key words Ethylene oxide · Propylene oxide · Hemoglobin adducts · Biomarkers · Exposure monitoring

P.J. Boogaard (✉) · N.J. van Sittert
Department of Molecular Toxicology,
Shell Research and Technology Center,
Amsterdam Shell International Chemicals B.V.,
P.O. Box 38000,
1030 BN Amsterdam,
The Netherlands
Tel.: +31 20 630 2907
Fax: +31 20 630 2219
e-mail: Peter.J.Boogaard@opc.shell.com

P.S.J. Rocchi
Safety and Health Department,
Shell Nederland Chemie/Raffinaderij B.V.,
P.O. Box 3000,
3190 GA Hoogvliet (Rotterdam),
The Netherlands

Introduction

Ethylene oxide (EO, oxirane; CAS 75-21-8) and propylene oxide (PO, methyloxirane; CAS 75-56-9) are important industrial chemicals that are produced mainly for chemical synthesis such as the production of glycols and glycol ethers. EO is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and various other authoritative bodies. Recently, the Dutch occupational exposure limit (OEL) for EO was lowered to 0.84 mg m^{-3} (0.5 ppm) as the 8-h time-weighted average (8-h TWA; SZW 1995a). PO is carcinogenic to rodents as shown in the studies of the United States National Toxicology Program (NTP 1985). Several authoritative bodies have evaluated the genotoxicity of PO and have concluded that there is insufficient (IARC 1994) evidence, if any (ACTS 1992), for the carcinogenic effects of PO in humans. As a consequence, IARC downgraded the carcinogenic clas-

sification of PO from a probable (2A) to a possible (2B) human carcinogen. In 1996 the American Conference of Governmental Industrial Hygienists (ACGIH) classified PO as an animal carcinogen (3A). Despite the similarity of the reaction kinetics of EO and PO (Ehrenberg and Hussain 1981), the mutagenic potency and genotoxic effects of PO are much lower than those of EO (recently reviewed by Ríos-Blanco et al. 1997). Nevertheless, pressure remains to lower the occupational exposure limit (OEL) for PO. Recently the Dutch Expert Committee on Occupational Standards (DECOS) reviewed the health risks associated with exposure to PO. On the basis in this review a reduction in the OEL to a level between 0.1 and 10 mg m^{-3} (8-h TWA) is currently being investigated (DECOS 1987, 1997).

Estimation of exposure to alkylating compounds such as EO and PO by air monitoring is complicated if the exposure is variable and occurs intermittently. Moreover, air monitoring data refer to potential exposure rather than to the actual dose received by the exposed operators. These problems can be solved by the determination of biomarkers such as adducts to proteins or DNA. One type of human material that may be available for such measurements is blood, and the blood proteins albumin and hemoglobin (Hb) have been used to determine adduct levels (Van Welie et al. 1992). Adducts to Hb are especially suitable because small molecules such as EO and PO form chemically stable adducts with the *N*-terminal valine of Hb, *N*-(2-hydroxyethyl)valine (HOEtVal) and *N*-(3-hydroxypropyl)valine (HOPrVal), respectively, and these adducts do not appear to affect the average life span of the erythrocytes. The exposure dose as measured by determination of Hb-adduct concentrations is thus integrated over the average life span of human erythrocytes, which is approximately 4 months.

The aim of this study was to establish a relationship between ambient exposure to EO and PO and the formation of their respective adducts to Hb by correlation of the Hb-adduct data with the corresponding personal air monitoring (PAM) data in various groups of operators potentially exposed to EO or PO. The determination of such a relationship allows the interpretation of Hb-adduct concentrations in terms of airborne levels of EO and PO and therefore permits the setting of biological exposure limit (BEL) values for EO and PO that are equivalent to airborne OEL values.

Subjects and methods

Chemicals

Control globin was obtained from workers with no known occupational exposure to EO, PO, ethylene, or propylene. Standards {[^{14}C]-*N*-(3-hydroxypropyl)valine, d_6-*N*-(3-hydroxypropyl)valine, and d_4-*N*-(2-hydroxyethyl)valine} prepared from Hb treated with ^{14}C-labeled PO (specific activity 43.3 μCi/mmol) or with perdeuterated PO or EO were gifts from Dr. S. Osterman-Golkar and Dr. M. Törnqvist, Department of Radiobiology, Stockholm University. The following chemicals were obtained from Fluka and were of analytical grade: ammonium hydroxide, concentrated hydrochloric acid (37%), diethyl ether, formamide, *n*-pentane, pentafluorophenyl isothiocyanate (PFPITC), isopropanol, sodium carbonate, sodium hydroxide, and toluene. All other chemicals were purchased from commercial sources and were of the highest grade available.

Study populations and design

Study 1

A total of 20 male operators of a chemical plant manufacturing glycols and glycol ethers from EO and PO took part in this study, which was conducted in 1990. The potential exposure to EO and PO was expected to be low since the glycols were produced in a completely closed system and during maintenance activities on EO- or PO-containing systems, respiratory protection (full face masks with fresh air supply) was applied. Once a month during a period of 4 consecutive months, potential exposure to EO and PO was quantified by PAM using gas-diffusion monitors in the breathing zone of the operators on randomly selected days. During this period of 4 months, EO was used as the starting material for 3 weeks, followed by a period of 2 weeks during which PO was used, after which the plant was shutdown for 3 weeks for maintenance. Following the startup, EO and PO were individually used as the starting material for glycol production for another month. At the end of this 4-month period, blood samples were collected from 17 operators for Hb-adduct determinations. Altogether, 36 operators from a different division of the same plant with no exposure to EO or PO served as controls for the Hb-adduct measurements.

Study 2

Another 18 operators from the same plant described for study 1 were monitored for exposure to EO during various activities during a maintenance and inspection shutdown of the plant in 1992. Hb adducts of EO were assessed in blood samples collected prior to and immediately after the termination of the activities. Airborne exposure was determined by PAM during the entire shift on all days where, as judged by the occupational hygienist, exposure might be expected. Effectively, this meant that the potential exposure to EO of 15 of the 18 operators was monitored every working day throughout the early phase of the maintenance shutdown.

