APPENDIX A6.1.3.6 ETHYLENE OXIDE - FACTSHEET

	HPHC	BoExp assessed by PMI (matrix)
IUPAC name	Oxirane	2-Hydroxyethyl mercapturic acid (in urine)
Usual abbreviation	EO, EtO	HEMA
Other names	Ethylene oxide, 1,2-epoxyethane, dimethylene oxide, oxacyclopropane	N-Acetyl-S-(2-hydroxyethyl)-L-cysteine, S-(2-hydroxyethyl)-N-acetyl-L-cysteine, (2R)-2-acetamido-3-(2-hydroxyethylsulfanyl)propanoic acid (IUPAC), 2-Hydroxyethylmercapturic acid
CAS number	75-21-8	15060-26-1
Formula and molecular weight	C_2H_4O MW: 44.05	$C_7H_{13}NO_4S$ MW: 207.25

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Main characteristics

Chemical class	Epoxide
HPHC lists	FDA-93 [1], PMI-58
Source in cigarettes	Amount in MSS (3R4F): 9.24 µg/cigarette (ISO); NM (HCI) [2] Smoke phase: gas/vapor phase [3] Presence in tobacco / pyrosynthesis (T°): not present in uncombusted tobacco [4] / It is generally agreed that the main source of ethylene oxide is the decomposition of propylene glycol, which occurs at temperatures of around 500°C [5, 6].
Other sources [7, 8]	Occupational exposure: ethylene oxide is an important raw material used in the manufacture of chemical derivatives that are the basis for major consumer goods in virtually all industrialized countries. Conversion of ethylene oxide to ethylene glycols represents a major use for ethylene oxide in most regions. It can be used as sterilizing agent (e.g., in industries, hospitals), fumigant and insecticide, either alone or in non-explosive mixtures with nitrogen, carbon dioxide or dichlorofluoromethane. Environmental exposure is mainly due to release of occupational ethylene oxide into the atmosphere. Other non-occupational sources of exposure to ethylene oxide (e.g., residues in spices and other food products and in skin-care products) are expected to be minor. Ethylene oxide is formed during the combustion of fossil fuel, but the amount is expected to be negligible. Hospital patients may be exposed during dialysis when the equipment has been sterilized with ethylene oxide. Ethylene oxide occurs endogenously as a metabolite of ethylene in certain plants and micro-organisms. Ethylene oxide can be generated from water-logged soil, manure and sewage sludge, but emissions are expected to be negligible. Ethylene is metabolically converted to ethylene oxide and thus also contributes to the exposure to ethylene oxide Ethylene is ubiquitous in the environment, arising from both natural and man-made sources [9]. Major natural sources are emissions from vegetation of all types, where it functions as a plant hormone. The main anthropogenic sources are from combustion of gas, fuel, coal and biomass. The highest exposure concentrations to humans are due to ethylene from car motors. Ethylene is also produced endogenously [10].

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Toxicity class (FDA) [1]	CA, RT, RDT
Toxicity – CA	IARC: group 1: carcinogenic to humans [8] In epidemiological studies of exposure to ethylene oxide, the most frequently reported association has been with lymphatic and haematopoietic cancers. There is limited evidence in humans for the carcinogenicity of ethylene oxide, but there is sufficient evidence in experimental animals for the carcinogenicity of ethylene oxide. In making the overall evaluation, the IARC Working Group took into consideration the following supporting evidence. Ethylene oxide is a directly acting alkylating agent that: (1) induces a sensitive, persistent dose-related increase in the frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes and micronuclei in bone marrow cells of exposed workers; (2) has been associated with malignancies of the lymphatic and hematopoietic system in both humans and experimental animals; (3) induces a dose related increase in the frequency of hemoglobin adducts in exposed humans and dose related increases in the numbers of adducts in DNA and hemoglobin in exposed rodents; (4) induces gene mutations and heritable translocations in germ cells of exposed rodents; and (5) is a powerful mutagen and clastogen at all phylogenetic levels. There is some evidence of an association between exposure to ethylene oxide and the development of haematological cancers in epidemiological studies of occupationally exposed populations, limitations of the data preclude definitive conclusions. An internal analysis in a study of 7500 women showed a significant exposure-response relationship between ethylene oxide exposure and breast-cancer incidence, with the risk doubled among women with higher cumulative exposures [11]. An epidemiological investigation of ethylene oxide and cancer risk studied more than 18000 employees at 14 industrial facilities where ethylene oxide was used to sterilize medical supplies or food spices, or to test the sterilizing equipment [8]. Mortality from lymphatic and haematopoietic cancer was greatest in the group with the highest category of cumulative exposure to ethylene oxide, but the trend across three categories of cumulative exposure was weak.
Toxicity – RT	Inhalation of ethylene oxide is irritating to mucous membranes including those associated with the respiratory system [12]. Concentrations as low as 200 ppm produce rapid onset of nose and throat irritation. Higher concentrations may cause inflammation of the lining in the trachea and bronchi, narrowing

