Arch Toxicol (1992) 66: 157–163

Archives of
Toxicology

Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene

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Received 24 June 1991/Accepted 7 October 1991

Abstract. The inhalation pharmacokinetics and the endogenous production of ethylene has been determined in healthy volunteers with respect to the formation of the carcinogen ethylene oxide. Ethylene showed a low degree of accumulation in the body determined in six subjects, the thermodynamic partition coefficient "body/air" being 0.53 ± 0.23 (mean ± SD) and the accumulation factor "body/air" at steady-state being 0.33 ± 0.13 (mean ± SD). The rate of metabolism was directly proportional to the exposure concentration. Only 2% of ethylene inhaled was metabolized to ethylene oxide, whereas 98% of ethylene was exhaled unchanged. The rate of the endogenous production of ethylene was 32 ± 12 nmol/h (mean ± SD), as calculated from exhalation data from 14 subjects. The resulting body burden was 0.44 ± 0.19 nmol/kg (mean ± SD). By analyzing published data on ethylene oxide in man its half-life was estimated to be 42 min. Using the pharmacokinetic parameters of ethylene and ethylene oxide, the body burden of ethylene oxide due to the sum of the exposure to environmental ethylene of about 15 ppb and to endogenous ethylene exposure of 0.44 nmol/kg was predicted to be 0.25 nmol/kg. In the blood of five non-smokers and one smoker the hemoglobin adduct resulting from the reaction of ethylene oxide with the N-terminal valine, N-(2-hydroxyethyl)valine, was quantified by gas chromatography/mass spectrometry. The value of 20 ± 5 pmol/g Hb (mean ± SD) found in the non-smokers corroborated the steady-state level of 18 ± 3 pmol/g Hb (mean ± SD) calculated from the pharmacokinetic approach.

Key words: Ethylene – Ethylene oxide – Pharmacokinetics – Hemoglobin adduct – Man

Introduction

Ethylene is of considerable industrial importance and is ubiquitous in the environment (Abeles and Heggestad 1973). It is endogenously produced in plants (Leopold 1972) and has been identified in the exhaled air of rat and man (Ram Chandra and Spencer 1963; Conkle et al. 1975; Sagai and Ichinose 1980; Harrison 1981). The rate of the endogenous production has been quantified for both species by Shen et al. (1989). The origin of endogenous ethylene in mammals is still unclear. Four sources are being discussed: lipid peroxidation (Frank et al. 1980; Sagai and Ichinose 1980; Törnqvist et al. 1989b), oxidation of free methionine (Lieberman and Mapson 1964; Kessler and Remmer 1990), oxidation of hemin in hemoglobin (Kessler 1987) and metabolism of intestinal bacteria (Gelmont et al. 1981; Törnqvist 1989).

Ethylene is metabolized to ethylene oxide as has been demonstrated in vitro with rat liver microsomes (Schmiedel et al. 1983) and in vivo in mice (Ehrenberg et al. 1977) and in rats (Filser and Bolt 1983). Ethylene oxide is mutagenic (reviewed in: Ehrenberg and Hussain 1981; ECETOC 1984) and carcinogenic in rodents (Dunkelberg 1982; Lynch et al. 1984; Snellings et al. 1984; Garman et al. 1985; NTP 1987) and probably in man (IARC 1985; Hogstedt et al. 1986). Due to its electrophilicity ethylene oxide alkylates cellular macromolecules as DNA and proteins (Ehrenberg and Osterman-Golkar 1980; Ehrenberg and Hussain 1981; Segerbäck 1983). Identical reaction products which have been observed after exposure of mice to ethylene have been attributed to the metabolite ethylene oxide (Ehrenberg et al. 1977; Segerbäck 1983).

In inhalation experiments with ethylene oxide in rats, the degree of alkylation of hemoglobin could be related directly to that of alkylation of DNA (Osterman-Golkar et al. 1983; Potter et al. 1989). Therefore, from measurements of hydroxyethyl adducts to amino acids in hemoglobin the dose of ethylene oxide delivered to the tissue DNA can be estimated. However, low levels of hydroxyethyl adducts were also found in human hemoglobin which had not been deliberately exposed to ethylene oxide or ethylene (Van

Abbreviations: HOEtVal, N-(2-hydroxyethyl)valine; PFPTH, pentafluorophenylthiohydantoin

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Sittert et al. 1985; Törnqvist 1989; Törnqvist et al. 1989b). As measured by adducts to N-terminal valine in hemoglobin there seems to be a true background of ca. 20 pmol/g Hb of N-(2-hydroxyethyl)valine (HOEtVal) in non-smokers (Törnqvist 1989). These adducts are probably reaction products with ethylene oxide formed metabolically from endogenous ethylene and from exogenous ethylene ubiquitously present in the environment.

