Exposure to Ethylene Oxide in Hospitals: Biological Monitoring and Influence of Glutathione *S*-Transferase and Epoxide Hydrolase Polymorphisms

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Abstract

Ethylene oxide is considered as a human carcinogen. A biomarker of exposure would be a useful instrument to assess the risk in occupationally exposed workers. This cross-sectional study aimed at examining (*a*) whether the urinary excretion of a metabolite of ethylene oxide, 2-hydroxyethyl mercapturic acid (HEMA), could be used for monitoring occupational exposure and (*b*) whether glutathione *S*-transferase (GST) and epoxide hydrolase genotypes influenced biological monitoring. Exposure to ethylene oxide was measured by personal sampling in 80 hospital workers (95% of those eligible). HEMA concentrations were determined in three urine samples (baseline, end of shift, and next morning) by liquid chromatography with tandem mass spectrometry. GSTs (*GSTT1*, *GSTM1*, and *GSTP1*) and epoxide hydrolase (*EPHX1*) were also genotyped. The influence of exposure, genotypes, and several other factors was examined in multiple regression analyses. Exposure was always <1 parts per million. On a group basis, exposure and a non-null *GSTT1* genotype increased the HEMA concentrations in the urine sample collected at the end of the shift and these factors remained statistically significant after considering possible confounding or modifying factors. (Cancer Epidemiol Biomarkers Prev 2007;16(4):796–802)

Introduction

Ethylene oxide is used, among others, for sterilizing heat- or moisture-sensitive medical supplies. The use of this gas necessitates strict precautions as it is considered to be a human carcinogen capable of increasing the incidence of leukemia and/or lymphoma (1-5). Besides its carcinogenic properties, ethylene oxide is an irritant (6) and may induce abortion (7).

The uptake of ethylene oxide occurs mainly through inhalation and is critically dependent on the alveolar ventilation rate (8). Whether dermal absorption is significant is unclear (2, 9-11). Ethylene oxide is a directly alkylating agent. It is also metabolized by conjugation to glutathione by glutathione *S*-transferase (GST) T1 resulting in *S*-(2-hydroxyethyl)glutathione and *N*-acetyl-*S*-(2-hydroxyethyl)-L-cysteine [hydroxyethyl mercapturic acid (HEMA)]. Another possible pathway for ethylene oxide detoxification is the formation of ethylene glycol partly by epoxide hydrolase (EPHX1; refs. 2, 12). Thus, carriers of GST or epoxide hydrolase genotypes with high enzyme activity may form more HEMA or ethylene glycol, respectively, and be better protected against ethylene oxide.

To protect workers, the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health recommend threshold limit value time-weighted average exposures for an 8-h work shift of 1 and 0.1 parts per million (ppm) ethylene oxide, respectively (13). However, both alveolar ventilation rate and skin resorption cannot be assessed by air monitoring. Thus, a biological monitoring method, which would take the total uptake into account, is desirable and several approaches have been proposed to this end: determination of blood ethylene glycol (14), hemoglobin adducts (15, 16), and glutathione derivatives, such as thioethers metabolites (17) or HEMA in urine (18). However, for some of these biomarkers, a disadvantage may be the occurrence of genetic polymorphisms of the enzymes metabolizing ethylene oxide, such as GSTT1 and EPHX1, potentially leading to a high interindividual variability in metabolite excretion rate for a similar level of exposure.

Therefore, the first purpose of this study was to determine the value of the urinary HEMA concentration for the biological monitoring of exposure to ethylene oxide and to examine whether GSTT1 or EPHX1 polymorphisms had an influence on this biomarker. An additional purpose was to assess the risk of exposure to ethylene oxide in the participating hospitals.

Materials and Methods

Population. Owing to the scarcity of published results (17-19), power calculations were impossible. An expert opinion based on similar studies suggested that a true difference in metabolite concentrations could be detected by comparing 20 exposed and 20 control subjects. However, as the lack of power was a main threat to the study success, power was increased by doubling the number of participants and using each subject as its own control with respect to HEMA excretion rate. About GSTT1, assuming a prevalence of ~15% for the *GSTT1*-null genotype (20, 21) and a population of 80 subjects, 8 to 16 subjects with the null genotype had to be expected, which would have been enough to make some comparisons between carriers of both *GSTT1* genotypes.

Eligible subjects were all subjects occupationally exposed to ethylene oxide because of their work in sterilization units. Eligible sterilization units were those from hospitals with own surgery department according to the official list of Swiss hospitals. They were approached one by one, beginning with

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the region of Zurich, and asked for participation until enough subjects had entered the study. The small sterilization unit of the veterinary faculty was included as well. One sterilization unit was excluded because of a quite unusual work organization making exposure measurements questionable, another unit closed before the beginning of the study, and there was no sterilization with ethylene oxide in nine further hospitals, leaving seven sterilization units with 84 eligible subjects. The study was conducted between March 2003 and March 2004.

