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Review

Genotoxic effects of ethylene oxide, propylene oxide and epichlorohydrin in humans: update review (1990–2001)

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Abstract

Ethylene oxide (EtO), propylene oxide (PO) and epichlorohydrin (ECH) are important industrial chemicals widely used as intermediates for various synthetic products. EtO and PO are also environmental pollutants. In this review we summarize data published during the period 1990–2001 concerning both the genotoxic and carcinogenic effects of these epoxides in humans. The use of DNA and hemoglobin adducts as biomarkers of exposure and the role of polymorphism, as well as confounding factors, are discussed. We have also included recent in vitro data comprising genotoxic effects induced by EtO, PO and ECH in mammalian cells. The uncertainties regarding cancer risk estimation still persist, in spite of the large database collected.

Keywords: Ethylene oxide; Propylene oxide; Epichlorohydrin; Cytogenetic effects; Carcinogenic effects; DNA and hemoglobin adducts; Epidemiological studies

1. Introduction

The genotoxic effects of three important aliphatic epoxides, ethylene oxide (EtO), propylene oxide (PO) and the halogenated epoxide epichlorohydrin (ECH), are described. These compounds are widely used in chemical industries as intermediates for the synthesis of such products as ethylene glycol (from EtO), propylene glycol (from PO), polyurethane foam, epoxy resins, synthetic glycerin and surfactants. EtO and PO are also used as fumigants for pharmaceutical and agricultural products. Moreover, EtO is widely used for sterilization of heat-sensitive disposable medical supplies and materials. According to the International Agency for Research on Cancer (IARC), the total world production of EtO and PO is estimated as about 5.5 and 4.0 million tonnes per year, respectively [1]. ECH is produced in many countries, but its total production is unknown. To provide an example,

Abbreviations: CA, chromosomal aberration; CHP, 3-chloro-2-hydroxypropyl; CI, confidence interval; DSB, DNA double-strand breaks; ECH, epichlorohydrin; EtO, ethylene oxide; HA, 2-hydroxyalkyl; HE, 2-hydroxyethyl; HFC, high frequency cells; HP, 2-hydroxypropyl or 2-hydroxypropano; HPRT, hypoxanthine phosphoribosyl transferase; IARC, International Agency for Research on Cancer; MN, micronuclei; NIOSH, National Institute for Occupational Safety and Health; OR, odds ratio; OSHA, Occupational Safety and Health Administration; PFGE, pulsed-field gel electrophoresis; PO, propylene oxide; SCE, sister chromatid exchanges; SMR, standardized mortality ratio; SSB, DNA single-strand breaks; 6-TG, 6-thioguanine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TWA, time-weighted-average; US EPA, US Environmental Protection Agency

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213 thousand tonnes of ECH were produced in the USA only in 1978 [2].

Occupational exposure mainly occurs in workplaces dealing with the production of the epoxides themselves, and also during the manufacturing of epoxide-based chemicals. At present, due to technical development and modernization of industrial processes, a majority of operations in chemical plants are performed in closed systems; however, exposure is still inevitable during some procedures. In the case of EtO, the additional sources of occupational exposure are sterilization plants and sterilization facilities in hospitals. According to the National Institute for Occupational Safety and Health (NIOSH), about 250,000 workers in the USA were annually exposed to EtO [3]. NIOSH also reported that 421,000 workers were potentially exposed to PO (between 1981 and 1983), although only 2% of them were estimated to be exposed directly in the workplace; other people were exposed to materials containing PO [1]. The extent of occupational exposure to EtO and PO in Europe and Asia is not available, but the amounts of the epoxides produced in these parts of the world indicate that many hundreds of thousands of individuals might be exposed. Exposure to ECH is also extensive; about 25,000 employees in European countries and 80,000 in the USA were annually exposed (data from 1990–1993 and 1981–1983, respectively) [2].

Moreover, unlike PO and ECH, which are not natural products, EtO is produced metabolically in the body from ethylene [4–6]. Among the endogenous sources of ethylene, metabolic processes in intestinal microorganisms, lipid peroxidation and oxidation of methionine can be mentioned [7,8]. Ethylene is also produced by soil microorganisms, and by combustion of organic compounds. Additionally, ethylene is a component of cigarette smoke [1]. Practically everyone is, therefore, exposed to some extent to ethylene and EtO during their lifetime.

The harmful effects of EtO to human health were brought into focus after publishing of alarming data demonstrating increased frequencies of chromosomal aberrations (CA) in peripheral blood lymphocytes of workers occupationally exposed to high concentrations of EtO [9]. The first comprehensive review dealing with the genotoxic effects of epoxides in living organisms and with quantitative aspects of risk assessment was published 20 years ago by Ehrenberg and Hussain [10]. During the past two decades, several reviews devoted to the carcinogenic and mutagenic effects of EtO and some of its derivatives have been published [1,2,11–21].