Study 3

A total of 28 male workers potentially involved in the operation during a maintenance shutdown of a styrene-PO plant were selected for this study, which was carried out in 1997. Blood samples for Hb-adduct measurements were taken prior to the shutdown activities and at the very end of the activities, whereas PAM was performed on every working day from the beginning of the activities until the day of the second blood sample. As it appeared that 7 persons would have a minimal risk of potential exposure to PO, no PAM was applied in these cases, nor was a second blood sample collected at the end of the study. The PAM data were incomplete for 5 operators, and no second blood sample was collected from 2 other operators at the end of the shutdown period.

For each study, details on smoking behavior were obtained from all the operators and the controls by means of a self-administered questionnaire.

Air monitoring

Sampling

Potential exposure to EO and PO was determined by PAM. For all workers, air samples were collected using gas-diffusion monitors (3M, Minneapolis, USA), which were attached to one of the lapels

of their overalls. In studies 1 and 2, 3M Ethylene Oxide Monitors (type 3551) were used; according to the information of the manufacturer at that time, they were also suitable for PO monitoring. In study 3, gas-diffusion monitors (type 3500) were used as recommended by 3M (1986) and, in a selected number of workers, air samples were also collected simultaneously by active pumping of air from the breathing zone over charcoal tubes (SKC, type 226-01) according to NIOSH method 1612 to enable comparison of the two methods (NIOSH 1985). Air measurements were carried out according to the recommendations of Bianchi et al. (1997) during the full shift. Details about exposure times and circumstances were recorded by the plant's occupational hygienist.

When completed, the air samples were closed airtight and transported to the laboratory in a cooling box with ice packs. Upon arrival the samples were immediately stored frozen at −20 °C until analysis.

Analyses

Studies 1 and 2

The 3M 3551 gas-diffusion monitors contain hydrogen-bromide-coated charcoal on which EO and PO are instantaneously converted to bromoethanol and bromopropanol, respectively. The bromoalcohols were desorbed from the monitors with 2.0 ml methanol and subsequently analyzed by gas chromatography with electron-capture detection using 1,2,3-trichloropropane as the internal standard according to the instructions supplied by the manufacturer of the monitors (Esposito et al. 1984). The efficiency of desorption was determined at five different levels in duplicate; for the duplicate determination, monitors from a different lot were used.

Study 3

The gas-diffusion monitors were desorbed with 1.5 ml carbon disulfide and analyzed by gas chromatography with flame-ionization detection using *n*-octane as the internal standard according to standard procedures for the determination of PO. The efficiency of desorption was determined at five different levels in duplicate; for the duplicate determination, monitors from a different lot were used.

The charcoal tubes were desorbed with 1.0 ml carbon disulfide and analyzed by gas chromatography with flame-ionization detection using *n*-octane as the internal standard. The efficiency of desorption was determined at five different levels in triplicate according to NIOSH method 1612 (NIOSH 1985).

Calculations

All values were expressed as time-weighted average air concentrations over 8 h (8-h TWA). Most workers had potential exposure to EO or PO during a small number of consecutive days. In all cases in studies 2 and 3, more than 75% of the total exposure during the study occurred on a single day. For the correlation with the actual exposure as determined by Hb-adduct determination it was assumed that the total exposure occurred on that particular day.

Biological monitoring

Sampling

Blood samples of approximately 5 ml were collected from the antecubital vein into a Vacutainer tube containing sodium edetate as an anticoagulant using a γ-radiation-sterilized syringe and needle. The samples were immediately transported to the laboratory for isolation of the erythrocytes. The cells were isolated by centrifugation (10 min, 1000 *g*, room temperature) and washed twice by gentle suspension in isotonic saline followed by centrifugation. The washed cells were suspended in deionized water and then subjected to four cycles of freezing at −80 °C and thawing at 37 °C. The lysed cell suspension was centrifuged at 6000 *g* for 90 min at room temperature to remove cell debris and nonlysed red cells. The supernatant was transferred into polypropene tubes and stored frozen at −80 °C until analysis.

Analysis

The supernatant was added to a freshly prepared solution of HCl in 2-propanol for dissociation of the heme from the globin. The precipitated heme was separated by centrifugation. The globin was subsequently precipitated from the supernatant by the addition of ethyl acetate, collected by centrifugation, washed with fresh ethyl acetate and *n*-pentane, and then dried in a desiccator under vacuum overnight. The dried globin samples were stored at −80 °C until further analysis.

The dry globin (100–200 mg) was dissolved in formamide at a concentration of 33 mg/ml and, following the addition of the appropriate internal standard, either d_4-2-hydroxyethylvaline or d_6-3-hydroxypropylvaline, the sample was adjusted to a pH of 6.8 by the addition of 1 *M* NaOH. For calibration, known amounts of the calibration standard solution were added to control globin prior to neutralization. After the addition of derivatization reagent (PFPITC, 14 µl/100 mg) the samples were left overnight in a shaking mixer in darkness at room temperature. For completion of the derivatization the samples were finally warmed at 45 °C for 1.5 h. Deionized water was added to the samples (2 ml/100 mg), which were subsequently extracted twice with diethyl ether (4 ml/100 mg). The combined ethereal phases were evaporated to dryness by a gentle stream of N_2 and the residue was redissolved in toluene. The toluene solution was subsequently extracted with 2-ml aliquots of deionized water and 0.1 *M* aqueous sodium carbonate solution (twice) and again with water. The toluene extract was evaporated to dryness at ±50 °C in a water bath using a gentle stream of N_2, and the residue was redissolved in toluene (50–100 µl). The samples were kept in darkness at 4 °C and were analyzed immediately.