Medical Examination. The study was approved by the ethics committee of the Canton of Zurich and took place in the frame of an obligatory assessment of occupational risks required by the Swiss law. All workers were informed about the purposes of the study and gave written consent. An extensive clinical and occupational history considering previous evaluations (22-25) was taken by an occupational physician according to a check list and written rules. The coding of the answers was reviewed by a second occupational health practitioner and divergences resolved by consensus. Smoking and alcohol consumption were assessed by using questions from the questionnaire of the European Community of Steel and Coal (revision 1967) and according to Rollason et al. (26), respectively. Blood pressure was measured twice and hypertension was defined as a mean blood pressure >140 and/or 90 and/or treatment for hypertension. Three socioeconomic classes were defined by the highest education level attained at age 20 years (no apprenticeship, apprenticeship, and university). Creatinine in serum and urine and serum γ-glutamyl transferase were measured on a Beckman Synchron LX 20 analyzer (Beckman Coulter GmbH, Krefeld, Germany).

Air Measurements. Ethylene oxide was collected separately during the first and the second half of the work shift (~4 h each) by a sampling train consisting of a pump (SKC model 210-2002, SKC, Inc., Eighty Four, PA) and a charcoal sorbent tube coated with hydrobromic acid (SKC 226-38-03) clipped on the collar of each worker. Pumps (flow rate of ~100 mL/min) were calibrated before and after each 4-h time period. Sorbent tubes were transported and stored at 4°C and analyzed within 4 weeks according to National Institute for Occupational Safety and Health method 1614 (27). Pump failures were registered and their effect was considered in the statistical analyses. Concentrations are given in ppm. Whole-shift concentrations are the sum of the mass of both halves of the shift divided by the sum of the corresponding volumes.

Biological Monitoring. The baseline urine sample (urine 1) was collected in a morning before work, after at least 2 days without exposure (either before or after the air measurements). The end of shift urine sample (urine 2) was collected at the end of the work shift (after ~4 h in subjects working half time). On the day following the air measurements, the first urine sample after getting up was discarded and the second morning sample collected before work was used for determination of HEMA (next morning sample; urine 3). Urine samples were kept at 4°C before and during transport and then frozen at −20°C until analysis, which was carried out from February to May 2006. Samples from days with and without exposure were analyzed in the same run, and HEMA was determined without knowing the results of the air measurements. HEMA concentrations were expressed without (microgram per liter) and with correction for the effect of diuresis by dividing the HEMA concentration by the creatinine concentration of the same urine sample (microgram per gram creatinine). The liquid chromatography assay for HEMA determination was developed on a liquid chromatography with tandem mass spectrometry MicroQuattro system from Waters-Micromass Ltd. (Zellik, Belgium) based on a method described by Barr and Ashley (28), with minor modifications. Samples below the limit of detection (1 μg/L) were attributed to a concentration equal to half that limit. Three samples had to be excluded because the limit of detection could not be attained (too low peak of deuterated internal standard).

Genotype Analyses. The determination of genotypes was done on DNA obtained from a blood sample collected during the medical examination (EDTA blood, transport at 4°C, conservation at −20°C after DNA extraction). The *GSTT1* and *GSTM1* genotypes were determined by a previously described multiplex PCR method. Briefly, albumin-specific signal was amplified as an internal control, whereas the GSTT1- and GSTM1-specific signals revealed the presence of these genes, respectively (29). The *GSTP1* genotype ($Ile^{105}Val$) was determined by restriction fragment-length polymorphism analysis, using *Sna*BI restriction enzyme digestion; the presence of the *Sna*BI restriction site identified the GSTP1 Val^{105} allele (30). The *EPHX1* genotypes ($Tyr^{113}His$ and $His^{139}Arg$) were also determined by restriction fragment-length polymorphism analysis as described previously (31). Based on *in vitro* functional expression of variant alleles at respective residues 113 and 139, three predicted EPHX1 enzymatic activity levels were assigned as follows (32): low activity for individuals His^{113}/His^{113}-His^{139}/His^{139}, His^{113}/His^{113}-His^{139}/Arg^{139}, Tyr^{113}/His^{113}-His^{139}/His^{139}, or His^{113}/His^{113}-Arg^{139}/Arg^{139}; medium activity for individuals Tyr^{113}/Tyr^{113}-His^{139}/His^{139}, Tyr^{113}/His^{113}-His^{139}/Arg^{139}, or Tyr^{113}/His^{113}-Arg^{139}/Arg^{139}; and high activity for individuals Tyr^{113}/Tyr^{113}-Arg^{139}/Arg^{139} or Tyr^{113}/Tyr^{113}-His^{139}/Arg^{139}.