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	of the bronchi, and partial lung collapse [13].
Toxicity – CT	-
Toxicity – RDT	Data in humans are limited. In an epidemiological study by Hemminki et al. [14], the spontaneous abortion rates in ethylene oxide sterilizer personnel in hospitals in Finland were found to be significantly higher than those of non-exposed workers. Various adverse reproductive effects have also been noted in animal studies, including a decreased number of implantation sites in rats, decreased testicular weights and testicular degeneration [12]. Risk of teratogenicity, and fetal/embryo toxicity is also probable.
Toxicity – AD	-
Toxicity - others	All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [13].

ADME properties

Absorption [12] - Animal studies have shown that ethylene oxide is rapidly absorbed by the respiratory systems of the rat, mouse, and rabbit. In a study of hospital workers, alveolar ethylene oxide concentrations were highly correlated with ambient ethylene oxide concentrations, indicating absorption after inhalation by the lungs. The average alveolar retention of ethylene oxide was approximately 75% of the ethylene oxide inhaled from ambient air.

Distribution [12] - No studies were located regarding the distribution of ethylene oxide in human tissue after inhalation exposure. In mice, 75 minutes after exposure by inhalation, the highest concentrations of ethylene oxide were observed in the lungs, liver and kidneys. Lesser amounts were found in the spleen, brain and testes. In rats administered ^{14}C-ethylene oxide, the highest concentrations of 14 C-activity were found in the urinary bladder, liver, packed blood cells, and adrenal glands, with the lowest concentration found in the fat.

Metabolism [12, 15] - In animals and humans, there are two routes of ethylene oxide catabolism, both of which are considered to be detoxification pathways [16]. The first involves hydrolysis to ethylene glycol (1,2-ethanediol), with subsequent conversion to oxalic acid, formic acid, and carbon dioxide. The second involves conjugation with glutathione, with subsequent metabolic steps yielding S-(2-hydroxyethyl)cysteine [S-(2-carboxymethyl)cysteine] and N-acetylated derivatives (i.e., N-Acetyl-S-(2-hydroxyethyl)-L-cysteine, also called 2-hydroxyethylmercapturic acid or HEMA) [12]. Based upon available data, the route involving conjugation with glutathione appears to predominate in rats and mice; in larger species (rabbits, dogs), the conversion of ethylene oxide is primarily via hydrolysis through ethylene glycol. Ethylene oxide may also be formed from the metabolism of ethylene [17]. Ethylene glycol was identified in the plasma and urine of beagle dogs one hour after intravenous administration of ethylene

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oxide. In this species, ethylene glycol was the major metabolite of ethylene oxide, with 7 to 24% of the administered dose excreted in the urine within 24 hours.

Results of a study on pre-exposed and non-pre-exposed rats indicate that prolonged exposure of rats to ethylene oxide has little effect on the metabolism of the chemical. After chronic exposure of rats to ethylene oxide for three months, hepatic cytochrome P450 and protoheme activity have, however, been shown to decrease by 28% and 19%, respectively. Studies in rats and mice clearly indicate an involvement of glutathione in the lung and liver in the detoxification of ethylene oxide. Decrease of GSH concentrations was also observed in blood, bone marrow and testis.