To confirm this assumption, we investigated the pharmacokinetics of ethylene and ethylene oxide in humans and determined the body burden of both substances caused by endogenous and exogenous ethylene. Using these data, the expected levels of HOEtVal were calculated for six volunteers and compared with the HOEtVal levels measured in blood samples from the same subjects.

Materials and methods

Exposure system. Pharmacokinetic data for ethylene in healthy volunteers of 22–65 years (eight males, six females) were obtained in gas uptake studies. A modified spirometer was used as closed exposure system (for detailed description see Filser 1991). In each experiment one volunteer was linked via a special breathing mask to the gas room of the spirometer (12 l). Ethylene uptake and metabolism was studied in six volunteers (five non-smokers, one smoker) by adjusting initial atmospheric concentrations of ethylene (99.95% pure, Messer Griesheim, Düsseldorf, FRG) to approximately 5 and 50 ppm (v/v), respectively, in the gas phase of the spirometer and recording the decline of the concentration up to 2 h by gas chromatography (inhalation experiments). By exposing volunteers (eight males, six females) to synthetic air (hydrocarbon free, Messer Griesheim) only, the enrichment of exhaled endogenous ethylene in the spirometer atmosphere was measured up to 2.5 h (exhalation experiments). From the outcome of these experiments the pharmacokinetic parameters of ethylene were calculated.

Gas chromatography. Determination of ethylene in the atmosphere of the exposure system and in the environment was carried out on Shimadzu GC-8A gas chromatographs equipped with 5 ml injection loops and flame ionisation detectors. Separation was done isothermally on stainless steel columns of 1/8″ × 2.5 m packed with Tenax GC 60-80 mesh (Alltech, Unterhaching, FRG) and of 1/8″ × 3.5 m filled with Porapak Q 50-80 mesh (Alltech, Unterhaching, FRG), respectively. The oven temperatures and the corresponding retention times were 70° C /1.2 min and 80° C /2.3 min, respectively. The gas flows were : H_2, 50 ml/min; air, 456 ml/min; N_2, 54 ml/min (column containing Tenax GC) and 62 ml/min (column containing Porapak Q). The detection limit of ethylene was 7 ppb on both columns with a signal-to-noise ratio of 3. Areas of the peaks were determined by means of Varian 4290 integrators. Since ethylene has been found to be omnipresent in the environment, the background concentration in the air of the laboratory was monitored routinely before starting any experiment. The mean background concentration was 15 ppb (range: not detectable – 58 ppb; $n = 20$). The enrichment of exhaled endogenous ethylene was determined on those days only when the ambient concentration was below the detection limit of 7 ppb. Furthermore, these experiments were always carried out on other days than the experiments with exposure to exogenous ethylene.

Ethylene oxide in the ambient air was also determined by gas chromatography using a Shimadzu GC-8A gas chromatograph equipped with a 5 ml injection loop and flame ionisation detector. Separation was done isothermally on stainless steel columns of 1/8″ × 2.5 m packed with Tenax GC 60-80 mesh (Alltech, Unterhaching, FRG). The oven temperature was 110° C and the gas flows were: H_2, 50 ml/min; air, 456 ml/min; N_2, 54 ml/min. The retention time of ethylene oxide was 1.1 min. The detection limit of ethylene oxide was 15 ppb with a signal-to-noise ratio of 3. Concentrations of ethylene oxide in the ambient air were always below this value.