Statistical Analyses. The normality of the distributions was tested, and logarithmic transformations or nonparametric tests were used when appropriate. Univariate analyses used paired tests when indicated. Linear multiple regression models were laid down before the beginning of the study and included the following seven main variables: sex (male, 0; female, 1), age (years), exposure (ppm), baseline HEMA concentration (microgram per liter or microgram per gram creatinine) when HEMA in urine 2 was the dependent variable (HEMA in urine 2 was used when urine 3 was the dependent variable), *GSTT1* and *EPHX1* genotypes, and smoking (number of cigarettes per day). Further possible confounding or modifying factors were listed before beginning the study too and added to the model if there was some suspicion of an association in univariate analyses or for pathophysiologic reasons: nationality, socioeconomic level [two dummy variables: no apprenticeship (0/0), apprenticeship (1/0), and university (0/1)], alcohol consumption, diet, hypertension, liver, kidney and skin disease, regular drug intake, menopause, skin contact with ethylene oxide (in minutes, subjective estimate of the worker), urine creatinine concentration of the sample (only when HEMA as dependent variable was expressed in microgram per liter), and *GSTP1* and *GSTM1* genotypes. About genotypes, codes were 0 and 1 for *GSTT1* as well as *GSTM1* null-null and positive-positive (or null-positive), respectively; 0, 1, and 2 for *GSTP1* $Ile^{105}Ile$, $Ile^{105}Val$, and $Val^{105}Val$, respectively; and 0, 1, and 2 for *EPHX1* low, medium, and high, respectively. Unexpectedly, shift work (day work, 0; work between 12 and 22 h, 1) was associated with HEMA concentration in univariate analyses and was, therefore, added to the model as well.

Distribution of residuals and collinearity diagnostics were examined for each model. All calculations were done with SAS statistical software (version 8.2, SAS Institute, Inc., Cary, NC). The calculated two-tailed *P* values are indicated in text but should be interpreted cautiously. Indeed, each subject had six HEMA concentrations (urine 1, 2, and 3, each expressed as microgram per liter and microgram per gram creatinine), four genotype determinations, and mostly three ethylene oxide measurements (first and second half of the shift and whole shift), making chance findings likely.

Table 1. Main characteristics of the study population

	Male workers, n = 26	Female workers, n = 54	All workers, N = 80
Age, median (range), y	45.5 (25-65)	45.5 (21-63)	45.5 (21-65)
Education, n (%)			
No apprenticeship	3 (11)	24 (45)	27 (34)
Apprenticeship	14 (54)	26 (48)	40 (50)
University	9 (35)	4 (7)	13 (16)
Current smoking,* n (%)	12 (46)	18 (33)	30 (38)
Cigarettes/d, median (range)	12.5 (1-20)	14 (3-30)	14 (1-30)
Serum creatinine, median (range), mg/100 mL	0.89 (0.72-1.27)	0.72 (0.55-1.16)	
γGT (IU/L), median (range)	22.5 (11-60)	14.5 (8-47)	
Menopausal status, n (%)		18 (33)	
Diseases, n (%)			
Kidney	0	4 (7)	4 (5)
Liver	2 (8)	4 (7)	6 (8)
Hypertension	12 (46)	9 (17)	21 (26)
Drugs,† n (%)	5 (19)	27 (50)	32 (40)
GSTT1 null-null, n (%)	4 (15)	11 (20)	15 (19)
GSTM1 null-null, n (%)	8 (31)	28 (52)	36 (45)
GSTP1, n (%)			
Code 0	11 (42)	25 (46)	36 (45)
Code 1	11 (42)	21 (39)	32 (40)
Code 2	4 (15)	8 (15)	12 (15)
EPHX1, n (%)			
Low	9 (35)	21 (39)	30 (38)
Medium	9 (35)	21 (39)	30 (38)
High	8 (31)	12 (22)	20 (25)
Shift work,‡ n (%)			
Late shift	14 (54)	29 (54)	43 (54)
Years in current job, median (range)	9.5 (0.5-33)	7.5. (0.5-34)	8 (0.5-34)

NOTE: Because of rounding totals, percentage may not amount exactly to 100%. See text for nationality and alcohol consumption.
Abbreviation: γGT, γ-glutamyl transferase.
*Smoking: all subjects smoked only cigarettes.
†Drugs: regular intake of any drug.
‡Shift work: either day or late shift (no case of night shift).