Samples from studies 1 and 2 were analyzed by gas chromatography-mass spectrometry (GC-MS) as described elsewhere (Van Sittert et al. 1993; Van Sittert and Van Vliet 1994). Samples from study 3 were also analyzed by GC-MS-MS. A Carlo Erba SFC 3000 series GC equipped with an AS800 autosampler was used for separation on a J&W DB5MS fused-silica capillary column (dimensions: 30 m × 0.25 mm, 0.25-µm film thickness) using the following temperature gradient: from 90° to 190 °C at 30°C/min, then from 190° to 230 °C at 3 °C/min, and, finally, from 230° to 320 °C at 30 °C/min. Aliquots of 2 µl were injected ''cold-on-column''. A VG Quattro I triple-quadrupole MS in the negative-ion electron-capture ionization mode with methane as the reagent and argon as the collision gas was applied for detection using multiple reaction monitoring (MRM) of the following ions: *m*/*z* 368 to 320 (fragments from d_6-HOPrVal-PFPTH) and *m*/*z* 362 to 318 (fragments from HOPrVal-PFPTH).

Calculations

Quantification of the Hb adducts was performed as described above for the samples from studies 1 and 2 (Van Sittert et al. 1993). Quantification of the Hb adducts of PO in study 3 was based on measurements of the internal reference standard. For calibration samples the peak areas of the HOPrVal-PFPTH (*m*/*z* 362 to 318) were compared with the peak area of the d_6-HOPrVal-PFPTH (*m*/*z* 368 to 320). The concentration of d_6-HOPrVal in the globin used for an internal standard was 2.43 µmol/g globin as determined by comparison of its peak area, as an HOPrVal-PFPTH derivative, to the peak area of the HOPrVal-PFPTH derivative of the [^{14}C]-HOPrVal in the reference globin, which contained 1.7 µmol HOPrVal/g globin. Various quantities of the internal standard solution were added as appropriate to control samples and the test samples for quantification of the HOPrVal adducts.

Results

Comparison of personal PO air-sampling methods

No detectable amount of PO could be eluted from the blank monitors ($n = 5$) or the blank tubes ($n = 4$). Carbon disulfide eluted PO from the monitors and the tubes with an efficiency of 78 ± 2% and 80 ± 3%, respectively. All values were corrected for the recovery. The values obtained by the nine measurements from the charcoal tubes using the NIOSH method correlated very well with the results obtained from the 3M type 3500 monitors (Fig. 1). On one occasion the charcoal tube was overloaded, as the backup tube contained 27% of the amount found in the first tube (in all other tubes the backup tube contained less than 2% of the total quantity of PO). Since the result recorded for the total concentration was reported as unreliable, this data point was not included in the comparison of the results (Fig. 1). Linear regression analysis of the data set showed a highly significant correlation ($P < 0.0001$; correlation coefficient 0.996) with the following equation:

$$[\text{PO}] \text{ on tubes } = 0.893(0.034) \times [\text{PO}] \text{ on monitors} + 0.090(0.065),$$

where the concentration PO is expressed in milligrams per cubic meter of air (8-h TWA) and the figures in parentheses denote the standard errors. This equation shows a slightly higher estimate (approximately 11%) for the airborne PO concentrations obtained using the 3M type 3500 gas-diffusion monitors as compared with the charcoal tubes. The intercept was not significantly different from zero ($P = 0.214$). The data obtained using gas-diffusion monitors were used for all calculations and correlations.

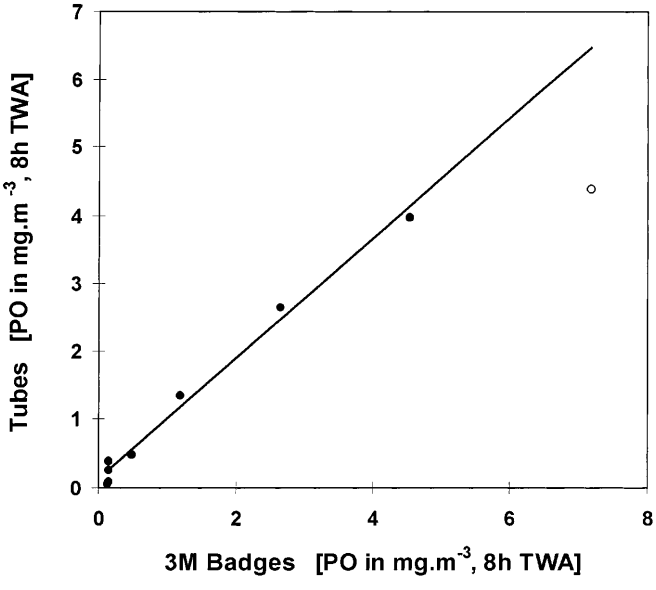

Fig. 1 Personal air monitoring of airborne exposure to propylene oxide (*PO*): comparison between charcoal tubes and 3M gas-diffusion monitors for sampling ($n = 8$). The *open circle* represents the data for the overloaded charcoal tube that were excluded from the regression analysis (see Subjects and methods for details)

Personal air monitoring

Study 1

In all, 70 measurements were made on 20 workers. On 27 monitors (38.6%) the concentration of EO was below the limit of detection (0.01 mg m^{-3}, 8-h TWA). PO could be detected on only 4 of the monitors (5.7%; limit of detection 0.02 mg m^{-3}, 8-h TWA). The highest concentration of EO measured was 8.3 mg m^{-3} (8-h TWA), and the median value was 0.01 mg m^{-3} [mean 0.30 ± 0.16 (SE) mg m^{-3}, 8-h TWA]. The concentrations of PO measured were 0.02, 0.05, 0.10, and 7.3 mg m^{-3} (8-h TWA).

Study 2

PAM was applied to 15 operators during all days; for 3 operators, not all days with potential exposure were monitored. In all, 57 air measurements were made. On 20 monitors the concentration was below the limit of detection (0.01 mg m^{-3}, 8-h TWA). The highest concentration measured was 4.4 mg m^{-3} (8-h TWA), and the median value was 0.02 mg m^{-3} [8-h TWA; mean 0.38 ± 0.12 (SE) mg m^{-3}, 8-h TWA].