A physiologically based pharmacokinetic (PBPK) model for the dosimetry of inhaled ethylene oxide was first developed for rats and included binding of ethylene oxide to hemoglobin and DNA in addition to tissue distribution, metabolic pathways (i.e., hydrolysis by epoxide hydrolase and conjugation by glutathione-S-transferase), and depletion of hepatic and extra-hepatic glutathione. The model was then refined and extended to mice and humans [18]. Simulations indicate that in mice, rats, and humans, about 80%, 60%, and 20% of inhaled ethylene oxide, respectively, would be metabolized via glutathione conjugation [18].

Ethylene oxide is an electrophilic agent that alkylates nucleophilic groups in biological macromolecules, including DNA and proteins. In hemoglobin, for example, adducts can be formed at cysteine residues, N-terminal valine, as well as N'-histidine [15]. Since ethylene oxide is formed during the metabolism of ethylene, a natural body constituent, endogenous as well as exogenous sources of ethylene and ethylene oxide contribute to background alkylation of proteins such as hemoglobin and albumin, as well as DNA [15]. N-(2-Hydroxyethyl)valine (HEVal) and hydroxyethylhistidine (HEHis) adducts have been frequently monitored in blood of workers exposed to ethylene oxide in occupational settings [15, 17].

Excretion - Tardif et al. [19] studied the qualitative and quantitative urinary disposition of some metabolites of ethylene oxide in three rodent species: mouse, rat and rabbit. Important differences were observed among the three species in the urinary metabolic disposition of ethylene oxide. The observed differences among the three species in the metabolic disposition of ethylene oxide were found to be qualitatively independent of the route of exposure (i.e., inhalation or intravenous injection). These results suggest that care should be exercised when using any single animal species as a model for human disposition of ethylene oxide. From occupational studies, the correlation between exposure to ethylene oxide and urinary levels of HEMA as well as the apparent absence of accumulation of HEMA with the number of days at work has suggested a half-life of less than 5h for HEMA [20].

Exposure levels in smokers and non-smokers

HEMA concentrations in smoker urine are approximately 3 times higher compared with those in nonsmokers (Table 1). A recent study in which smokers consuming 20 cigarettes per day (cpd) or more were forced to smoke exactly 20, 15, 5 or 0 cpd during a 5-day confinement period showed a small dose-relationship between the number of cigarettes

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and the levels of urinary HEMA, even (i.e. 8.3, 7.8, 4.4 and 4.8 µg/24h, respectively) [21].

Smoking cessation studies of 3 to 8 days showed a 3- to 4-fold decrease of HEMA levels (Table 2).

Determination of blood ethylene glycol, hemoglobin adducts, and glutathione derivatives, such as thioethers or HEMA in urine have been proposed as biomonitoring approaches for exposure to ethylene oxide [20]. 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. For example, studies of smokers exposed to ethylene oxide in cigarette smoke [22] have revealed higher levels of hemoglobin HEVal adducts among individuals with a GSTT1 "null genotype" (i.e., homozygous deletion of GSTT1 gene) than among those with a GSTT1 "positive genotype" (i.e., having at least one copy of the GSTT1 gene). HEVal adducts have been evaluated to be in a range of 132 pmol/g globin in smokers and 21.1 pmol/g in non-smokers [23]. High variability is seen between subjects, due to the metabolic conversion of exogenous and endogenous ethylene to ethylene oxide, and to genetic polymorphisms.

Urinary HEMA for the biological monitoring of exposure to ethylene oxide and the potential influence of GSTT1 or EPHX1 polymorphisms was studied by Haufroid et al. [20]. Although the GSTT1 genotype was found to have a non-negligible influence on the urinary HEMA concentration, it did not appear to have a major clinical influence. HEMA is not a metabolite specific of ethylene oxide, as a major pathway of acrylonitrile metabolism comprises the formation of 2-cyanoethylene oxide via epoxidation, which is followed by a variety of reactions leading to a spectrum of urinary metabolites, including HEMA [24]. Also, a substantial part of the smoking-related increase in urinary HEMA levels might come, besides from acrylonitirile, from ethylene, the amount of which in mainstream smoke is about 100 times higher than that of ethylene oxide (levels of 0.2-0.3 mg per cigarette vs ca. 10 µg/cigarette of ethylene oxide) [2, 25]. Because ethylene, ethylene oxide and acrylonitrile are all found in cigarette smoke, HEMA remains a useful BoExp, but the contribution of the three compounds will influence the levels of HEMA measured in smokers. A study by Wu et al. found an insignificant correlation between HEMA concentration and cotinine concentration, but they had only two smokers in their 36 subjects [26]. In a study on healthy Chinese subject, HEMA was detectable in the urine of 33% of the non-smoker samples and 20% of the smoker samples [27].