Results

Seven sterilization units (six in hospitals and one in the veterinary faculty) with 84 workers (2-26 workers per unit) were eligible. All eligible units and 80 workers (95%) participated. Nonparticipation was due to sick leaves (n = 3) and holiday (n = 1). Thus, this population includes nearly all ethylene oxide–exposed workers in hospitals of northeast Switzerland (population of 2,040,700).

Main characteristics are presented in Table 1. Seven workers drank alcohol daily (1-2 and 3-5 ''glasses'' in six and one cases, respectively), 52 drank alcohol only socially, and 21 did not drink any alcohol. Whereas seven subjects reported a skin disease, skin examination was normal in all but four cases (eczema and small superficial skin wounds in two cases each). More than 11 different nationalities were present. Protective respiratory masks were never used, and among the workers with skin contact, only one worker used gloves. The workers reported three minor incidents during the shift, all with skin contact (5-15 min).

Air concentrations were measured during both the first and the second part of the shift in 68 workers (Table 2). Twelve workers had part-time jobs or were exposed only during part of the shift because they had additional tasks. Therefore, exposure could only be assessed during one half of the shift in those workers. Intensity of exposure was never >1 ppm in either shift half. The exposure limit of <0.1 ppm recommended by the National Institute for Occupational Safety and Health was exceeded in 4, 10, and 6 subjects during the first and second half of the shift and the whole shift, respectively. Current cigarette smoking had no significant influence on airborne ethylene oxide concentrations determined by personal sampling (-0.03 < Spearman ρ < -0.27; $0.2 < P < 0.8$).

Urine 1 (baseline) was collected either before or after the day of exposure (n = 19 and 60, respectively). Number of days without exposure before collecting urine 1 was 2 and 3 to 14 in 70 and 9 workers, respectively. No evidence of accumulation of HEMA during the week was found. Indeed, before collecting urine 2, number of days at work since the last day off was 1, 2, 3, 4, and 5, in 7, 2, 42, 15, and 13 workers, respectively. After merging subjects with 1 and 2 workdays, no association between number of days at work and HEMA concentration (either in microgram per liter or in microgram per gram creatinine) in urine 2 or 3 was found.

Urine creatinine concentrations ranged from 0.10 to 3.39, 0.10 to 3.13, and 0.15 to 2.91 g/L in urine 1, 2, and 3, respectively. In each urine sample, correlation between HEMA (μg/L) and creatinine was fairly strong (Spearman ρ = 0.4-0.7).

Table 2. Characteristics of the exposure

Ethylene oxide in air (ppm)	n	Median (range)
First part of shift	79	0.023 (0.00-0.32)
Second part of shift	69	0.033 (0.014-0.59)
Whole shift	68	0.028 (0.009-0.18)
Urinary HEMA (μg/L)	**n**	**Median (range)**
Urine 1, baseline	77	2.1 (0.5-28.0)
Urine 2, end of shift	78	1.95 (0.5-55.0)
Urine 3, next morning	76	1.7 (0.5-23)
Urinary HEMA (μg/g creatinine)	**n**	**Median (range)**
Urine 1, baseline	77	1.77 (0.19-12.39)
Urine 2, end of shift	78	2.54 (0.25-32.54)
Urine 3, next morning	76	1.79 (0.24-19.58)
HEMA concentration below limit of detection (1 μg/L), n (%)		
Urine 1, baseline		26 (34)*
Urine 2, end of shift		29 (37)*
Urine 3, next morning		29 (38)*

*Only 10 (13%) workers had all three samples with HEMA concentrations below limit of detection.

The HEMA concentrations (microgram per gram creatinine) increased significantly from urine 1 to 2 (median, 0.68; range, −6.39-30.63 μg/g creatinine; $P = 0.02$, signed rank test). No such increase was found with the HEMA concentrations expressed in microgram per liter ($P = 0.4$). The distribution of HEMA concentrations was shifted toward higher values after exposure (Table 2). When considering workers exposed to ethylene oxide during a whole 8-h work shift ($n = 66$), airborne ethylene oxide concentrations correlated with HEMA (microgram per gram creatinine) in urine 2 (Spearman $\rho = 0.31$; $P = 0.01$), and when excluding the subjects with pump failure, the correlation was improved ($\rho = 0.39$; $P = 0.007$, $n = 46$). No correlation appeared when HEMA was expressed in microgram per liter ($P = 0.3$). Meaningful statistical analyses restricted to the workers who only worked half a shift could not be done because of the small sample size ($n = 12$).