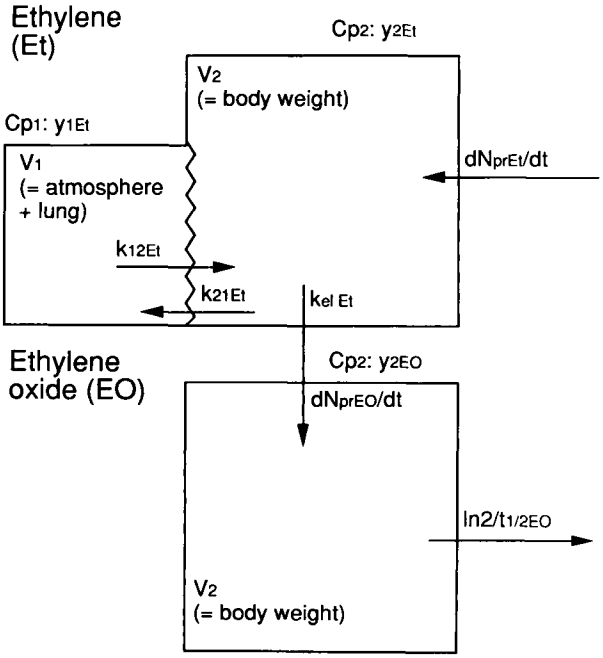

Fig. 1. Linear pharmacokinetic compartment model for the closed exposure chamber.

Cp_1:	compartment 1 representing the atmosphere of the closed chamber + lung (expiratory reserve volume + residual volume)
Cp_2:	compartment 2 representing the exposed organism
V_1:	volume of Cp_1
V_2:	volume of Cp_2
y_{1Et}:	concentration of ethylene in Cp_1
y_{2Et}:	concentration of ethylene in Cp_2
y_{2EO}:	concentration of ethylene oxide in Cp_2
k_{12Et}:	microconstant of the uptake process of ethylene into Cp_2
k_{21Et}:	microconstant of the exhalation process of ethylene from Cp_2
k_{elEt}:	microconstant of the metabolic elimination process of ethylene within Cp_2
k_{elEO}:	microconstant of the metabolic elimination process of ethylene oxide within Cp_2
dN_{prEt}/dt:	rate of endogenous production of ethylene (constant value)
dN_{prEO}/dt:	rate of endogenous production of ethylene oxide (constant value)
$t_{1/2EO}$:	half-life of ethylene oxide; $\ln 2/t_{1/2EO} = \kappa$

Determination of the pharmacokinetics of ethylene. The atmospheric concentration-time data of ethylene obtained in the inhalation and exhalation experiments were analyzed using a linear two-compartment pharmacokinetic model as presented in Fig. 1. The analytical procedure and the mathematical equations related to the model have been published in several publications which are reviewed with a detailed discussion of this model by Filser (1991).

Using the model, a series of pharmacokinetic parameters were determined as: the thermodynamic equilibrium constant "body/air" (K_{eq}), the bioaccumulation factor "body/air" at steady state (K_{st}) and the clearances of uptake, exhalation and metabolism. The equivalent mathematical expressions are outlined in Table 1 and Table 2.

Clearance of uptake, when multiplied by the actual atmospheric concentration of ethylene, gives the corresponding amount taken up by the organism per unit of time. Since both, clearance of exhalation and of metabolism, are related by K_{st} to the atmospheric concentration of ethylene, their products with this concentration give the amounts exhaled and metabolized per unit of time at steady-state conditions. Furthermore, the half-life, the ratio of exhaled and of metabolized ethylene to the amount taken up by the organism and the alveolar retention were calculated. The

Table 1. Pharmacokinetic constants for distribution, metabolism, and endogenous production of ethylene in man (70 kg) determined from the individual experiments.

Constant		Value mean ± SD ($n = 6$)	Dimension
Name	Expression		
Clearance of uptake (related to atmosph. concn.)	$k_{12} \times V_1$	25 ± 7.6	l/h
Microconstant of exhalation	k_{21}	0.68 ± 0.17	1/h
Microconstant of metabolic elimination	k_{el}	0.39 ± 0.14	1/h
Endogenous production rate[a]	dN_{prEt}/dt	32 ± 12	nmol/h

[a] Calculated using the means of $k_{12} \times V_1$, k_{21} and k_{el} from the inhalation experiments and concentration-time courses of 14 exhalation experiments

Table 2. Pharmacokinetic parameters of ethylene in man (70 kg)