Study 3

Altogether, 112 measurements were made on 15 workers. On 89 monitors (79.5%) the PO concentration was below the detection limit (0.2 mg m^{-3}, 8-h TWA). The concentrations of PO in air ranged from <0.1 to 10.0 mg m^{-3} (8-h TWA), with the median value being <0.3 mg m^{-3} [8-h TWA; mean 0.8 ± 0.2 (SE) mg m^{-3}, 8-h TWA].

Biochemical effect monitoring

Study 1

In virtually all the Hb samples collected from the 36 control workers with no occupational exposure to EO or PO, significant background values of HOEtVal were found. These background values correlated closely with smoking behavior. The median background concentration of HOEtVal detected in the 23 nonsmokers of the control group was 19 pmol/g globin [average 22 ± 5 (SE) pmol/g globin; range 6–49 pmol/g globin]. Smoking caused on average an increase of 9.4 pmol HOEtVal/g globin for each cigarette smoked per day, which corresponds very well with the value of 11 pmol/g globin

reported by other investigators (Bader et al. 1995). In some of the controls, low background levels of HOPrVal were detected.

The HOEtVal concentrations measured in the blood samples collected from the operators from the ethylene glycol/glycol ether plant ranged from 0 to 239 pmol/g globin after correction for smoking and normal background. The median HOEtVal concentration detected in these operators was 16 pmol/g globin [average 46 ± 17 (SE) pmol/g globin]. The values recorded without correction for smoking and normal background ranged from 12 to 320 pmol/g globin, with the median value being 46 pmol/g globin [average 92 ± 25 (SE) pmol/g globin].

Study 2

In all, 15 of the 18 operators supplied a blood sample both prior to their work and immediately after they had finished their work. The increases in the HOEtVal concentrations measured during this period ranged from 0 to 213 pmol/g globin in the 15 operators. The median increase in HOEtVal concentration was 58.4 pmol/g globin [average 64 ± 15 (SE) pmol/g globin]. In the same operators the increase in the HOPrVal concentrations measured during this period ranged from 0 to 12.2 pmol/g globin, with the median value being 0.1 pmol/g globin [average 1.3 ± 0.9 (SE) pmol/g globin].

Study 3

A total of 46 measurements were made in 27 operators, including the 15 operators involved in the PAM. For all 27 operators a measurement was made prior to the maintenance activities the median HOPrVal concentration was 24.4 pmol/g globin [average 40.2 ± 8.0 (SE) pmol/g globin]. For 19 operators the concentration of Hb adducts of PO was also determined at the end of the shutdown period. The median concentration detected in these samples was 45.7 pmol/g globin [average 45.3 ± 8.0 (SE) pmol/g globin].

Correlation of PAM and biological monitoring

Study 1

No significant correlation ($r = 0.116$; $P = 0.656$) was obtained by regression analysis of the concentration of HOEtVal detected in the blood of the operators as a function of the concentrations of EO measured in air (Fig. 2).

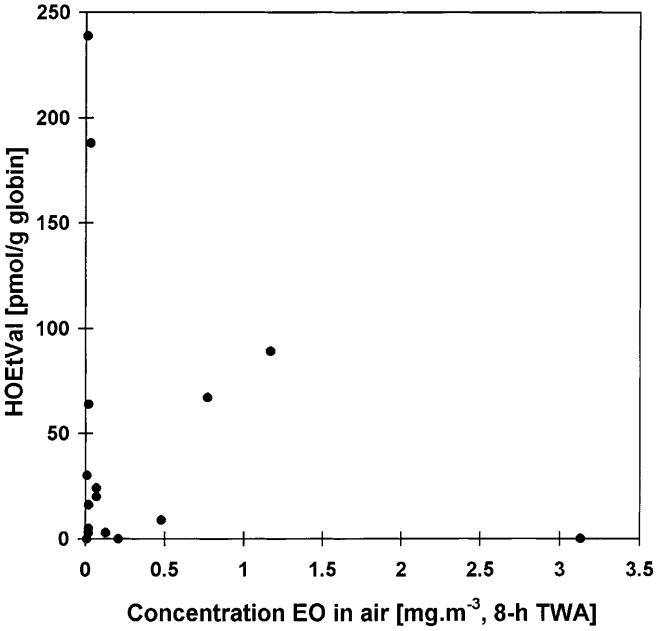

Fig. 2 Correlation between 8-h TWA respiratory exposure data as measured in study 1. Airborne levels of EO were determined once a month over a period of 4 months. For each operator ($n = 17$) the average exposure over this period was calculated from his individual exposure data. Hb adducts of EO were determined at the end of this period as HOEtVal. Linear regression analysis showed no significant correlation ($P = 0.656$)

Study 2

The increments in the concentration of Hb adducts of EO, expressed as HOEtVal, detected in the operators during the maintenance shutdown correlated strongly ($P < 0.0001$) with the total airborne concentration of EO expressed in milligrams per cubic meter of air per hour during this shutdown (Fig. 3). The correlation could be expressed as:

$$\text{Increment in Hb adduct [pmol/g globin]} = 7.53\ (0.90) \times \text{Concentration EO in air } \left[\text{mg m}^{-3}\ \text{h}\right],$$

where the figure in parentheses denotes the standard error.

Study 3

The increments in the concentration of Hb adducts of PO, expressed as HOPrVal, detected in the operators during the maintenance shutdown correlated strongly ($P = 0.0004$) with the total airborne concentration of PO expressed in milligrams per cubic meter of air times hour during this shutdown period (Fig. 4). The correlation could be expressed as:

$$\text{Increment in Hb adduct [pmol/g globin]} = 1.06\ (0.14) \times \text{Concentration PO in air } \left[\text{mg m}^{-3}\ \text{h}\right]$$

where the figure in parentheses denotes the standard error.