The influence of possible confounding or modifying factors on the HEMA concentration (microgram per gram creatinine) in the three urine samples is summarized in Table 3. An effect was suggested for *GSTT1* genotype and shift work. The effect of smoking did not appear on the day of exposure. Results were quite similar with HEMA in microgram per liter. HEMA concentrations (both microgram per liter and microgram per gram creatinine) did not differ in a consistent and statistically significant way (5% significance level) according to age (10-year subgroups), menopause, hypertension, regular drug intake, or duration of skin contact with ethylene oxide (subjective estimate). No relevant correlation between body mass index, γ-glutamyl transferase activity, or serum creatinine and HEMA (microgram per liter or microgram per gram creatinine) appeared. The effect of nationality, alcohol consumption, diet, and skin, liver, or kidney disease could not be tested because of too small and/or heterogeneous subgroups.

About HEMA, the main results of the multiple linear regressions are summarized in Table 4. Importantly, the distribution of the residuals was clearly less good with HEMA expressed in microgram per liter than in microgram per gram creatinine. The results were consistent across several models (without HEMA concentration in urine 1; with additional possible confounding factors). Specifically, *GSTM1* and *GSTP1* genotypes had no effect. According to the equation presented in the second column of Table 4, two workers with a non-null or null *GSTT1* genotype exposed to 0.1 ppm ethylene oxide during the whole shift would have a HEMA concentration in urine 2 of 1.30 and 0.96 μg/g creatinine, respectively (assuming a 40-year-old nonsmoking female working day shift, a median HEMA concentration in urine 1 of 1.77 μg/g creatinine, a medium EPHX1 activity, and an intermediate education level). Smoking 20 cigarettes/d would increase both concentrations by 0.2 μg/g creatinine.

The variables associated with the change (increase or decrease) in HEMA concentrations (microgram per gram creatinine) between urine 2 and baseline urine were examined using the same set of independent variables as for HEMA (microgram per gram creatinine; model presented in column 2 of Table 4). The difference in HEMA concentrations was expressed as an absolute change, as a change in percentage of the concentration in baseline urine and on a log scale (change = log concentration in urine 2 minus log concentration in baseline urine). The results were affected by the selection of the units of the dependent variable (absolute or relative increase, log scale; details not shown). However, the influence of exposure to ethylene oxide was always statistically significant ($0.0003 < P < 0.04$). In the whole group, the model with both the highest adjusted R^2 (adjusted $R^2 = 0.42$) and the best distribution of residuals (model 2) showed an association between change in HEMA concentration expressed on a log scale and exposure to ethylene oxide (ppm) during the whole day ($P = 0.03$) and *GSTT1* genotype ($P = 0.009$).

About urine 3, results were fairly consistent across all models (data not shown). The influence of concentration in urine 2 on concentration in urine 3 was obvious. The increase in HEMA concentration due to smoking was mostly at least of borderline significance ($P < 0.10$) and the influence of exposure to ethylene oxide and *GSTT1* was nonsignificant. In the model with HEMA in microgram per liter, the effect of urinary creatinine was again significant.

Table 3. HEMA concentrations (microgram per gram creatinine) in urine samples 1, 2, and 3 according to some possible confounding factors

	HEMA concentration (μg/g creatinine)								
	Urine 1 (baseline)			Urine 2 (end of shift)			Urine 3 (next morning)		
	n	Median (range)	*P**	*n*	Median (range)	*P**	*n*	Median (range)	*P**
Gender									
Male	24	1.05 (0.27-8.07)		26	1.69 (0.25-32.54)		25	1.65 (0.29-12.73)	
Female	53	1.96 (0.19-12.39)	0.08	52	2.81 (0.40-19.85)	0.10	51	1.85 (0.24-19.58)	0.9
Current smoking									
No	48	1.42 (0.29-11.18)		49	2.38 (0.25-32.54)		49	1.28 (0.24-12.73)	
Yes	29	3.33 (0.19-12.39)	0.007	29	2.75 (0.26-19.85)	0.3	27	3.29 (0.33-19.58)	0.003
Shift work									
Day shift	35	1.83 (0.27-11.18)		35	4.10 (0.49-32.54)		34	1.89 (0.24-12.73)	
Late shift	42	1.71 (0.19-12.39)	0.8	43	2.17 (0.25-19.85)	0.02	42	1.69 (0.33-19.58)	0.7
GSTT1									
Null-null	14	2.04 (0.38-6.44)		15	1.44 (0.33-6.77)		14	1.35 (0.24-4.08)	
Null-positive and positive-positive	63	1.74 (0.19-12.39)	0.6	63	2.63 (0.25-32.54)	0.09	62	2.03 (0.35-19.58)	0.04
GSTM1									
Null-null	35	1.77 (0.19-11.07)		35	2.44 (0.33-19.85)		33	1.14 (0.29-19.58)	
Null-positive and positive-positive	42	1.78 (0.27-12.39)	0.6	43	2.63 (0.25-32.54)	0.6	43	2.53 (0.24-12.73)	0.03
GSTP1									
$Ile^{105}Ile$	35	1.96 (0.27-12.39)		35	2.82 (0.25-19.85)		35	2.36 (0.30-19.58)	
$Ile^{105}Val$	31	1.61 (0.19-11.18)		31	2.63 (0.33-32.54)		29	1.49 (0.29-12.73)	
$Val^{105}Val$	11	1.77 (0.40-10.32)	1.0	12	1.41 (0.26-7.74)	0.3	12	1.35 (0.24-11.11)	0.4
EPHX1									
Low	29	1.77 (0.55-12.39)		29	3.57 (0.33-19.85)		29	1.35 (0.29-19.58)	
Medium	28	1.78 (0.19-9.21)		29	2.63 (0.26-32.54)		28	1.76 (0.24-12.73)	
High	20	2.08 (0.29-11.18)	0.8	20	1.71 (0.25-18.33)	0.6	19	1.85 (0.41-11.41)	0.7