In this review we summarize and discuss data published predominantly during 1990–2001 about carcinogenic, cytotoxic and genotoxic effects of EtO, PO and ECH in humans. Data concerning DNA and hemoglobin adduct formation in humans are also included, as well as recent data about genotoxic effects of these epoxides in cultured mammalian cells in vitro.

2. Carcinogenic effects in humans

The carcinogenicity of EtO was reported for the first time in three Swedish cohort studies ([22–24], updated and summarized in [25]). A significant increase in cancer mortality as well as an excess of stomach cancer and certain types of leukemia (especially chronic lymphatic leukemia and acute myeloid leukemia) were demonstrated. These findings stimulated numerous epidemiological investigations of occupationally exposed people. In the end of the 1980s and beginning of the 1990s, several large-scale cohort studies were initiated to verify the previous observations (Table 1). Only one [26] out of nine large cohort studies quoted here [26–35] showed a statistically significant increase of cancer mortality. The authors also reported a significant increase of mortality due to lymphosarcoma and reticulosarcoma [26]. However, two-thirds of the cohort members (1334 of 1971 subjects) had potential exposure to other toxic substances (27 chemicals are listed in the paper). Moreover, a documentation of the exposure levels of EtO and of other chemicals is missing. In two studies [31,32], an increased risk to non-Hodgkin's lymphoma among men was observed.

Shore et al. [15] critically reviewed 10 epidemiological studies published between the years 1979 and 1993 [22,23,26–29,31–33,36]. The overall standardized mortality ratios (SMR) were evaluated with the help of meta-analysis of the combined data, with respect to exposure intensity or frequency, duration of exposure and to latency (time past from the first exposure). There was a slight indication that duration of exposure and longer latency can increase the risk of leukemia (non-significant data). At the same time,

Table 1
Occupational exposure of humans to ethylene oxide, propylene oxide or epichlorohydrin (data collected during period 1990–2001)

Population/character of exposure	Concentration of epoxide in air	Observed effect	Reference
Ethylene oxide			
Workers ($n = 2658$; men) from eight chemical plants; at least 1 year of exposure between 1928 and 1981 (FRG)	No data available	No significant increase in total and cancer mortality compared to the local state rates	[27]
Workers ($n = 2174$; men) from a chemical company producing EtO, employed between 1940 and 1978 (USA)	1–5 ppm[a] (8 h TWA)	No significant increase in mortality due to all causes and for total cancers compared to USA death rates	[28]
Workers ($n = 2170$) from two plants producing disposable medical equipment, employed between 1972 and 1990; at least 1 year of employment (Sweden)	>0.2 ppm (8 h TWA), occasionally up to 75 ppm	No significant correlation between EtO exposure and cancer morbidity; non-significantly increased risk of leukemia	[29,30]
Workers ($n = 18{,}254$) employed in 14 plants producing medical supplies and spices; average 4.9 years of exposure between 1976 and 1985 (USA)	Average 4.3 ppm for sterilizer operators and 2 ppm for other workers (8 h TWA)	Overall, no significant increase in mortality compared with the rate in the general population; increased rate of death due to non-Hodgkin's lymphoma and leukemia among men	[31]
Workers ($n = 18{,}728$; 8709 men and 10,019 women); the same population that Steenland et al. [31] studied; the follow-up was for 1 year past the closing date of Steenland et al.'s study; at least 90 days of exposure (USA)	20% of the workers (sterilizer operators): 4–16 ppm; other workers: 2–5 ppm	No significant excess of any type of cancer. An excess of non-Hodgkin's lymphoma among men, but not among women	[32]
Workers ($n = 1896$; men) employed in two chemical plants producing EtO; average duration of exposure was over 5 years; follow-up from 1940 to 1988. Update of the study by Greenberg et al. [28] (USA)	After 1976, <1 ppm (8 h TWA); in the 1960s, 3–20 ppm (8 h TWA)	No significant increase of cancer mortality	[33]
Workers ($n = 1971$) in chemical industry; 44 years of follow-up, 1940–1984 (Italy)	No exposure data	Increased mortality from hematopoietic tissue cancers (lymphosarcoma, reticulosarcoma)	[26]
Workers ($n = 1361$; men) employed in production of EtO and PO through ethylene and propylene chlorohydrin process; minimum of 30 days of workplace experience in 1940–1992 (USA)	No exposure data	No significant increase of pancreatic cancer or lymphopoietic and hematopoietic cancer	[34]