Parameter[a] Name	Expression	Value mean ± SD[b]	Dimension
Thermodynamic equilibrium coefficient "body/air" (K_{eq})	$k_{12} \times V_1/k_{21} \times V_2$	0.53 ± 0.23[c]	nl gas/ml tissue/ppm in atmosph.
Bioaccumulation factor "body/air" at steady state (K_{st})	$k_{12} \times V_1/(k_{el}+k_{21}) \times V_2$	0.33 ± 0.13	nl gas/ml tissue/ppm in atmosph.
Clearance of exhalation (related to atmosph. concn.)	$k_{21} \times V_2 \times K_{st}$	16 ± 5.4	l/h
Clearance of metabolism (related to atmosph. concn.)	$k_{el} \times V_2 \times K_{st}$	9.3 ± 3.8	l/h
Clearance of metabolism (related to concn. in organism)	$k_{el} \times V_2$	27 ± 9.6	l/h
Half-life	$\ln 2/k_{el}+k_{21}$	0.65 ± 0.14	h
% metabolized (Amount taken up = 100%)	$k_{el} \times 100/k_{el}+k_{21}$	36 ±11	%
% exhaled (Amount taken up = 100%)	$k_{21} \times 100/k_{el}+k_{21}$	64 ±11	%
Alveolar retention	$k_{el} \times V_2 \times K_{st} \times 100$/Alveol. Ventilation	2 ± 0.8[d]	%
Body burden of ethylene due to endogenous production	$y_{2stEt\ end}$[e]	0.44 ± 0.19	pmol/ml tissue

[a] Valid for atmospheric concentrations below 50 ppm
[b] SD calculated from values of Table 1 by means of Monte Carlo Simulation (4500 runs)
[c] Values are independent of the atmospheric concentration
[d] Alveolar ventilation was set to be 450 l/h at rest (own measurements)
[e] Body burden with endogenous produced ethylene $y_{2stEt\ end}$ was calculated with the equation:
$y_{2stEt\ end} = (dN_{prEt}/dt)/[(k_{el}+k_{21}) \cdot V_2]$; $V_2 = 70$ l

endogenous production rate (dN_{prEt}/dt [pmol/h]) was determined according to Dogra et al. (1988) by analyzing the concentration-time profiles of endogenous ethylene exhaled into the atmosphere of the spirometer system. The best fits to the measured data were computed on an Apple II plus computer (Apple Computer Inc., Cupertino, CA, USA) running an iterative nonlinear regression program based on the least square method (Dynacomp 1981). In this program a function [equation (12) in Filser and Bolt 1983] was inserted in which only dN_{prEt}/dt was variable. The values of all the other parameters were known from the pharmacokinetic analysis of the inhalation experiments with ethylene conducted previously. The production rate dN_{prEt}/dt was obtained from the best fit.

The body burden of ethylene at steady state [y_{2stEt} (pmol/ml tissue)] due to the exogenous and endogenous compound was calculated for exposures to ambient constant concentrations of ethylene [y_{1amb} (ppm)]. At room temperature the molar volume of an ideal gas is about 25 l depending on the actual air pressure. Under such conditions y_{2stEt} is determined by the rates of uptake of ethylene from the atmosphere [$k_{12} \times V_1 \times y_{1amb}/0.025$ (pmol/h)] and of endogenous production (dN_{prEt}/dt) and by the rates of elimination due to metabolism [$k_{el} \times V_2 \times y_{2stEt}$ (pmol/h)] and exhalation [$k_{21} \times V_2 \times y_{2stEt}$ (pmol/h)]:

eq. 1 $$y_{2stEt} = [k_{12} \times V_1 \times y_{1amb}/0.025+dN_{prEt}/dt]/[(k_{el}+k_{21}) \times V_2]$$

This equation was also used to calculate y_{2stEt} resulting from endogenous ethylene alone by setting $y_{1amb} = 0$.

Determination of the pharmacokinetics of ethylene oxide. To obtain the half-life for ethylene oxide in man we used data published by Brugnone et al. (1986). These authors monitored occupational exposure of ten workers to ethylene oxide every hour during a workshift by measuring the concentrations in environmental air (C_i) and alveolar air (C_a). Then, they determined the ratios of C_a to C_i for each time point by linear regression analysis of the obtained ten data pairs. We plotted these ratios versus the corresponding exposure times and fitted a curve given by the function $y = y_\infty \times (1-e^{-\kappa t})$ through the data points by means of an iterative nonlinear regression program (Dogra et al. 1988) (Fig. 2). From the value obtained for κ [1/h] the half-life ($t_{1/2EO}$) was calculated as $\ln 2/\kappa$ [h].