HEMA

In non-smokers	Ranges: 6.51-38.8 nmol/24h [1349.20-8041.30 ng/24h] [23]
In smokers	Ranges: 19.1-102 nmol/24h [3958.48-21139.50 ng/24h] [23]

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Table 1 Representative urinary HEMA levels in smokers and non-smokers

Author (year)	Levels in non-smokers (or ex-smokers)	Levels in smokers	Units; variability
Calafat (1999) [28]	1.1 [1100]	2.8 [2800]	µg/g creat [pg/mg creat]; geometric mean
Schettgen (2008) [29]	1.7 (0.7-4.2) [1700 (700-4200)]	4.0 (1.3-6.0) [4000 (1300-6000)]	µg/g creat [pg/mg creat]; median (ranges)
Ding (2009) [30]	ND-1.05 [1050]	ND-16.0 [16000]	µg/g creat [pg/mg creat]; median ranges
Eckert (2011) [31]	1.6 (0.6–8.1) [1600 (600-8100)]	4.9 (1.1–67.7) [4900 (1100-67700)]	µg/g creat [pg/mg creat]; median ranges
Hou (2012) [27]	4.4 (<LOQ–60.6) [912 (<LOQ-12559)]	15.5 (3.98–944) [3,212 (825-195644)]	nmol/24h; mean (ranges) [converted into ng/24h]
Xiaotao (2014) [24]	0.72 (0-10.0)	1.18 (0.00-11.20) 8mg tar	ng/ml; mean (ranges)
Xiaotao (2014) [24]	0.72 (0-10.0)	2.54 (0.00-18.8) 10mg tar	ng/ml; mean (ranges)
Xiaotao (2014) [24]	0.72 (0-10.0)	1.24 (0–7.28) 13mg tar	ng/ml; mean (ranges)
Pluym (2015) [32]	1.1 (0.11-38.3)	2.0 (1.1-4.2)	µg/g creat; median (min-max)
Alwis (2015) [33]	0.66 ± 1.16	1.90 ± 3.70	ng/mL; mean ± SD

Table 2 Representative urinary HEMA levels after smoking cessation

Author (year)	Levels in quitters	Levels in smokers	Units; variability
Carmella (2009 and 2012) [34, 35]	12 ± 16.8 [a] [2487 ± 3482]	51 ± 47.1 [10570 ± 9761]	nmol/24h; mean ± SD [ng/24h]
Scherer (2010) [36]	6.79 ± 3.05 [b] [6790 ± 3050]	25.8 ± 8.8 [25800 ± 8800]	µg/24 h [ng/24h]; mean ± SD
Theophilus (2015) [21]	4.8 ± 2.2 [c] [4800 ± 2200]	14.3 ± 9.6 [14300 ± 9600]	µg/24 h [ng/24h]; mean ± SD

[a] Smoking cessation for 3 days

[b] Smoking cessation for 8 days

[c] Smoking cessation for 5 days

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [37]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion

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CA	Carcinogen
CAS number	Chemical Abstracts Service number
Creat	Creatinine
CT	Cardiovascular toxicant
EPHX1	Epoxide Hydrolase 1
FDA	Food and Drug Administration
GSH	Glutathione
GSTT1	Glutathione S-transferase theta-1
HC	Health Canada
HCI	Health Canada Intense regime [38]
HEHis	Hydroxyethylhistidine
HEMA	2-hydroxyethylmercapturic acid
HEVal	N-(2-Hydroxyethyl)valine
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [39]
IUPAC	International Union of Pure and Applied Chemistry
LOQ	Limit of quantitation
MSS	Main stream smoke
MW	Molecular weight
ND	Not detectable
PBPK	Physiologically based Pharmacokinetic
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

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