*Level of significance of the difference between HEMA concentration according to the respective variable (Wilcoxon two-sample or Kruskal-Wallis test).

Table 4. HEMA (after logarithmic transformation) in urine 2: multiple linear regression models

	Model 1*		Model 2*		Model 3*		Model 4*		Model 5*	
	Dependent variable									
	HEMA (μg/g creatinine)	*P*	HEMA (μg/g creatinine)	*P*	HEMA (μg/g creatinine)	*P*	HEMA (μg/L)	*P*	HEMA (μg/L)	*P*
Sample size	66		64		45		45		64	
Independent variables †										
Gender	0.27	0.03	−0.04	0.8	−0.04	0.8	0.29	0.2	−0.09	0.6
Age (y)	−0.004	0.5	−0.008	0.1	−0.004	0.5	0.007	0.4	−0.003	0.7
Exposure (whole shift; ppm)	2.88	0.06	2.82	0.03	5.85	0.001	6.11	0.003	3.62	0.009
HEMA in urine 1 (log transformed)	NI		0.49	0.0003	0.59	<0.0001	0.38	0.01	0.49	0.0004
GSTT1	0.35	0.02	0.34	0.009	0.22	0.09	0.16	0.3	0.18	0.2
EPHX1	−0.08	0.3	−0.07	0.3	−0.04	0.6	−0.09	0.3	−0.08	0.2
Cigarettes/d (number)	0.01	0.2	0.01	0.1	0.0007	0.9	−0.0006	1.0	0.004	0.7
Shift work	NI		−0.31	0.006	−0.13	0.3	NI		−0.28	0.02
Creatinine in urine 2	NI		NI		NI		0.51	0.0002	0.38	0.0001
Education level										
Intermediate vs low	NI		−0.22	0.08	−0.20	0.2	NI		−0.22	0.09
High vs low	NI		−0.034	0.07	−0.68	0.005	NI		−0.40	0.04
Adjusted R^2	0.15		0.42		0.51		0.50		0.60	
Pr > F	0.01		<0.0001		<0.0001		<0.0001		<0.0001	

NOTE: Dependent variable is always HEMA in urine 2 (either microgram per gram creatinine or microgram per liter) after logarithmic transformation. In every model, HEMA concentrations are consistently expressed with the same units (microgram per liter or microgram per gram creatinine, respectively). Figures indicate partial regression coefficients and corresponding significance levels. Pr > F: statistical significance of the R^2. Sample size is 45 after exclusion of all cases with possible underestimation of exposure because of pump failure.

Abbreviation: NI, variable not included in this run.

*Overall, the effect of exposure is consistent across all models even if additional independent variables are included (comparison between models 1 and 2), the effect of pump failures is considered (comparison between models 2 and 3), or different units are used to express the HEMA concentrations (comparison between models 2 and 5). About *GSTT1*, statistical significance is more model dependent. Urine creatinine concentration is included only when the effect of diuresis is not taken into account (microgram per liter).

†The following codes or units were used: sex (male, 0; female, 1), age (years), exposure (ppm), smoking (number of cigarettes per day), shift work (day shift, no; late shift = 1), creatinine (gram per liter), and socioeconomic level [two dummy variables: no apprenticeship (0/0), apprenticeship (1/0), and university (0/1)]. About genotypes, codes were 0 and 1 for *GSTT1* null-null and positive-positive (or null-positive), respectively, and 0, 1, and 2 for *EPHX1* low, medium, and high, respectively.