Table 1 (*Continued*)

Population/character of exposure	Concentration of epoxide in air	Observed effect	Reference
Workers ($n = 1132$; 82% women) employed between 1974 and 1980 at different plants; follow-up to 1987 (USA)	No exposure data	No significant increase in breast cancer and other cancer forms compared to age- and sex-specific incidence rates	[35]
Sterilization workers ($n = 34$), about 8 years of employment; control ($n = 23$) (USA)	<0.3–1 ppm (8 h TWA)	Increase of SCE frequency; increased level of EtO–hemoglobin adducts; suppression of DNA repair capacity; the levels of MN, CA and SSB were not significantly elevated	[50]
Hospital workers involved in sterilization of medical equipment, group EI ($n = 9$) and control group ($n = 8$). Workers from a plant involved in the production of EtO-sterilized medical equipment, group EII ($n = 15$) and control group ($n = 15$) (The Netherlands)	Group EI: 0.025 ppm (40 h TWA); group EII: 5 ppm (40 h TWA); average exposure levels during 4 months were estimated from hemoglobin adduct levels[b]	A significant increase (60%) in the *HPRT* mutant frequency in lymphocytes in group EII, but not in EI. A significant increase in CA in EI and EII (130 and 260%, respectively); MN significantly enhanced only in EII (217%). SCE significantly elevated in EI and EII (20 and 100%, respectively). Increased percentage of cells with high frequency of SCE (3–4 times in EI and 17 times in EII)	[51]
Hospital workers ($n = 73$), US group I ($n = 32$); US group II ($n = 11$); US control ($n = 8$); Mexican group I ($n = 9$); Mexican group II ($n = 13$); Mexican control ($n = 1$) (USA)	Cumulative exposure during 4 months. US group I: >0–32 ppmh; US group II: >32 ppmh; Mexican group I: >0–32 ppmh); Mexican group II >32 ppmh	Increase of hemoglobin adducts and SCE frequency; no effect on MN frequency. In the Mexican groups: increase of hemoglobin adducts, but data on SCE are inconclusive	[52]
Hospital workers ($n = 10$) engaged in sterilization of medical instruments; control ($n = 10$) (Italy)	60–69 ppm (8 h TWA)	A significant increase in SCE and CA frequencies	[53]
Hospital nurses: group I ($n = 9$), and group II ($n = 27$); control ($n = 24$) (Hungary)	Group I: 5–20 mg/m^3; group II: 5–100 mg/m^3	Slightly elevated and significantly increased levels of SCE in groups I and II; significantly increased CA frequency in both groups	[54]
Hospital sterilization workers ($n = 25$); standardized control group ($n = 25$)	Peak concentrations up to 417 ppm	SCE frequency lower than in the standardized control group but higher than in a historic control	[55]
Sterilizer operators and supervisors ($n = 94$); matching control group (Canada)	0.1 ppm during several years (accidental levels of up to 1000 ppm were recorded 5–6 years prior to blood sampling)	No increase in SCE and mutation frequency in the *HPRT* locus	[56]
Industrial workers from a chemical manufacturing plant: groups I–III ($n = 7$ each group); group IV—control ($n = 7$) (The Netherlands)	Group I: incidentally acute high doses; groups II and III: chronic exposure to low levels in the range <0.01–0.06 mg/m^3 for <5 and >15 years, respectively	Increased hemoglobin adduct levels in groups I–III. No significant differences between groups I–IV concerning *HPRT* mutant frequency, MN and SCE	[57]
Sterilization workers ($n = 97$) (Germany)	Mean concentration 1.47 ± 0.52 mg/m^3; maximal concentration 16.5 mg/m^3 (4 h TWA)	In non-smokers exposed to 0.5–2 mg/m^3, a significantly higher mean DNA elution rate (119%) than in workers exposed below 0.1 mg/m^3 in peripheral mononuclear blood cells; similar tendency but not significant found in smokers	[58]