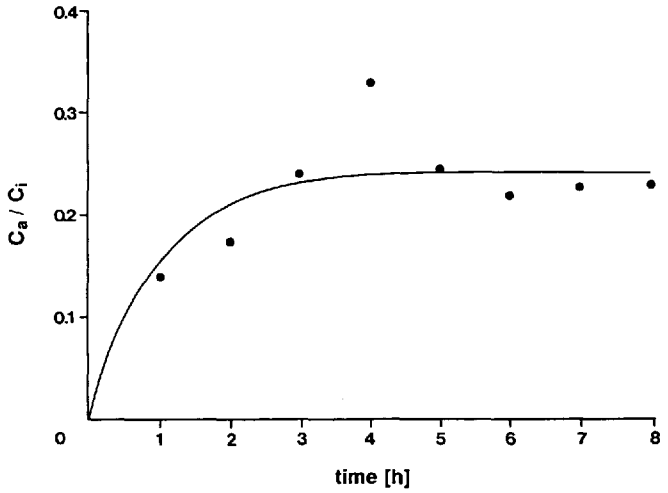

Fig. 2. Exposure of man to ethylene oxide. Ratio of alveolar concentrations (C_a) to concentrations in the environmental air (C_i) in dependence of exposure time (data obtained from Brugnone et al. 1986). Symbols: data from Brugnone et al. 1986; each point calculated from 10 measurements. Lines:best fit

The endogenous production rate of ethylene oxide [dN_{prEO}/dt (pmol/h)] due to exogenous and endogenous exposure to ethylene was assumed to be equal to the rate of metabolism of ethylene given above:

eq. 2 $$dN_{prEO}/dt = k_{el} \times V_2 \times y_{2stEt}$$

Both κ and dN_{prEO}/dt determine the amount of ethylene oxide in the body at steady state due to exogenous and endogenous ethylene. To obtain the average ethylene oxide concentration in the body at steady state [y_{2stEO} (pmol/ml tissue)], the volume of the body (V_2) has to be respected:

eq. 3 $$y_{2stEO} = (dN_{prEO}/dt)/(\kappa \times V_2)$$

The steady state degree of alkylation of N-terminal valine in hemoglobin [HOEtVal (pmol/g Hb)] due to ethylene oxide resulting from chronic exposure to exogenous and endogenous ethylene was estimated according to Törnqvist (1989):

eq. 4 $$HOEtVal = y_{2stEO} \times LSE_{1/2} \times RC_{EO}$$

$LSE_{1/2}$ [h] signifies the half length of the life span of the erythrocytes. It is equal to $^1/_2 \times 126 \times 24 = 1512$ [h] (Osterman-Golkar et al. 1976).

RC_{EO} (ml/h × g Hb) is the rate constant of the reaction of ethylene oxide with N-terminal valine in Hb. Its value is 0.05 ml/h × g Hb (Segerbäck 1990).

y_{2stEO} was estimated to be the same for the organism and for the blood due to its high solubility in aqueous solutions (Schmiedel et al. 1983).

Clearance of uptake of ethylene from the atmosphere and microconstants of ethylene were calculated from the inhalation experiments. Means of these values were used.

Statistics. The data obtained in individual experiments for clearance of uptake, for the microconstants of exhalation and of metabolism, and for rates of endogenous production of ethylene were used to calculate the corresponding pharmacokinetic constants (means and standard deviations) standardized for a human subject of 70 kg body weight (V_2 set equal to 70 l) as described by Filser (1991). These data were used to compute the derived pharmacokinetic parameters of ethylene and their standard deviations by means of Monte Carlo Simulation using a program from Market Engineering Corporation (1988).