To obtain the correlations for both studies the regression lines were fitted without any intercept to the

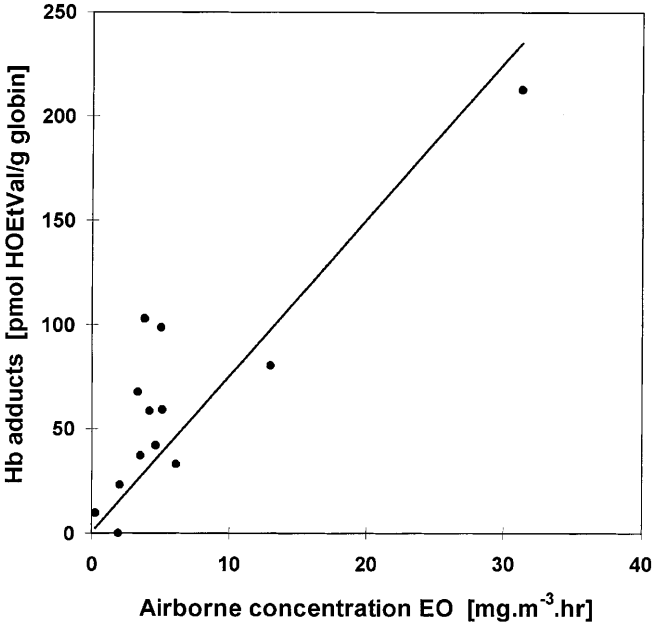

Fig. 3 Correlation between 8-h TWA respiratory exposure data as measured in study 2. Airborne levels of EO were determined daily over the entire shift during the maintenance shutdown, and the cumulative exposure over this period was calculated for each operator from his exposure data ($n = 13$). Hb adducts of EO were determined at the beginning and at the end of this period as HOEtVal. The increase in blood levels of HOEtVal are given as a function of the potential airborne exposure. Linear regression analysis showed a highly significant correlation ($P < 0.0001$)

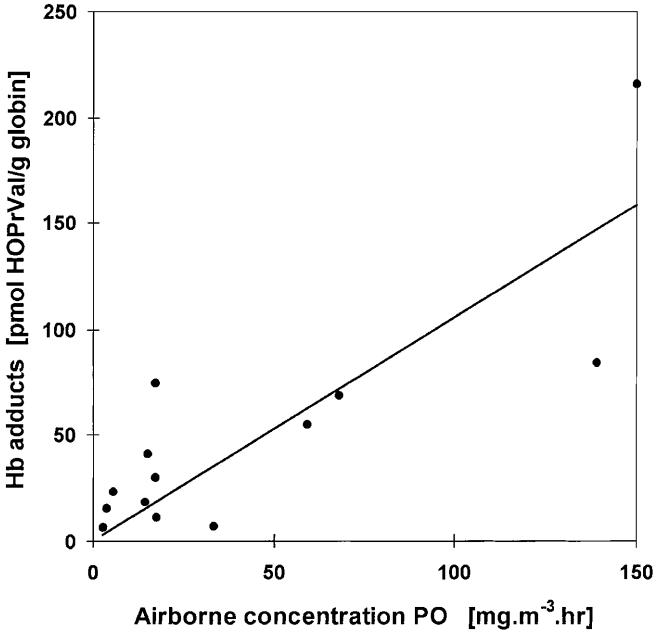

Fig. 4 Correlation between 8-h TWA respiratory exposure data as measured in study 3. Airborne levels of PO were determined daily over the entire shift during the maintenance shutdown, and the cumulative exposure over this period was calculated for each operator from his exposure data ($n = 13$). Hb adducts of PO were determined at the beginning and at the end of this period as HOPrVal. The increase in blood levels of HOPrVal are given as a function of the potential airborne exposure. Linear regression analysis showed a highly significant correlation ($P = 0.0004$)

data, since no adduct increment is expected if there is no exposure above the normal background. When the curve was fitted with an intercept a small positive value was found that was not significantly different from zero ($P = 0.06$ and $P = 0.39$ for EO and PO, respectively), indicating that this value is most probably due to the variation in natural background and to random errors in the analyses.

Estimation of steady-state levels at low exposure

The accumulation of stable Hb adducts in the blood during continuous exposure is the net result of daily increments (a) of the concentration of adduct and daily losses due to the removal of erythrocytes from the circulation. On exposure for a period exceeding the average life span of erythrocytes, t_{er}, which is 126 days in humans, a steady-state concentration of adducts (A_{ss}) is attained. This steady-state concentration, A_{ss}, is linked to the daily increment in adducts, a, by the following equation (Granath et al. 1992):

$$A_{ss} = a \times t_{er}/2,$$

or, for humans:

$$A_{ss} = 63 \times a.$$

The equations derived for the correlation between the PAM data and the biological monitoring data as obtained for EO in study 2 and for PO in study 3 allow the calculation of the daily increment when a person is continuously exposed to the occupational exposure limit (OEL).

For the present Dutch OEL for EO of 0.84 mg/m^3 as the 8-h TWA (0.5 ppm) the increment a equals 50.6 pmol HOEtVal/g globin and A_{ss} is 3.19 ± 0.38 (SE) nmol HOEtVal/g globin (95% confidence interval 2.54–4.29 nmol HOPrVal/g globin). For a concentration of 10 mg PO/m^3, as the 8-h TWA (4.15 ppm), which is currently being investigated as the new Dutch OEL, the increment a equals 84.4 pmol HOPrVal/g globin and A_{ss} is 5.32 ± 0.71 (SE) nmol HOPrVal/g globin (95% confidence interval 4.20–7.04 nmol HOPrVal/g globin). Increments in HOEtVal and HOPrVal corresponding to other OEL values are given in Table 1.