Sterilization workers ($n = 93$); controls ($n = 275$) (Germany)	Mean concentration ≤ 0.1 or >0.1 mg/m^3 (4 h TWA)	In non-smokers exposed to >0.1 mg/m^3, a significant increase of SSB in mononuclear blood cells compared to workers exposed below 0.1 mg/m^3; similar tendency, but not significant, found in smokers	[59]
Sterilization workers ($n = 6$) and assemblers ($n = 12$) (Sweden)	2 ppm (8 h TWA; for the sterilization workers)	Significant increase of CA and MN frequencies in lymphocytes compared to PO-exposed workers	[60]
Industry workers ($n = 75$); control ($n = 22$); 3-month sampling; confounding exposure to other chemicals (Brazil)	2–5 ppm (8 h TWA)	Significant increase of CA (with or without gaps) and MN frequencies in binucleated lymphocytes; significant increase of hemoglobin adducts	[61]
Workers ($n = 9$ [62]; $n = 10$ [63]) in a sterilization unit; three persons were acutely exposed (accidental EtO leakage); mean length of exposure 5 ± 4 years; control ($n = 27$ [62]; $n = 10$ [63]) (Italy)	0.025–0.38 ppm (6.5 h TWA). No data concerning the acutely exposed persons	A significant increase ($P < 0.01$) of MN frequency in nasal mucosa in two out of the three acutely exposed subjects [62]; a non-significant increase of MN frequency at chronic exposure, and no effect in lymphocytes and buccal mucosa [62,63]	[62,63]
Hospital nurses ($n = 10$) engaged in sterilization of medical instruments; control ($n = 10$) (Hungary)	No exposure data	Significant increase of H-*ras* and *p53* expression in white blood cells	[68]
Hospital nurses ($n = 20$) engaged in sterilization of medical instruments; two control groups (each $n = 20$) (Hungary)	No exposure data	Elevated N-*ras* and detectable *p53* expression in white blood cells	[69]
Propylene oxide			
Workers in a plant producing alkylated starch ($n = 20$; men); 1–20 years of exposure (Sweden)	0.33–11.4 ppm	CA and MN frequencies in lymphocytes were significantly lower than those in EtO-exposed individuals from the same study	[60]
Workers at a PO-producing plant ($n = 8$); control ($n = 8$) (Peoples Republic of China)	1–7 ppm	A significant increase in SCE frequency	[64]
Epichlorohydrin			
Enterline cohort: workers at two chemical plants ($n = 863$); employment during 1948–1965; follow-up to 1983 (USA)	Levels of exposure were classified as "heavy, moderate, light, nil, or unknown"	No significant increase of total cancer mortality; statistically significant increase of SMR for leukaemia and heart diseases at moderate to heavy exposure levels	[39]
Workers from the Enterline cohort ($n = 280$) still employed from 1981 to 1988	Exposure levels as stated above for the Enterline cohort	Enterline cohort: no increase in heart disease morbidity or mortality; no morbidity events for cancer of the respiratory system or leukemia	[40]

Table 1 (*Continued*)

Population/character of exposure	Concentration of epoxide in air	Observed effect	Reference
Shell cohort: workers ($n = 713$) from the same chemical plants as in the Enterline cohort; employment during 1981–1988 (USA)		Shell cohort: no increase in heart disease morbidity; a significant increase of morbidity from skin and subcutaneous tissue disorders	
Enterline cohort: a 10-year extension of follow-up (up to 1993) (USA)	Exposure levels as stated above for Enterline cohort	No excess deaths from heart disease, lung cancer, or non-malignant respiratory disease for employees with 20 or more years after first exposure	[41]
Workers ($n = 1064$; men) employed in the production of ECH and ECH-based chemicals during 1957–1986; follow-up to1989 (USA)	1–5 ppm (8 h TWA)	No significantly elevated SMR for any malignant neoplasms and heart diseases	[42]
Delzell cohort: workers ($n = 2642$; men) employed at a dye and resin manufacturing plant where ECH was also produced; employment at least for 6 months; follow-up study 1952–1985 (USA)	No exposure data	The mortality rates lower than the rate in the general USA population. A significantly elevated mortality from lung cancer	[45]
A case-control study of persons from the "Delzell cohort" (see earlier): lung cancer cases ($n = 51$) and controls ($n = 102$) (USA)	Ten categories of potential chemical contact, from 0 (no potential contact) to 10 (maximum potential contact)	A significant increase of OR for lung cancer (OR = 2.4; 95% CI 1.1–5.2) in dye and ECH production workers; no significant trend with duration of employment	[43]
A case-control study of persons from the "Delzell cohort" (see earlier): CNS neoplasms ($n = 11$) and controls ($n = 44$) (USA)	Ten categories of potential contact (see above)	A significant increase of OR for lung cancer (OR = 2.4; 95% CI 0.7–26) compared with unexposed subjects. Association with duration of exposure	[44]
Factory workers ($n = 14$), control ($n = 14$)—office clerks (FRG and Sweden)	0.11–2.6 ppm, 12 h per day, 4 days per week	Increase in SCE frequency in lymphocytes	[65]
Workers from a resin synthesis plant ($n = 85$) (Taiwan)	0.1–2 ppm	Significantly increased SCE frequency at higher ($\geq$1 ppm) ECH concentration	[66]

[a] Parts per million; 1 ppm =1.83 mg/m^3 for EtO, 2.38 mg/m^3 for PO and 3.8 mg/m^3 for ECH.