Measurement of hemoglobin adduct. Two samples of 5 ml blood were collected with gamma-sterilized cannulae (special order, Becton-Dickinson, Meylan, France) in gamma-sterilized, heparinized vacuumtubes (Vacutainer; Becton-Dickinson) from the six subjects (five non-smokers, one smoker) of which the endogenous production rate of ethene was

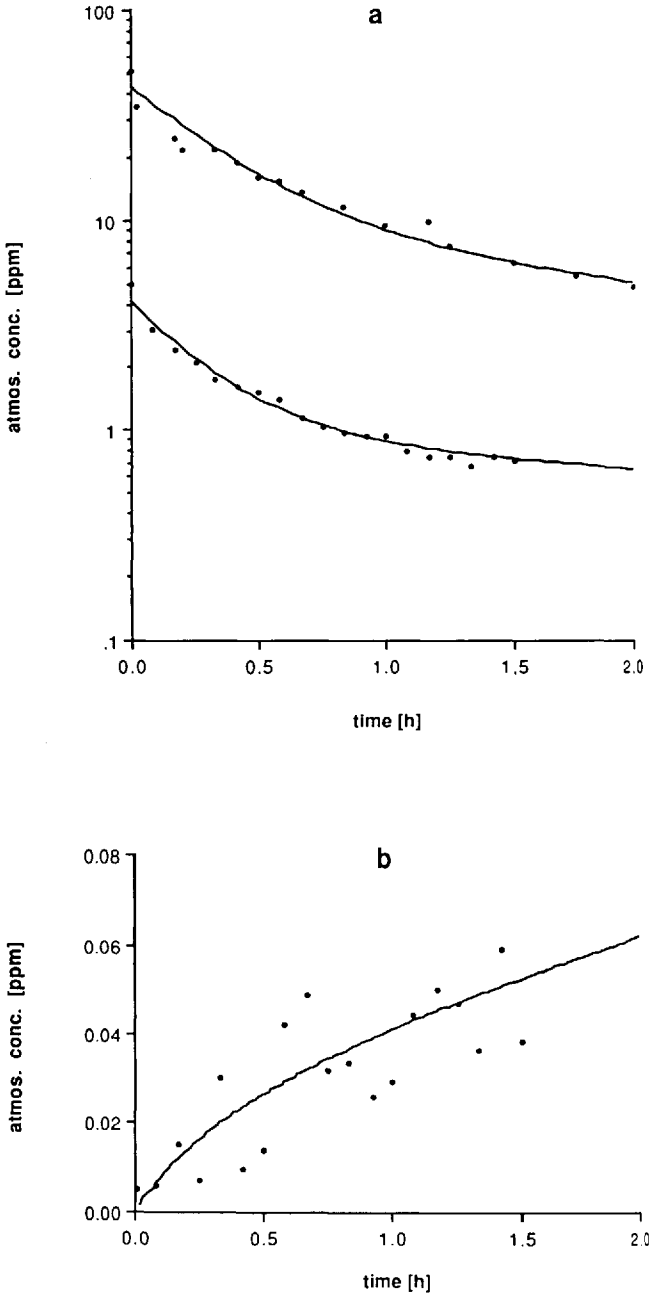

Fig. 3. Concentration-time courses of ethylene in three experiments in the atmosphere of a spirometer system to which volunteers were linked by a breathing mask. **a** Inhalation experiments; initial concentrations 5 and 50 ppm ethylene, respectively (semilogarithmic plot). **b** Exhalation experiment; initial concentration 0 ppm ethylene (linear plot). Symbols: measured values. Lines: calculated curves

pharmacokinetically determined as described above. Double samples were treated somewhat differently in order to exclude contribution from artefacts to the measured values (Törnqvist 1990). In one (series A) of the double samples, red cells and plasma were immediately separated by centrifugation and the red cells were washed twice with 0.9% sodium chloride solution. The samples of series B were left intact in the sample tubes. The samples, series A as red cells, series B as whole blood, were then sent to Sweden by air. Globin was prepared from series A immediately after receipt (2 days after collection of blood), samples from series B were stored at +4° C for 2 more days. Globin was precipitated with ethyl acetate from 2-propanol-HCl solutions according to Mowrer et al.