To exclude a bias due to the HEMA concentrations below the limit of detection, the multiple regression analyses were repeated in the subset of workers having both worked during the whole shift and HEMA concentrations above the limit of detection. Owing to the smaller population, only few variables could be included in the models and *GSTT1* genotype was not considered because only three to five *GSTT1*-null subjects remained in these calculations. The effect of exposure remained significant for urine 2. For urine 3, the effect of smoking was not detectable any more (Table 5). However, the number of smokers was limited to 16 or less in these analyses.

The associations between exposure during the first or the second half of the shift taken separately and HEMA concentrations in urine 2 and 3 were also examined with the same multiple regression models (data not shown). Exposure had an effect on urine 2 but not urine 3, whereas the reverse was true for smoking. The influence of exposure during the first half of the shift was consistently and clearly statistically significant, whereas it was positive but mostly nonsignificant for the second half of the shift and HEMA concentration in urine 2. The effect of HEMA concentration in the baseline urine on HEMA concentration in urine 2 (or of HEMA in urine 2 on urine 3) was confirmed. The influence of *GSTT1* genotype was less consistent.

None of the nine activities (loading or unloading the sterilizer, preparing medical supplies for sterilization, transport of sterilized material, working in the storage room, changing gas pressure cylinders, maintenance, management, and other tasks), which were looked at, lead to an increased

Table 5. HEMA in urine 2 and 3: multiple linear regression models in the subgroups of workers with HEMA concentrations above the detection limit

	Dependent variable							
	HEMA in urine 2				HEMA in urine 3			
	HEMA (μg/g creatinine)	*P*	HEMA (μg/L)	*P*	HEMA (μg/g creatinine)	*P*	HEMA (μg/L)	*P*
Sample size	31		31		25		25	
Exposure (whole shift)	6.51	0.0005	5.75	0.008	0.63	0.8	2.12	0.2
HEMA in urine 1	0.45	0.06	0.64	0.02	NI		NI	
HEMA in urine 2	NI		NI		0.48	0.04	0.30	0.08
Cigarettes/d (number)	−0.003	0.8	−0.02	0.1	0.006	0.5	0.006	0.5
Adjusted R^2	0.32		0.23		0.20		0.16	
Pr > F	0.004		0.02		0.05		0.09	

NOTE: All subjects had detectable HEMA concentrations in both urine samples used in each regression equation (urine 1 and 2 or urine 2 and 3). Dependent variable is HEMA either in urine 2 (end of shift) or in urine 3 (next morning). HEMA concentrations in every model are consistently expressed with the same units (microgram per liter or microgram per gram creatinine, respectively). With respect to the other variables, the following codes or units were used: exposure (ppm) and smoking (number of cigarettes per day). Figures indicate partial regression coefficients and corresponding significance levels. Pr > F: statistical significance of the R^2.

exposure, with the exception of changing gas pressure cylinders. Specifically, loading and unloading the sterilizer, transporting sterilized material, or working in the storage room were not causes of increased HEMA concentrations in urine samples 1, 2, or 3.

Discussion

In this study, a representative population of workers exposed to ethylene oxide in sterilization units was studied. HEMA was determined by isotope dilution high-performance liquid chromatography-tandem mass spectrometry in three urine samples (baseline, end of shift, and next morning). Airborne ethylene oxide concentrations at the workplace and genotypes were assessed too.

All samples were stored frozen at −20°C until analysis, which had some advantage. Indeed, HEMA was determined in all samples within a short period and within few analytic runs. Thus, shifts due to laboratory apparatus or different batches of reagents, which may occur over long times, were prevented. Furthermore, HEMA in frozen urine samples seems to be stable, as suggested by Barr and Ashley (28). Finally, the median concentrations found in this study were very comparable with those reported by Calafat et al. (19), which does not suggest that conservation over a 2-year period could have been a source of bias.

Median HEMA concentrations in urine 1 (baseline) were 1.42 and 3.33 μg/g creatinine in nonsmokers and smokers, respectively. This agrees well with the median concentrations of 1.1 and 2.9 μg/g creatinine, respectively, found by Calafat et al. (19) in the frame of the National Health and Nutrition Examination Survey by isotope dilution high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry.

Although exposure was always <1 ppm and often <0.1 ppm, a clear association between exposure and urinary HEMA at the end of the shift was found on a group basis. Therefore, it seems that the present method is suitable for monitoring workers exposed to ethylene oxide concentrations <1ppm. This method could also prove useful to document an inadvertent escape of ethylene oxide when air measurements are not available, especially if urine collected after at least 2 days without exposure can be used for comparison.