[b] The authors used the value 2.4 nmol/g globin for the accumulated adduct level from exposure to 1 ppm EtO during work hours (see Tables 3 and 4).

the meta-analysis indicated an increase in the overall SMR for stomach cancer (SMR = 1.28, 95% confidence interval (CI) 0.98–1.65). There was no increase in the overall SMR for pancreatic cancer, nervous system cancer, or total cancer (SMR = 0.94) [15].

Recently, Teta et al. [20] have presented an updated meta-analysis of findings from 10 EtO study cohorts, including nearly 33,000 workers [23–31,33,34,36–38]. No increased risk of cancer of the brain, stomach, or pancreas was detected by this analysis, and the cumulative findings on leukemia and non-Hodgkin's lymphomas were considered inconclusive. The authors suggested, based on both animal and human studies, that cancers of the lymphopoietic tissues warrant additional epidemiological follow-up.

Epidemiological studies on carcinogenic effects of PO in humans have not been published as yet. Concerning ECH, data on carcinogenic effects in humans are less comprehensive than for EtO. Four large cohort studies were carried out during the 1990s (Table 1). Three of these cohorts included workers from the same "Enterline cohort" [39–41]. In the study by Enterline et al. [39], a significant increase of mortality due to leukemia (SMR = 5, $P < 0.05$) was demonstrated. Also, an increased mortality connected with heart diseases 20 or more years after the first exposure (SMR = 3.92, $P < 0.05$) was reported. However, in follow-up studies of the group of workers from this cohort [40,41], neither an increased leukemia incidence nor increased morbidity and mortality from heart diseases were confirmed (estimated SMR values were lower than 1). No increase in cancer mortality was found in workers employed in the production of ECH and ECH-based chemicals, such as epoxy resin, glycerin and allyl chloride [42]. In two case-control studies [43,44] of workers from the "Delzell cohort" [45] employed in anthraquinone dye and ECH manufacturing, an elevation of the odds ratios (OR) for incidence of lung cancer and central nervous system neoplasm were observed. Unfortunately, exposure levels were not determined; exposure estimation was done only on the basis of interviews with the employees. It is also possible that workers were exposed not solely to ECH, but also to other chemicals used in the chemical manufacturing facilities.

Studies on carcinogenicity of EtO in experimental animals are outside the scope of this review and are reported elsewhere ([46,47], reviewed in [1,11,12]). Chronic bioassays in rodents have served as a basis for cancer risk assessment. Among tumors induced in rats, mononuclear cell leukemia, peritoneal mesotelioma and glioma (brain tumor) were observed [47]. In the case of PO, tumors in the forestomach were induced after oral administration [46] and nasal tumors upon inhalation [47]. In similarity with EtO and PO, ECH is tumorigenic in rodents [48]. Forestomach hyperplasia, papilloma and carcinoma were reported in ECH-treated rats [49].

3. Cytogenetic effects in humans

An overview of recent cytological investigations in occupationally exposed humans is presented in Table 1. Earlier data concerning EtO are reviewed by Dellarco et al. [12]. Induction of sister chromatid exchanges (SCE), CA and micronuclei (MN) was the main focus. In five out of eight cytological studies [50–54] included in Table 1, SCE frequency was significantly elevated in the lymphocytes of EtO-exposed persons. Two of the studies, where exposure levels were high, showed a dose–response relationship for the SCE frequency [51,53]. The investigation by Popp et al. [55] of hospital workers exposed to high EtO levels showed a non-significant increase of the SCE frequency when the exposed group was compared to a historic control, but a decrease when compared to a standardized control group. In the studies by Tomkins et al. [56] and Tates et al. [57], there were no differences between exposed workers and controls. Quantitative evaluations of the data are hampered by uncertainties in exposure records and individual differences in metabolism, as well as by uncertainties concerning the persistence of the DNA damage that may result in SCE [19] and to individual differences in metabolism.

Fuchs et al. [58] studied DNA single-strand break (SSB) induction in peripheral blood cells of workers exposed to EtO by using the alkaline elution method. A time-weighted-average (TWA) of the concentration of EtO in air for the last 4 h prior to blood sampling was calculated for each worker. The authors showed that the elution rate of DNA (a measure of the level of SSB) from EtO-exposed workers increased with increasing exposure levels. Similar results were reported by Oesch et al. [59]. These results are seemingly at odds with the result presented by Popp et al.