Table 3. Endogenous production of ethylene and hydroxyethylation of N-terminal valine of hemoglobin (HOEtVal) in 6 individuals. Levels of HOEtVal were calculated for exposure to endogenous ethylene only and exposure to endogenous plus exogenous ethylene (15 ppb)

Subject	dN_{prEt}/dt [pmol/(h·kg)]	HOEtVal (pmol/g Hb) predicted from pharmacokinetic parameters due to		HOEtVal (pmol/g Hb) determined in blood samples
		endogenous ethylene	endogenous plus exogenous ethylene 15 [ppb][a]	sample A; B
6	530[b]	15	20	14; 18
1	514	14	20	25; 26
5	489	13	19	18; 14
3	462	13	19	24; 23
4	388[b]	11	17	18; 21
2 (smoker)	247	7.0	13	51; 52
x± SD	438±106[d]	12±2.9	18±2.7	20±4[c]
(n)	6	6	6	5[c]

[a] Mean concentration determined at the working place
[b] Mean of two experiments
[c] From means of A and B, smoker not considered
[d] Statistical uncertainty, not considering systematic error (SD in Table 1)

(1986). The globin samples were stored at −20° C for 5 months before derivatization.

For the determination of the levels of 2-hydroxyethylation of the N-terminal valines (HOEtVal) in hemoglobin, samples were derivatized and purified using an updated version (Törnqvist et al. 1988) of the N-alkyl Edman method (Törnqvist et al. 1986). Globin samples (50 mg) were dissolved in formamide and NaOH was added for adjustment of pH. The internal standard, globin alkylated with deuterium-substituted ethylene oxide, and the reagent, pentafluorophenyl isothiocyanate, were added and the derivatization was carried out during a time period of 18 – 20 h.

HOEtVal was cleaved off as pentafluorophenylthiohydantoin (PFPTH), extracted from the globin and purified by a washing procedure as described earlier. The analyses of HOEtVal-PFPTH were done using a Finnigan 4500 GC/MS instrument in the negative ion chemical ionization mode, using methane as reactant gas, and monitoring characteristic ions as described by Törnqvist et al. (1986, 1988). On-column injection was carried out into a DB-5 fused silica capillary column. A linear calibration curve was established in the range 8 – 110 pmol/g Hb from calibration samples prepared according to Törnqvist et al. (1988).

Results

Concentration-time courses of exogenous ethylene inhaled from the atmosphere of the spirometer system and of endogenous ethylene exhaled into it are shown as examples in Fig. 3. The best fits to these data using the two-compartment model are plotted as curves.

The initial concentration decline of the curves following administration of 50 and 5 ppm (Fig. 3*a*) is determined mainly by the clearance of uptake. During this time period the inhaled ethylene accumulates in the body. Thereafter, concentrations decrease more slowly and the further courses are determined mainly by the metabolism of ethylene.

The endogenous production of ethylene was shown by the exhalation of ethylene into the atmosphere of the spirometer (Fig. 3*b*). With increasing concentration of ethylene in the atmosphere its concentration in the organism is enhanced, and consequently the rate of its metabolism increases, too. Therefore, the "exhalation curve" flattens to finally reach a plateau at which the rate of metabolism equals that of the endogenous production.

The pharmacokinetic constants computed by analyzing such concentration-time courses for all subjects are summarized in Table 1. Pharmacokinetic parameters calculated by means of these values and the body burden by ethylene produced endogenously are given in Table 2. The data differ slightly from those published earlier (Shen et al. 1989), but this deviation can be explained by the lower number of measurements in the former study.

The mean half-life of ethylene oxide in man was determined using data of Brugnone et al. (1986) to be 42 min. With this value the body burden of ethylene oxide due to the endogenous production of ethylene (y_{2EOend}) was calculated to be 0.17 pmol/ml tissue. Assuming an additional exogenous exposure to 15 ppb atmospheric ethylene, the body burden of ethylene oxide was calculated to be 0.25 pmol/ml tissue.

Table 3 contains for six individuals the calculated endogenous production rates of ethylene and the levels of HOEtVal expected to result from the endogenous production of ethylene and from exposure to environmental ethylene of 15 ppb. These expected values are compared with the levels of HOEtVal determined directly in blood samples from the same persons.

Discussion

Pharmacokinetics of ethylene and of ethylene oxide

Ethylene is nearly insoluble in water and poorly soluble in blood; the partition coefficient blood/air has been determined to be 0.15 (Steward et al. 1973). Due to its low solubility the enrichment in the organism was marginal only as can be seen from the thermodynamic partition coefficient K_{eq}, which describes the maximal possible accumulation in the body. For the same reason, the values of the clearances for uptake, exhalation and metabolism were very small, being a few percent of the alveolar ventilation only.

