

Occupational exposure to ethylene oxide

Relation between in vivo dose and exposure dose

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OSTERMAN-GOLKAR S, BERGMARK E. Occupational exposure to ethylene oxide: Relation between in vivo dose and exposure dose. *Scand J Work Environ Health* 14 (1988) 372–377. As a basis for risk estimations for ethylene oxide (EtO) exposure and for the establishment of occupational exposure limits in work environments it is important to know the ratio between the in vivo dose and the exposure dose of this compound. For an assessment of this ratio, data on hemoglobin adduct levels in occupationally exposed workers and exposure levels in the work environment have been collected. The in vivo dose is directly proportional to the product of the uptake and retention time ($1/\lambda$) of EtO in the body. The rate of clearance (λ) of EtO has been calculated for individual workers from adduct levels and estimated EtO uptake. The wide range of λ values found ($\sim 1-65 \text{ h}^{-1}$) can only partly be ascribed to a true variation between individuals with respect to clearance rates. One uncertainty results from the difficulties to estimate EtO uptake. A better estimate of λ ($\approx 3 \text{ h}^{-1}$) is probably derived from the measurements of environmental and instantaneous blood concentrations of EtO in exposed workers by Brugnone et al [Int Arch Occup Environ Health 58 (1986) 105–112].

Key terms: clearance rate, dosimetry, hemoglobin adducts.

Ethylene oxide is a directly alkylating agent with mutagenic and carcinogenic properties. (For reviews see, eg, references 7, 14, 26.)

Several studies have demonstrated cytogenetic effects, including chromosome aberrations (5, 11), sister chromatid exchanges (10, 28), and micronuclei in bone marrow cells (11), among workers occupationally exposed to ethylene oxide. In a Swedish epidemiologic study on cancer an increased frequency of leukemia (8 cases against 0.8 expected) and ventricle cancer (6 cases against 0.65 expected) was observed (12) that suggested that ethylene oxide is carcinogenic in man.

It may be possible to tie early cytogenetic effects, as well as the risk of late effects, to the tissue doses² (or exposure levels) of ethylene oxide. Such dose-risk/dose-response relationships would be useful not only for the risk assessment of ethylene oxide (and its precursor ethene), but might also be of value for assessments of other alkylating agents or intermediates.

Current exposures can be monitored through the determination of hydroxyethylated amino acids in hemoglobin (2, 9, 24) for a reliable measure of the average in vivo dose obtained during the last months of exposure (2, 18). However, the control of work environ-

ments and occupational exposure limits is still based on the air concentrations of ethylene oxide. Furthermore, assessments of past exposure have to rely on available data on such concentrations. Therefore, the in vivo dose:exposure dose ratio, and the possible variation between the individuals, are important pieces of information.

In this report available data on the adduct levels in the hemoglobin of workers exposed to ethylene oxide and the exposure levels in the work environment (though limited) have been collected in order that the ratio in vivo dose:exposure dose can be estimated. This ratio was also calculated from the data of Brugnone et al (1) on air concentrations and instantaneous concentrations of ethylene oxide in blood.

Materials and methods

Sample collection

Plant I. During the summer of 1976 the air levels of ethylene oxide were monitored in plant I, which used ethylene oxide to sterilize medical equipment (3). The blood samples were collected from the workers at the end of 1977. No known alterations in the work conditions had been made during the intervening time. Callemann et al (2) measured the alkylation of histidine- N^ϵ in hemoglobin. The study included two workers from plant II [subjects F(I) and G(I)].

Plant II. A mixture of ethylene oxide and methylformate was used in plant II for sterilizing medical equipment. The work conditions have been described by Högstedt et al (11). The air levels of ethylene oxide

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² Dose (D) is defined as the time integral of concentration (level, C),

$$D = \int C dt.$$

Dose thus has the dimension concentration (level) $\times$ time (eg, molar-hours or parts per million-hours (ppmh)).