[55] where a decreased elution rate was observed in EtO-exposed disinfectors. This was interpreted as an increase in DNA cross-links, especially with proteins. Thus, SSB and cross-links, with opposite influence on the DNA elution rate, may both exist [55].

Increased CA levels after EtO exposure were reported in several studies [53,54,60,61]. Lerda and Rizzi [53] found that increased levels of CA (chromatid and chromosome breaks, acentric fragments and chromatid fragments) remained in lymphocytes 3 months after EtO exposure.

Elevated levels of MN in lymphocytes of workers exposed to EtO were reported by Högstedt et al. [60]. Sarto et al. found a significant increase of MN in nasal mucosa [62], but not in lymphocytes and in buccal exfoliated cells [62,63]. The reason for the increased MN frequency in lymphocytes in the first study [60] may be higher EtO exposure levels than in the latter studies (see Table 1).

Högstedt et al. [60] investigated three groups of workers from two different factories: (a) EtO-exposed sterilizers from the factory producing medical equipment; (b) workers exposed to PO in the factory manufacturing alkylating starch; and (c) a control group of workers assembling electronic equipment. However, analysis of hemoglobin adducts showed that also the assemblers were exposed to EtO. Namely, the assemblers worked in the same factory, only 100 m from the sterilizers. Therefore, this study is lacking control data from unexposed persons. The frequencies of CA (gaps and breaks) and MN were somewhat higher in the EtO-exposed group, assemblers excluded, compared to the PO-exposed group (mean values of groups, total numbers of CA per 100 metaphases were 5.8 and 4.7, respectively). The MN frequencies in EtO-exposed workers were about two-fold higher compared with the PO-exposed group (mean values of groups, 5.4 against 2.6, respectively, per 100 metaphases).

Recently, new data indicating a capability of PO to induce SCE in humans have been published. Czene et al. [64] have demonstrated a significantly increased SCE frequency ($P = 0.011$) in workers exposed to this epoxide (PO levels in the range of 1–7 ppm) at a PO-producing plant.

SCE and high frequency cells (HFC; cells with a number of SCE which is higher than the 95% upper confidence limit of the SCE distribution for the pooled control data) were studied in T-lymphocytes of ECH-exposed workers [65]. The mean frequencies of SCE per cell were 7.0 and 5.6 for exposed and unexposed persons, respectively. The corresponding numbers for HFC per 50 cells were 10.2 and 0.6 (a highly significant difference). The same authors studied the effect of ECH on MN, but the difference between exposed and unexposed persons was not statistically significant. Cheng et al. [66] found a significantly increased SCE frequency in workers exposed to high concentrations of ECH (up to 4 ppm), compared to those with low or no ECH exposure ($P < 0.05$).

The knowledge about the mutagenicity of the epoxides in humans is limited. The hypoxanthine phosphoribosyl transferase (*HPRT*) gene [67] was used for studies of the mutagenic effect of EtO. In a study by Tates et al. [51], a significant increase (over 60%) of the *HPRT* mutant frequency, compared to the control group, was found at relatively high exposure levels (5 ppm, 40 h TWA). In two later studies [56,57], where the chronic exposure levels were considerably lower (see Table 1), no association between 6-thioguanine (6-TG)-resistant mutant frequency and exposure was found.

Interesting observations were made by a team of Hungarian scientists [68,69] who detected higher expression of the N-*ras* oncogene and the *p53* tumor suppressor gene in lymphocytes of EtO-exposed hospital nurses than in non-exposed hospital controls. Unfortunately, exposure data are lacking in these studies.

4. Reactions with DNA

EtO, PO and ECH are direct-acting alkylating agents that can react with nucleophilic sites in cellular macromolecules [10]. In contrast to EtO and PO, which are monofunctional alkylating agents, ECH is bifunctional, and is thus able to form a cross-link between two nucleophilic sites [70].

Our knowledge about the reactions of epoxides with DNA is mainly based on in vitro studies. The epoxides react with DNA predominantly at ring nitrogen atoms, leading to formation of 2-hydroxyalkyl (HA) adducts. Reaction with the *N*7-position of guanine is the most prevalent event due to the nucleophilicity and steric availability of the position. However, many other sites in DNA may be alkylated, mainly the *N*1-

Table 2
Amounts of DNA adducts formed by the reaction of the epoxide with double-stranded DNA in vitro at neutral pH and 37 °C[a]

	Ethylene oxide		Propylene oxide		Epichlorohydrin	
*N*7-HA-Gua	1 [78]	1 [79]	1 [73]	1 [74]	1 [70]	1 [77]
O^6-HA-Gua	0.005	–	–	–	–	–
*N*3-HA-Ade	0.044	0.118	–	0.105	–	–
*N*1-/N^6-HA-Ade	+	0.104	0.035	0.008	–	0.09[b]
*N*7-HA-Ade	+	–	–	–	–	–
*N*3-HA-Cyt/Ura	–	0.012	0.017	0.098	–	0.04
*N*3-HA-dThd	–	0.006	–	–	–	–

+, Detected but not quantified; –, not analyzed.
[a] The results shown are relative to the *N*7-HA-Gua.
[b] Data on *N*1-/N^6-HA-Ade in case of ECH represent formation of the 1,N^6-HP-Ade.

and *N*3-position of adenine and the *N*3-position of cytosine.