In the concentration range between 1 ppm and 50 ppm the metabolism of ethylene was found to follow first-order kinetics. However, at higher concentrations a saturation of human ethylene metabolism has to be expected, similarly to observations in rats (Andersen et al. 1980; Bolt and Filser 1987).

In our studies with humans at rest, the mean alveolar ventilation rate was 450 l/h and the clearance of metabolism of ethylene about 2% of this value (Table 2). From determinations of HOEtVal levels in fruit-store workers exposed to an average concentration of 0.3 ppm ethylene it was estimated that about 3 (1–10)% of ethylene inhaled was metabolized to ethylene oxide (Törnqvist et al. 1989a) and this relationship has been corroborated in later studies of occupational exposure to some 5 ppm ethylene (Törnqvist, to be published). From these observations it can be deduced that at least at concentrations of ethylene below 5 ppm the fraction of ethylene metabolized is completely systemically available as ethylene oxide. In contrast, in studies with rats exposed to concentrations of ethylene higher than 1000 ppm it was found that only about 30% of ethylene metabolized were systemically available as ethylene oxide (Filser and Bolt 1984). The authors explained these findings by an intrahepatic first-pass metabolism for the ethylene oxide formed in the liver. From the unusual concentration-time course of the endogenous ethylene oxide in the study of Filser and Bolt (1984, Fig. 3) one can assume a fast induction of ethylene oxide-metabolizing enzymes. However, such an induction should not be expected for the very low levels of ethylene oxide resulting from exposure to low concentrations of ethylene.

Both enzymes, monooxygenase metabolizing ethylene and epoxide hydrolase detoxifying the formed ethylene oxide, are located within the endoplasmic reticulum. A complex containing both enzymes has been discussed (Oesch 1973). This complex could lead to an intrahepatic first-pass effect for ethylene oxide produced from ethylene. However, from the high solubility of ethylene oxide in water (Schmiedel et al. 1983), one can expect a fast diffusion into the cytoplasm. Therefore, and according to the outcome of our experiments in humans, we presume that an intrahepatic first-pass effect for ethylene oxide formed metabolically under such conditions can be neglected.

To calculate the half-life of ethylene oxide, data from Brugnone et al. (1986) were used in this study. The value obtained, 42 min, corresponds to several other estimates. Using a physiologically based pharmacokinetic model, Hattis (1987) computed from the same data a half-life of 41 min. A similar value of 39 min was obtained (Denk 1990) by extrapolating allometrically the half-life of 6 min determined in rats (Filser and Bolt 1984). Hattis (1987) estimated that about 75% of ethylene oxide inhaled would be taken up by the human organism. This uptake was assumed by Denk (1990) to be 40% as has been determined in rats. Similar values have also been found in the rat and in man for other water soluble vapors as acetone, ethanol and 1,3-butadiene monoxide (Nomiyama and Nomiyama 1974; Hallier et al. 1981; Wigaeus et al. 1981; Filser and Bolt 1984). With other polar solvents it has been experimentally shown that the relative respiratory uptake in man varied between about 40 and 80% (referred in Johanson 1991). Assuming a relative respiratory uptake of 40% and a half-life of 42 min, Denk (1990) computed the bioaccumulation factor "body/air" at steady state to be 2.7 in man. This value fits the ratio "venous blood/air" of 2.5 determined by Brugnone et al. (1986) for this rapidly metabolized water-soluble compound (Schmiedel et al. 1983).

HOEtVal due to endogenous and exogenous ethylene

The endogenous production of ethylene of 32 ± 12 nmol/h is calculated to give rise to a dose of ethylene oxide that would result in some two-thirds of the observed background level of HOEtVal in non-smokers (Table 3). The residual one-third of this background is of a magnitude estimated to be due to exposure to exogenous ethylene at the average level, 15 ppb, determined in the work environment of the participants in the study.

There is thus agreement between the levels of HOEtVal measured in hemoglobin from non-smokers and the levels of this adduct expected from the pharmacokinetic model applied to calculate in vivo doses of ethylene oxide as the primary metabolite of ethylene from endogenous and exogenous sources.

It is thus satisfactory that this first step of treating one and the same problem by two different approaches leads to conclusions in acceptable accordance.

Acknowledgement. We are due thanks to Ms. Anna-Lena Jönsson, Stockholm, for technical assistance. The work was supported financially by the Swedish Environment Protection Agency.

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