Discussion

The analysis of Hb adducts has proved to be a useful tool for monitoring of the occupational and environmental exposure of individual workers to industrial chemicals that are intrinsically electrophilic or become so as a result of metabolism. The method used for determination of Hb adducts by application of the Edman degradation procedure as developed by Törnqvist et al. (1986) for EO also appeared applicable for the measurement of Hb adducts of PO (Högstedt et al. 1990;

Table 1 OEL and corresponding BEL values

OEL value		BEL value nmol HOEtVal/g globin[a]	Comment
mg m^{-3}	ppm		
Ethylene oxide (EO):			
0.84	0.5	3.2	Current Dutch MAC (SZW 1995a)
1.8	1	6.8	Current U.S. TLV (ACGIH 1998); current German TRK (DFG 1997)
9.2	5	34.8	UK permissible exposure limit (UKHSE 1995)

OEL value		BEL value nmol HOPrVal/g globin[a]	Comment
mg m^{-3}	ppm		
Propylene oxide (PO):			
5	2	1.1	Current Swedish OEL (NBOSH 1993)
6	2.5	3.2	German TRK (DFG 1997)
10	4.15	5.3	Advised Dutch MAC (DECOS 1997)
12	5	6.4	UK permissible exposure limit (UKHSE 1995)
(48)	20	26	Current U.S. TLV (ACGIH 1998)
240	100	128	Current Dutch MAC (SZW 1995b)

[a] Increment in Hb adducts

Kautiainen and Törnqvist 1991) using the same internal standard, d_4-*N*-(2-hydroxyethyl)valine, used for EO-Hb adduct determination. This internal standard was also applied in the determination method for PO-Hb adducts, assuming that there would be no difference in response factors in the MS for the Hb adduct of EO and that of PO. This is probably true for single MS in the SIM mode, and exposure monitoring by determination of Hb-adduct concentrations by GC-MS-SIM has successfully been applied for a number of chemicals, including EO, PO, and butadiene monoxide, the primary metabolite of butadiene (Van Sittert et al. 1993; Van Sittert and Van Vliet 1994; Tates et al. 1995; Richardson et al. 1996].

However, the specificity and the sensitivity of this method do not allow the quantification of Hb-adduct concentrations corresponding to low, i.e., sub-ppm, levels of airborne PO or butadiene. Therefore, a GC method with tandem MS detection was developed. During the development of this method it was observed that the concentration of Hb adducts of butadiene monoxide was underestimated about seven times if d_4-*N*-(2-hydroxyethyl)valine was used instead of d_6-*N*-(4-hydroxy-2-butenyl)valine, due to differences in the response factor in the MRM mode (unpublished observation). Thus, we deemed it essential to validate the method for HOPrVal with d_6-HOPrVal, and the concentration of HOPrVal was determined in all samples by both methods. The results indicate that the values obtained by the GC-MS-MS-MRM method using d_6-HOPrVal as the internal standard are indeed slightly higher (5.6%) than those obtained by the GC-MS-SIM method using d_4-*N*-(2-hydroxyethyl)valine as the internal standard.

One of the essential aspects of risk analysis and control is human exposure assessment. Estimation of individual workers' exposure to low and variable concentrations of volatile compounds is not only cumbersome but also prone to errors, especially in the case of intermittent exposure. This was clearly demonstrated in study 1, where single exposure measurements carried out at random once a month were not representative of the overall exposure received by the operator during a 4-month period. Measurement of chemically stable Hb adducts of genotoxic compounds allows a time-integrated determination of variable and intermittent exposure at low concentrations and is therefore highly suitable for individual exposure monitoring and health surveillance of such chemicals. We had been determining levels of Hb adducts of EO and PO in our operators having potential exposure to these epoxides since 1988. However, we had not yet been capable of relating the results to airborne concentrations. The present studies therefore aimed at the establishment of such correlations.

In the first study, no clear correlation was found between ambient air concentrations of EO as determined by monthly PAM and the concentration of HOEtVal detected in the blood of the operators. As can be seen in Fig. 2, in one case a relatively high average level of exposure to EO was recorded (3.13 mg m^{-3}, 8-h TWA), with no concomitant increase in the concentration of HOEtVal being detected in the blood. This high level of potential exposure was due to a single day associated with a high degree of exposure (8.34 mg m^{-3}, 8-h TWA) during the draining of a pipeline. A possible explanation is that during these activities, some EO-containing liquid was spilled on the gas-diffusion monitor. Figure 2 also shows that in several operators the opposite was observed, which may be of greater concern: no indication of potential exposure but significant increases in HOEtVal concentrations. Since the most probable explanation for this observation is that exposure had occurred on days without PAM, we decided to change the setup of the studies.

In the next series of studies (studies 2 and 3) the operators were subjected to PAM on each working day during the entire shift to ensure that all potential exposure during the period of the maintenance shutdown would be monitored. Blood samples for Hb-adduct determinations were taken immediately before and after the shutdown. The results of the PAM show variable and intermittent exposure, but, as shown in Figs. 3 and 4, the total potential airborne exposure in the interval between the two blood samples correlated well with the increment in Hb adducts of both EO and PO during this period.

Using these correlations, the average airborne concentration of EO or PO during several days can be calculated from the observed increase in Hb-adduct

concentration over that period. As demonstrated by Granath et al. (1992) and Fennell et al. (1992), such a correlation can also be used for stable Hb adducts to estimate the daily increment and the steady-state concentration of Hb adducts if a person is more or less continuously exposed during a period that exceeds the average life span of erythrocytes, which is approximately 126 days (4 months). This means that a biological exposure limit value (BEL) can be calculated that corresponds to the average level of exposure to an OEL over 4 months, 5 days/week, 8 h/day. In Table 1, some values corresponding to OELs for EO and PO are listed. The value corresponding to the recently lowered Dutch OEL for EO (0.84 mg m^{-3}, 8-h TWA) is 3.19 nmol HOEtVal/g globin. The highest HOEtVal values measured in the operators in studies 1 and 2 were 0.21 and 0.22 nmol/g globin, indicating that the average exposure to EO had been well below 0.5 ppm. The values corresponding to the current Dutch OEL for PO (240 mg m^{-3}) and the value of 10 mg m^{-3} that is currently being investigated as the new OEL are 12.8 and 5.3 nmol/g globin, respectively. The actual values measured during the maintenance shutdown in study 3 varied from 0.005 to 0.161 nmol/g globin, indicating that the level of exposure during the maintenance shutdown had also been well below the OEL values.