The majority of the alkylation products (adducts) are unstable, and undergo chemical transformation to yield the following secondary DNA adducts: (i) apurinic/apyrimidinic sites, generated following depurination of *N*7-HA-dGuo or *N*3-HA-dAdo; (ii) imidazole ring-opened derivative of *N*7-HA-dGuo [71]; (iii) N^6-HA-dAdo created from *N*1-HA-dAdo in a Dimroth rearrangement [72,73]; and (iv) *N*3-HA-Ura, a product of hydrolytic deamination of *N*3-HA-Cyt [73–75]. The primary adducts from ECH (3-chloro-2-hydroxypropyl; CHP) may undergo secondary reactions. Thus, Plná et al. [76] were able to detect *N*7-(2,3-dihydroxypropyl)guanine which is formed from the primary adduct through reaction with water. In addition, ECH can form cyclic adducts, e.g. 1,N^6-(2-hydroxypropano)adenine [70,77]. Table 2 lists various adducts of EtO, PO and ECH with DNA and their relative amounts obtained under neutral conditions (pH 7.0 and 37 °C) [73–75,78,79].

The formation of EtO-induced adducts with DNA in vivo has been extensively studied in experimental animals [80,81]. There are also in vivo animal data about PO [18,82] and ECH [83]. In unexposed humans, there are several studies concerning the background of *N*7-(2-hydroxyethyl)guanine (*N*7-HE-Gua) in DNA. Wu et al. [81] reported background levels of about 2–19 adducts per 10^7 normal nucleotides (measured by means of gas chromatography/mass spectrometry) in lymphocytes of 23 individuals. The levels reported in other studies, where the ^{32}P-post-labeling was applied, range between about 0.4–10 adducts per 10^7 normal nucleotides [84,85]. The high background limits the possibility to detect adduct increments resulting from EtO exposure. van Delft et al. [86] used an immunochemical assay to analyze *N*7-HE-Gua (the ring-opened form) in white blood cell DNA from individuals exposed to 2–5 ppm EtO and from controls. The exposure did not result in a statistically significant increase of the adduct level. However, in the study by Zhao and Hemminki [85], a somewhat higher level of *N*7-HE-Gua was indicated in smokers than in non-smokers. The source of the background adducts needs to be clarified—there is no corresponding high background of EtO-induced adducts in hemoglobin.

Czene et al. [64] used an extremely sensitive ^{32}P-post-labeling technique to determine *N*1-(2-hydroxypropyl)adenine (*N*1-HP-Ade) in a small group of PO-exposed workers. There were significant relationships between the levels of DNA adducts (mean 0.66 adducts per 10^9 normal nucleotides), hemoglobin adducts (mean 2700 pmol HP-Val/g globin) and SCE. The DNA adduct levels in control subjects were below 0.1 adducts per 10^9 normal nucleotides. The same group of researchers studied DNA adducts in employees of a chemical industry where ECH was used. *N*7-CHP-Gua (0.8–7.1 adducts per 10^9 normal nucleotides) was detected in DNA from 7 out of 16 workers exposed or potentially exposed to ECH, but not in any of the 13 control subjects (<0.4 adducts per 10^9 normal nucleotides) [76].

5. Hemoglobin adducts as biomarkers of exposure

EtO and PO form chemically stable adducts with reactive sites in hemoglobin, including the N-terminal

Table 3
Estimation of the steady state hemoglobin adduct level resulting from occupational chronic exposure to 1 ppm of ethylene oxide

Type of exposure	Air monitoring method	Exposed populations	Measured air concentration (ppm)	Hemoglobin adduct level (pmol HE-Val/g globin)	Adduct level per ppm in ambient air during work hours (pmol HE-Val/g globin)	Reference
Sterilization	Personal and stationary ambient air measurements	Regularly exposed workers ($n = 9$)	0.2–8.5; mean: 4.2	5219–32,738; mean: 15,500 (steady state adduct levels)	4000[a] (11 pmol/g per ppmh)[b]	[91]
Maintenance at a chemical plant	Personal air monitoring during the entire shift on every working day during a period of 3 weeks. Blood samples were collected before and immediately after this period	Operators ($n = 15$)	0.21 ± 0.07 (mean ± S.E.) (0.38 ± 0.12 mg/m^3)	64 ± 15 (mean increase above background level ± S.E.)	4600 ± 550 (mean ± S.E.) (13 ± 2 pmol/g per ppmh)[b]	[90]