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were measured in December 1975. The sterilizing personnel was exposed to ethylene oxide when loading and unloading the sterilizers (personal sampling 7.6 ppm, area sampling 5.0 ppm), which took a total of 1–2 h a day, and when the sterilizer was closed (personal sampling 1.3 ppm). In the packing room the exposure level was 0.3–1 ppm (personal sampling). Blood samples were obtained in October 1978 in connection with an investigation of the cytogenetic effects of ethylene oxide (11). The sterilizing personnel began to wear protective respiratory devices in 1977. No further changes were made in the work routines in the intervening time. If the ethylene oxide concentration, the work routines, and the use of protective devices are taken into consideration, it may be concluded that all the subjects were exposed to ≤ 8 ppmh a day. The alkylation of N-terminal valine in hemoglobin was determined for six exposed workers. One was a sterilizer exposed 2–3 d a month [subject 1(II)], four were packing personnel, one cleaned the packing room [subject 3(II)], and five were referents from another department of the same factory.

Plant III. Blood samples were obtained in 1981 from four workers in plant III, which produced medical equipment sterilized with ethylene oxide within the plant. The process and the work conditions have been described in the context of an earlier investigation of the same individuals (11). Based on personal sampling, the ethylene oxide exposure was estimated to be 8 ppmh/d for one of the workers. According to repeated measurements the exposure during total shifts was estimated to be about 16 ppmh/d for the three others. The referents were from another department of the factory.

Plant IV. Ten employees, seven exposed persons and three referents, from an establishment (plant IV) which bottled ethylene oxide gas were studied with respect to the alkylation of histidine- N^ϵ and N-terminal valine in their hemoglobin (9). The exposure levels were characterized as high, intermediate, and low. The records of the air concentrations of ethylene oxide in the work environment were not made available.

Plant V. Five workers were exposed acutely to a high level of ethylene oxide (> 70 ppm) in connection with a leakage at plant V, which produced ethylene oxide. One of them stayed only a short while in the room, and the exposure was considered low. Blood samples were collected one week and four months after the exposure.

Analysis of hemoglobin alkylation

Blood samples from plants II, III, and V were analyzed with respect to adduct levels of N-terminal valine in hemoglobin according to the following procedure. The samples were collected in heparinized vacutainer tubes with cannulae sterilized with gamma radiation. Globin

was isolated according to Mowrer et al (17). Globin samples (50 mg) were dissolved in formamide (1.5 ml) and internal standard globin (20 μ g containing 2.5 nmol d_4 -hydroxyethylvaline/mg globin) was added. Pyridine (5 μ l) was added to neutralize the samples. Pentafluorophenyl isothiocyanate (7 μ l) was added, and the samples were then derivatized by incubation overnight at room temperature (23, 24). The samples were extracted with ether, and the extracts evaporated to dryness under nitrogen, redissolved in toluene, and washed with 0.1 M disodium carbonate and water. The toluene was carefully evaporated under nitrogen at 60°C, and the samples were redissolved in 50 μ l of toluene for analysis with gas chromatography-mass spectrometry.

The analyses were carried out with a Finnigan 4500 gas chromatograph-mass spectrometer in the negative ion chemical ionization mode. The operating parameters for the mass spectrometer were as follows: methane reagent gas at an ion source pressure of 0.50 torr (67 Pa), ion source temperature 100°C, ionization energy 130 eV. The operating parameters for the gas chromatograph were helium carrier gas at a constant pressure of 10 pounds per square inch (69 kPa); temperature programming 20°C/min from 100 to 200°C, followed by an increase of 10°C/min to 300°C. A 30-m DB-5 (0.33 mm inner diameter, 1 μ m phase thickness) fused silica capillary column was used (J&W Scientific, Inc, California, the United States).

One microliter of the toluene solutions was injected directly (on-column injector OCI-3, SGE, Ltd, Australia). The pentafluorophenylthiohydantoin of hydroxyethylvaline and its deuterated analogue were monitored at m/z 348 (M-HF) $^-$ and 352, respectively. Calibration was carried out as described by Törnqvist et al (23).