The present data also allow a comparison of the potential genotoxic effects of EO and PO in humans. Several animal studies have indicated that PO is 3–4 times less mutagenic than EO (Farooqi et al. 1993; Vogel and Nivard 1997). The concentration of Hb adducts was approximately 6 times higher in rats exposed to EO than in mice exposed to the same molar concentration of PO (Svensson et al. 1991). In monkeys that had been given an equimolar mixture of EO and PO at low concentrations, the levels of Hb adducts of PO were 4 times lower than those of EO (Couch et al. 1996). Using the equations developed by Granath et al. (1992), it can be calculated from our data that the daily increment in Hb adducts of EO and PO at an exposure level of 1 ppm (8-h TWA) would be 97.9 and 20.3 pmol/g globin, respectively. This indicates that EO forms adducts at a 4.8-fold higher rate than does PO, which corroborates the findings in animal studies.

Although Hb adducts are widely used to assess exposure to alkylating compounds, only very few studies have thus far been reported on the quantitative relationship between Hb adducts in the blood of workers and airborne concentrations of the alkylating compound. An early pilot study reported a concentration of HOEtVal of 2.4 nmol/g globin at exposures of 40 ppm h $week^{-1}$ (Duus et al. 1989). The airborne concentrations of EO in that study, however, were not based on concomitant measurements but were estimated from recorded stationary and personal air measurements combined with personal statements on work routines. Therefore, the value of 2.4 nmol/g globin was considered by the investigators to be uncertain by a factor of 3 (Ehrenberg, personal communication). On the basis of the same data set a value of 3.4 nmol/g globin was more recently estimated for an average exposure to 1 ppm during working hours (Osterman-Golkar and Bond 1996). On the basis of statistical extrapolations of data from animal studies a comparable value was advised as the BEL value in Germany (DFG 1997). In a recent study this data set was extended with an additional data point corresponding to a relatively high-level exposure to EO of approximately 4 ppm, which was estimated by occasional stationary and personal air measurements. The additional data point fitted very well in the existing data set, and it was estimated that exposure to 1 ppm EO should yield approximately 4 nmol HOEtVal/g globin (Angerer et al. 1998).

Our data, which are based on actual PAM data obtained during the complete period bridging the collection of blood samples for Hb-adduct measurements, result in an even higher BEL for an average exposure to 1 ppm EO: 6.2 nmol/g globin. However, it should be noted that apart from the study design, there is another fundamental difference between the two studies. In our study, calibration was based on globin that had been isolated from blood treated with ^{14}C-labeled EO or PO. The *N*-terminal valine-adduct concentrations in these globin samples were determined by scintillation counting after hydrolysis of the protein and separation of the modified amino acids by high-performance liquid chromatography. Angerer and co-workers applied a different method of calibration using a modified dipeptide. It is well known that the yield of derivatization by the modified Edman degradation method differs for free and protein-bound amino acids and is also dependent on the type of globin involved (Törnqvist et al. 1992; Van Sittert 1997), which might explain the difference in results.

These studies show that time-integrated exposure to EO and PO can readily and reliably be assessed by measurement of the concentration of their *N*-terminal valine adducts in Hb from a small blood sample and that there is a good correlation between these adduct levels and the airborne concentrations of EO and PO detected in the blood of workers occupationally exposed to low concentrations of EO or PO. In addition, these correlations allow the calculation of tentative BELs for EO and PO. At the current Dutch OEL for EO (0.5 ppm, 8-h TWA) this value is 3.2 nmol HOEtVal/g globin (limits of the 95% confidence interval 2.5–4.3 nmol/g globin). For the value of 4.15 ppm (8-h TWA) that is currently being investigated as the new Dutch OEL for PO the tentative BEL is 5.3 nmol HOPrVal/g globin (limits of the 95% confidence interval 4.2–7.0 nmol/g globin).

Acknowledgements The technical assistance by C.D. van der Giesen (Department of Occupational Health and Hygiene, Shell Nederland Chemie BV, Moerdijk, The Netherlands) and H. van der Waal (Safety and Health Department, Shell Nederland Chemie/Raffinaderij, Pernis, The Netherlands) is gratefully acknowledged.

References

3M (1986) Organic vapour monitors sampling and analysis guide. 3M, Minneapolis, Minnesota

ACGIH (1998) 1998 TLVs and BEIs. Threshold limit values for chemical substances and physical agents. Biological exposure indices. ACGIH, Cincinnati, Ohio

ACTS (1992) Propylene oxide. Criteria document for an occupational exposure limit (draft, report ACTS/33/92). Advisory Committee on Toxic Substances, UK Health and Safety Executive, Bootle, Merseyside

Angerer J, Bader M, Krämer A (1998) Ambient and biochemical effect monitoring of workers exposed to ethylene oxide. Int Arch Occup Environ Health 71: 14–18

Bader M, Lewalter J, Angerer J (1995) Analysis of *N*-alkylated amino acids in human hemoglobin: evidence for elevated *N*-methylvaline levels in smokers. Int Arch Occup Environ Health 67: 237–242

Bianchi A, Boyle B, Harrison P, Lawrie P, Le Lendu T, Rocchi P, Taalman R, Wieder W (1997) A review of analytical methods and their significance to subsequent occupational exposure data evaluation for 1,3-butadiene. CEFIC, Brussels/IISRP, London

Couch R, Ehrenberg L, Magnusson A-L, Nilsson R, Rosa ME de la, Törnqvist M (1996) In vivo dosimetry of ethylene oxide and propylene oxide in the cynomolgus monkey. Mutat Res 357: 17–23

DECOS (Dutch Expert Committee on Occupational Standards) (1987) Health-based recommended occupational exposure limit for propylene oxide (draft, report WGD 87-119-8). Health Council of the Netherlands, The Hague

DECOS (1997) Propylene oxide. Health-based calculated occupational cancer risk values. Report 1997/02WGD, Dutch Expert Committee on Occupational Standards. Health Council of the Netherlands, The Hague