Attributing a fixed value to samples with HEMA concentrations below the detection limit may be a source of error (33, 34). Therefore, regression equations were carried out again in the subgroup with HEMA concentrations above the limit of detection in both urine samples included in the regression model (1 and 2, and 2 and 3, respectively). The results were not altered. It should also be emphasized that few subjects had HEMA concentrations below the limit of detection in all three samples (10 subjects overall and 9 subjects working full time).

The correlation between exposure (ppm) and urine 2 (end of shift) and the apparent absence of accumulation of HEMA with the number of days at work suggest a half-life of <5 h for HEMA (35). This would be compatible with an estimated half-life of 40 to 50 min for ethylene oxide in blood (8). Whether urine samples collected after 4 h of exposure could also be used could not be examined directly owing to the small group of individuals who worked part time. However, the partial correlation coefficient between exposure during the second half of the shift and HEMA concentration in urine 2 was clearly weaker than between exposure measured during the first half of the shift and HEMA concentration in urine 2. The weaker correlation could not be explained by a higher exposure during the first part of the shift [median (range) 0.023 (0.000-0.320) and 0.033 (0.014-0.587) ppm in the first and second part of the shift, respectively] but could suggest that sampling urine after 4 h of exposure is not optimal. However, this is an indirect argument and alternative explanations are also possible. Indeed, pump failures occurred mainly in the second half of the shift and may have caused some misclassification of exposures.

In this population, a prevalence of *GSTT1*-null genotypes of 18.8% was found as expected (20, 21). According to the calculations based on the regression equation (see above), *GSTT1* genotype had a nonnegligible influence on the HEMA concentration. However, a comparison of this *GSTT1*-induced increase with the background HEMA levels in urine 1 (Table 2) may suggest that *GSTT1* genotype does not have a clinically major influence. Finally, the regression coefficient for *GSTT1* genotype was always positive but did not reach the 0.05 significance level in each model. From a statistical point of view, the effect of *GSTT1* genotype was, therefore, less stable than that of exposure (Table 4). This agrees with the data reported by Yong et al. (15) on the association between *GSTT1* genotype and hemoglobin adducts in a similar population (exposure to <1 ppm; *GSTT1*-null prevalence, 17%). Overall, this suggests that, at least at these exposure levels, genotyping might be useful to refine the interpretation of urinary HEMA concentration but that exposure remains the main determinant of HEMA excretion. The absence of influence of *GSTM1* genotype was also expected (15). *EPHX1* and *GSTP1* genotypes, which do not seem to affect urinary HEMA concentrations in this study, had not yet been examined, to the best of our knowledge, in humans exposed to ethylene oxide.

Urine 3 (next morning) did not correlate significantly with exposure (ppm) on the previous day. This might have several reasons. First, if the half-lives of ethylene oxide and HEMA are short, exposure should not be any longer detectable in the next morning sample. Second, if smokers do not smoke during work but only after or before it, the influence of the smoking habits should increase after work. This would explain why smoking had no influence on HEMA concentration in urine 2 but well in urine 3. Third, it is quite difficult to standardize the collection of the second morning urine (urine 3) so that the number of hours between end of shift and time of collection of urine 3 is the same in every subject even in case of shift work. The resulting imperfect standardization could explain the significant influence of the factor ''shift'' and lead to misclassifications explaining the absence of correlation between exposure and HEMA in urine 3. In any case, collection of urine 3 seems to be more complicated to do without having practical advantages for biological monitoring.

About HEMA excretion, it was not known before this study whether diuresis should be taken into account by dividing the HEMA concentration by the creatinine concentration of the same urine sample. Therefore, statistical analyses were done with both units (microgram per liter and microgram per gram creatinine). Similar results were found. However, the correlations between HEMA (microgram per liter) and creatinine in the same urine sample did suggest that taking into account creatinine concentration should be done.

Assuming that using half the detection limit for nondetectable HEMA concentrations and correcting for creatinine content to disentangle the respective effects of diuresis and exposure results in acceptable approximations, no increase or even a decrease in HEMA concentrations were found in 30 (38%) workers during the work shift. Thus, occupational exposure was not detectable in these subjects. Overall, these results suggest that the risk in these workers, if any, was minimal.

Conclusion. Biological monitoring of exposure to ethylene oxide <1 ppm is possible on a group basis by measuring the HEMA concentration in urine at the end of the shift, preferably in association with information on baseline HEMA excretion and *GSTT1* genotype.

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