Results and discussion

Experiments with rodents have demonstrated that ethylene oxide (EtO) is effectively absorbed from the alveolar air and rapidly distributed in the body to give approximately the same dose in different organs (as demonstrated through protein and deoxyribonucleic acid (DNA) binding; see references 6, 21, 22, 27). Pharmacokinetic studies of the disposition of ethylene oxide in dog have shown that the elimination from plasma follows first-order kinetics and that the apparent volume of distribution is approximately 1 l/kg (16). Animal and human bodies may therefore be treated as a single compartment, and the tissue dose (D) is hence related to the absorbed amount per kilogram of body weight (bw) ie, (EtO) $^\circ$, according to

$$D = \frac{(\text{EtO})^\circ}{\lambda}, \quad \text{equation 1}$$

where λ is the first-order rate constant for the elimination (clearance) of EtO from tissues. (See references 6 and 8.)

With the assumption of an alveolar retention of 80 % of the inhaled EtO (1), the rate of uptake of EtO, ie, $d[\text{EtO}]^{\circ}/dt$, may be estimated from the air concentration and the rate of alveolar ventilation, $0.2 \text{ l} \cdot (\text{kg} \cdot \text{bw})^{-1} \cdot \text{min}^{-1}$, according to the following equation:

$$\begin{aligned} d(\text{EtO})^{\circ}/dt &= \\ \frac{0.80 \cdot (0.2 \text{ l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \cdot (60 \text{ min} \cdot \text{h}^{-1}) \cdot (10^{-6} \text{ mol} \cdot \text{ppm}^{-1})}{25 \text{ l}} \\ &= (0.38 \mu\text{mol} \cdot \text{kg}^{-1}) \cdot (\text{ppmh})^{-1}, \end{aligned} \quad \text{equation 2}$$

where 25 l = molar volume.

Brugnone et al (1) monitored occupational exposure to ethylene oxide by measuring the concentrations in environmental air, alveolar air, and blood³ during and at the end of the workshift. During work the ethylene oxide concentration in the blood was, on an average, 3.3 times the environmental concentration of ethylene oxide, ie, a 1 ppm (0.04 $\mu\text{mol/l}$ air) ethylene oxide concentration in the air would result in an ethylene oxide concentration of 0.13 $\mu\text{mol/l}$ in blood. The dose delivered (per hour) may thus be formulated as follows:

$$\begin{aligned} D &= \frac{0.38 \mu\text{mol} \cdot \text{kg}^{-1}}{\lambda} = (0.13 \mu\text{mol} \cdot \text{l}^{-1}) \cdot 1 \text{ h}, \\ \text{ie, } \lambda &= 3 \text{ h}^{-1}. \end{aligned} \quad \text{equation 3}$$

The measurements by Brugnone et al did not reveal any differences between individuals (nine exposed workers). The variation between the four measurements for each individual was considerable and may have been related to the fact that the blood level is built up during a period of time that may be long compared to fluctuations in air concentrations.

The rates of the formation of reaction products of ethylene oxide with tissue nucleophiles (Y_j) are determined by the rate constant $k_{\text{EtO}, j}$ for the following reaction:



where HOEt = hydroxyethyl.

The yields of the products are directly proportional to the rate constants and the dose delivered to the system, ie,

$$(\text{HOEt} - Y_j)/(Y_j) = k_{\text{EtO}, j} \cdot D. \quad \text{equation 5}$$

Hydroxyethylated products in hemoglobin are stable, and the stability of alkylated hemoglobin chains is determined by the life span (t) of the erythrocytes (er) (which equals 126 d in man). During long-term exposure ($\geq t_{er}$) a steady-state (ss) adduct level is established (18). This steady-state adduct level is related to the average daily increment in the adduct level,

$(\text{HOEt} - Y_j)_d/(Y_j)$, according to the following equation:

$$[(\text{HOEt} - Y_j)/(Y_j)]_{ss} = (\text{HOEt} - Y_j)_d/(Y_j) \cdot t_{er}/2. \quad \text{equation 6}$$

Thus the rate of clearance from tissues (λ) may be estimated from the measured hemoglobin adduct levels and estimates of the average daily uptake of ethylene oxide, ie, $(\text{EtO})_d^{\circ}$, according to equations 1, 5, and 6:

$$\lambda = \frac{t_{er} \cdot k_{\text{EtO}, j} \cdot (\text{EtO})_d^{\circ}}{2 \cdot [(\text{HOEt} - \text{Hb})/(\text{Hb})]_{ss}}. \quad \text{equation 7}$$

Data on hemoglobin and daily exposure, estimated from available measurements of air levels of ethylene oxide, are presented in tables 1 and 2. The adduct levels of the referents of plant II agree with those of non-smokers (0.03–0.07 nmol/g hemoglobin) and smokers (0.14–0.55 nmol/g hemoglobin) reported by Törnqvist et al (25). The adduct levels determined for the referents of plant III were elevated and indicate a leakage of ethylene oxide into the environment. This leakage is also reflected in a significantly increased level of cytogenetic changes in these workers as compared with a group of workers exposed to propylene oxide (unpublished results of Högstedt et al).