DFG (Deutsche Forschungsgemeinschaft) (1997) Maximale Arbeitsplatzkonzentrationen und biologische Arbeitsstoff Toleranzwerte 1997. Mitteilung XXXIII der Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe. VCH, Weinheim

Duus U, Osterman-Golkar S, Törnqvist M, Mowrer J, Holm S, Ehrenberg L (1989) Studies of the determinants of tissue dose and cancer risk from ethylene oxide exposure. In: Freij L (ed) Proceedings of the symposium on management of risk from genotoxic substances in the environment. Swedish National Chemicals Inspectorate, Solna, pp 141–153

Ehrenberg L, Hussain L (1981) Genetic toxicity of some important epoxides. Mutat Res 86: 1–113

Esposito GG, Williams K, Bongiovanni R (1984) Determination of ethylene oxide in air by gas chromatography. Anal Chem 56: 1950–1953

Farooqi Z, Törnqvist M, Ehrenberg L, Natarajan AT (1993) Genotoxic effects of ethylene oxide and propylene oxide in mouse bone marrow cells. Mutat Res 288: 223–228

Fennell TR, Sumner SCJ, Walker VE (1992) A model for the formation and removal of hemoglobin adducts. Cancer Epidemiol Biomarkers Prev 1: 213–219

Granath F, Ehrenberg L, Törnqvist M (1992) Degree of alkylation of macromolecules in vivo from variable exposure. Mutat Res 284: 297–306

Högstedt B, Bergmark E, Törnqvist M, Osterman-Golkar S (1990) Chromosomal aberrations and micronuclei in lymphocytes in relation to alkylation of hemoglobin in workers exposed to ethylene oxide and propylene oxide. Hereditas 113: 133–138

IARC (1994) Propylene oxide. IARC Monogr Eval Carcinog Risks Hum 60: 181–213

Kautiainen A, Törnqvist M (1991) Monitoring exposure to simple epoxides and alkenes through gas chromatographic determination of hemoglobin adducts. Int Arch Occup Environ Health 63: 27–31

NBOSH (1993) Occupational exposure limits. National Board of Occupational Safety and Health, Solna

NIOSH (1985) Manual of analytical methods 3rd edn, vol 2, method 1612, Department of Health and Human Services, Public Health Service, Centers for Disease Control, Cincinnati, Ohio

NTP (1985) Toxicology and carcinogenicity studies of propylene oxide in F344/N rats and B6C3F$_1$ mice (inhalation studies). NTP Technical report series 267. National Technical Information Service, Springfield, Virginia

Osterman-Golkar S, Bond JA (1996) Biomonitoring of 1,3-butadiene and related compounds. Environ Health Perspect 104 [Suppl 5]: 907–915

Richardson KA, Megens HJJJ, Webb JD, Van Sittert NJ (1996) Biological monitoring of butadiene exposure by measurement of hemoglobin adducts. Toxicology 113: 112–118

Ríos-Blanco MN, Plna K, Faller T, Kessler W, Hkansson K, Kreuzer PE, Ranasinghe A, Filser JG, Segerbäck D, Swenberg JA (1997) Propylene oxide: mutagenesis, carcinogenesis and molecular dose. Mutat Res 380: 179–197

Svensson K, Olofsson K, Osterman-Golkar S (1991) Alkylation of DNA and hemoglobin in the mouse following exposure to propene and propylene oxide. Chem Biol Interact 78: 55–66

SZW (1995a) Invoering wettelijke grenswaarde benzine, benzeen en ethyleenoxide (press release 95/176). Ministerie van Sociale Zaken en Werkgelegenheid (SZW), Den Haag

SZW (1995b) De nationale MAC-lijst 1995. Inspectiedienst van het Ministerie van Sociale Zaken en Werkgelegenheid (P145). Sdu Servicecentrum Uitgeverijen, Den Haag

Tates AD, Boogaard PJ, Darroudi F, Natarajan AT, Van Sittert NJ (1995) Biological effect monitoring in industrial workers following accidental exposure to high concentrations of ethylene oxide. Mutat Res 329: 63–77

Törnqvist M, Mowrer J, Jensen S, Ehrenberg L (1986) Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. Anal Biochem 154: 255–266

Törnqvist M, Magnusson A-L, Farmer PB, Tang Y-S, Jeffrey AM, Wazneh L, Beulink GDT, Van der Waal H, Van Sittert NJ (1992) Ring test for low levels of *N*-(2-hydroxyethyl)valine in human hemoglobin. Anal Biochem 203: 357–360

UKHSE (1995) Occupational exposure limits 1995. UK Health and Safety Executive, Sudbury (Suffolk). HSE Books 24: 41

Van Sittert NJ (1997) *N*-2-Cyanoethylvaline, *N*-2-hydroxyethylvaline, *N*-methylvaline (as evidence of exposure to acrylonitrile, ethylene oxide as well as alkylating agents). In: Angerer J, Schaller KH (eds) Analyses of hazardous substances in biological materials, vol 5. VCH, Weinheim, pp 181–210

Van Sittert NJ, Van Vliet EWN (1994) Monitoring occupational exposure to some industrial chemicals by determining hemoglobin adducts. Clin Chem 40: 1472–1475

Van Sittert NJ, Beulink GDJ, Van Vliet EWN, Van der Waal H (1993) Monitoring occupational exposure to ethylene oxide by the determination of hemoglobin adducts. Environ Health Perspect 99: 217–220

Van Welie RTH, Van Dijck RGJM, Vermeulen NPE, Van Sittert NJ (1992) Mercapturic acids, protein adducts, and DNA adducts as biomarkers of electrophilic chemicals. CRC Crit Rev Toxicol 22: 271–306

Vogel EW, Nivard MJM (1997) The response of germ cells to ethylene oxide, propylene oxide, propylene imine and methyl methanesulfonate is a matter of cell stage-related DNA repair. Environ Mol Mutagenesis 29: 124–135