On the basis of data from tables 1 and 2 and rate constants from Segerbäck [in reference 22 and a personal communication [$k_{\text{Hb-His}} = (0.38 \pm 0.05) \cdot 10^{-4} \text{ l} (\text{g Hb})^{-1} \text{h}^{-1}$ and $k_{\text{Hb-Val}} = (0.45 \pm 0.06) \cdot 10^{-4} \text{ l} (\text{g Hb})^{-1} \text{h}^{-1}$]] λ values have been calculated according to equation 7. No correction for smoking has been made. Values in the range of $\approx 1 - 65 \text{ h}^{-1}$ have been recorded (tables 1 and 2). Since, within each study, there seems to be some agreement between estimated exposure levels and the determined adduct levels in hemoglobin (also in the data for plants IV and V), only a part of this variation can be ascribed to true differences between individuals with respect to the clearance rate of ethylene oxide. Obviously, point measurements of ethylene oxide levels in the work environment do not give reliable information on exposure to individual workers over the extended period of time that is measured by hemoglobin adducts (≈ 4 months). The impact of peak exposures may be overestimated due to uncertainty with respect to duration (plant I?) or they may not be recorded (plant III?).

Values of λ , calculated from experiments with animals (table 3), indicate moderate differences between sexes and species. The collected data for man give, at present, no reason to doubt that the λ_{man} falls within the range of animal values — the estimated λ_{man} (3 h^{-1}) from the data of Brugnone et al (1) being the most reliable. The estimation of relative sensitivities of different species to a chemical is often based on the approximation that basal metabolic rates are roughly proportional to the body surface area (20). An application of the correction factor 13.2 from mouse to man would give $\lambda_{\text{man}} = 4.6/13.2 = 0.35 \text{ h}^{-1}$. This value is close to that expected in the absence of en-

³ The blood samples were incubated at 37°C for 2–3 h before the analysis. This procedure introduces an uncertainty with respect to the measured ethylene oxide concentrations since the half-time of ethylene oxide in blood is 3–4 h (Ulf Duus, personal communication). The error is however not serious since the blood/air partition coefficient in vitro was determined in the same way.

Table 1. Degree of alkylation of histidine-*N*^c (plant I) or N-terminal valine (plant II, III) in the hemoglobin of workers exposed to ethylene oxide and unexposed referents and the estimated λ values.

Subject ^a	Adduct level (nmol/g hemoglobin)	Daily exposure dose (ppmh)	Estimated λ (h ⁻¹)	Source	
<i>Plant I</i>					
Subject A +	6.4	250	33 ± 22 ^b	Calleman et al (2)	
Subject B +	3.6	250			
Subject C -	14.3	312			
Subject D (+)	2.6	62			
Subject E -	0.5	50			
Subject F - ^c	2.0	<8			<2.6
Subject G - ^c	0.4	33			54
Subject H -	<0.05	Referent			—
Subject I -	<0.05	Referent	—		
<i>Plant II</i>					
Subject 1 +	1.4	≤8	≤4.4	Present study and Högstedt et al (11 and unpublished results)	
Subject 2 -	2.1		≤2.9		
Subject 3 -	0.7		≤8.8		
Subject 4 -	2.1		≤2.9		
Subject 5 -	1.6		≤3.9		
Subject 6 -	1.7		≤3.6		
Subject 7—9 +	0.57 ^b	Referents	—		
Subject 10—11 -	0.15 ^b	Referents	—		
<i>Plant III</i>					
Subject 1 +	1.7	8	3.6	Present study and Högstedt et al (11 and unpublished results)	
Subject 2 +	≈ 10	16	≈ 1		
Subject 3 -	≈ 10		≈ 1		
Subject 4 -	≈ 10		≈ 1		
Subject 5—7 +	1.2 ^d	Referents	—		
Subject 8—13 -	1.4 ^d	Referents	—		

^a + = smoker, - = nonsmoker.

^b Mean ± standard deviation.

^c From plant II; see the Materials and Methods section.

^d Average values.

Table 2. Degree of alkylation of N-terminal valine (plants IV and V) in the hemoglobin of workers exposed to ethylene oxide and the unexposed referents.

Subject ^a	Adduct level (nmol/g hemoglobin)	Comment on exposure	Source
<i>Plant IV</i>			
Subject 1	1.5	High	Farmer et al (9)
Subject 2	7.7	High	
Subject 3	4.6	Intermediate	
Subject 4	0.98	Intermediate	
Subject 5	0.02	Low	
Subject 6	0.30	Low	
Subject 7	0.41	Low	
Subject 8	0.93	Referent	
Subject 9	0.14	Referent	
Subject 10	0.03	Referent	
<i>Plant V</i>			
Subject 1 –	0.335	Acute exposure	Present study
Subject 1 –	0.046 ^b	.	
Subject 2 (+)	0.684	Acute exposure	
Subject 3 –	0.761	Acute exposure	
Subject 4 +	0.968	Acute exposure	
Subject 5 –	0.152	Short duration	
Subject 5 –	0.030 ^b	.	

^a + = smoker; - = nonsmoker.

^b Four months after exposure.

Table 3. Rate constants (λ) for the clearance of ethylene oxide in different species. (CBA = Color Black/Brown Agouti, NMRI = Naval Medical Research Institute)

Species	λ (h^{-1})	Reference
Mouse, male (CBA)	4.6 ^a	Ehrenberg et al (6), Osterman-Golkar et al (18)
Mouse, female (CBA)	2.5 ^a	Segerbäck et al (unpublished results)
Mouse, male (NMRI)	5 ^a	Segerbäck (unpublished results)
Rat, male (Fischer 344)	$\approx 4^a$	Osterman-Golkar et al (19)
Dog, male	1.3 ^b	Martis et al (16), calculated
Man	$\approx 3^c$	Brugnone et al (1), calculated (see also table 1)

^a Calculated from absorbed/injected amounts of ethylene oxide and adduct levels in deoxyribonucleic acid or hemoglobin.

^b Calculated from direct determinations of the half-time ($t_{1/2}$) of the elimination of ethylene oxide from blood plasma ($\lambda = 0.693/t_{1/2}$).

^c Calculated from measurements of environmental concentrations of ethylene oxide and instantaneous concentrations of ethylene oxide in the blood of exposed workers.

zyme-catalyzed reactions and is certainly an underestimate (ie, will give an overestimate of dose, and of risk).

Assuming the same ratio between tissue dose and exposure dose as was found for the mouse (ie, $\lambda = 4.6 \text{ h}^{-1}$),⁴ Calleman et al (2) presented an estimate of the leukemia risk associated with ethylene oxide exposure. The estimate was based on the rad equivalence of ethylene oxide (an effectiveness ratio of about 80 rad low LET radiation per molar-hour of ethylene oxide is found in various test systems for genotoxic activity; see references 4 and 15 and unpublished results of Segerbäck et al) and on the human leukemia risk coefficient established for gamma radiation. The epidemiologic investigations by Hogstedt et al (12, 13) seem to corroborate the estimated risk (Ehrenberg, to be published).

The compiled data indicate that λ_{man} is of the order of 3 h^{-1} . However, before dose-response relationships for the genotoxic effects of ethylene oxide are established, it would be appropriate to define the λ value, and its possible variation among individuals, better.

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⁴ Calleman et al (2) calculated tissue doses and estimated the λ_{man} to be approximately 10 h^{-1} . He used a rate constant for the reaction of ethylene oxide with histidine in hemoglobin; the constant, according to repeated later measurements (22), is too low. Because of uncertainties in the estimates of exposure dose, Calleman et al found it prudent to base a tentative risk estimation on animal data.

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