[a] The steady state adduct level (A_{ss}) resulting from long-term exposure is calculated from $A_{ss} = 63 \times a_d$, where a_d is the average daily adduct increment. Occupational exposure during 5 days per week leads to $A_{ss} = (5/7) \times 63 \times a_d$.

[b] The adduct increment per ppmh (assuming 8 work hours per day) is calculated from $A_{ss} = 8 \times (5/7) \times 63 \times a_{ppmh} = 360 \times a_{ppmh}$, where a_{ppmh} is the adduct increment per ppmh.

Table 4
Estimation of ethylene oxide exposure levels based on measurements of air concentrations and calculated from hemoglobin adduct levels

Exposed group	Air monitoring method	Measured air concentration	Subjects	Hemoglobin adduct level (pmol HE-Val/g globin)	Calculated air concentration[a] (ppm)	Reference
Sterilization workers	Repeated monitoring 1–3 days once a year of air concentrations	>0.2 ppm (8 h TWA)	$n = 8$	1380	0.32	[29]
		<0.2 ppm (8 h TWA) (estimated to 0.1 ppm)	$n = 16$	230	0.05	
Sterilization workers	Repeated monitoring of air concentrations; 1–3 days once a year	~2 ppm (8 h TWA)	$n = 4$	1900 to ca. 10,000	~2	[60]
Assemblers working in the same factory but in another department		Assumed to be nil	$n = 9$	900–2300	~0.3	
Sterilization workers	Fifteen personal air samples taken during a work period (6.5 h)[b]	0.38 ppm (6.5 h TWA)	Workers in sterilization area; $n = 3$	270[c], 160 and 50	≤0.06	[62]
		0.025 ppm (6.5 h TWA)	In preparation area; $n = 4$	60, 50[c], 140 and 400[c]	≤0.1	
Sterilization workers	Stationary and personal monitoring	Near or below 1 ppm during the 8 years preceding the study	Workers: non-smokers; $n = 19$	2100[d]	<0.1	[50]
		<0.3 ppm (8 h TWA) during 2 weeks prior to blood sampling	Smokers; $n = 9$	3600[d]	–	
Controls		–	Non-smokers; $n = 16$	690[d]	–	
			Smokers; $n = 4$	2300[d]	–	
Sterilization workers (EI)		About 10 min exposure to 22–72 ppm once or twice a week (i.e. about 8–16 ppmh per week)	$n = 9$	179 ± 121 (mean ± S.D.)	0.05	[51]
Employees in production and sterilization of medical equipment (EII)		17 ppm (mean; peak exposures up to 400 ppm)	Daily exp. $n = 7$	13,200 ± 2550	3.1	
			Occasional exp. $n = 8$	2720 ± 1940	–	
Sterilization workers	Long-term area sampling and long-term personal sampling	4 months of cumulative exposure				[52]
		US group I: >0–32 ppmh; mean 0.04 ppm	$n = 32$	90[e]	0.02	
		US group II: >32 ppmh; mean 0.16 ppm	$n = 11$	160[e]	0.04	
		Mexican group I: >0–32 ppmh; mean 0.02 ppm	$n = 9$	60[e]	0.01	
		Mexican group II >32 ppmh; mean 0.54 ppm	$n = 12$	160[e]	0.04	
Industry workers	Stationary air sampling at the work sites	2–5 ppm (8 h TWA) during the 3-month sampling period	$n = 8$	294 ± 121 (mean ± S.D.)	0.07	[61]

[a] Levels of exposure during 4 months prior to blood sampling were calculated assuming that an average exposure to 1 ppm for 8 h per day, 5 days per week would lead to a steady state level of 4300 pmol HE-Val/g globin (see Table 3).
[b] No exposure data for three acutely exposed persons.
[c] Experienced an acute exposure a few days prior to blood sampling. The values are corrected for smoking and background.
[d] The values are overestimated by a factor of about 10.
[e] Means adjusted for confounding factors (background and smoking).

amino group, histidine-N and cysteine-S. In contrast to adducts with DNA, hemoglobin adducts are not repaired but are eliminated as a consequence of turnover of the erythrocytes (the life span of erythrocytes in humans is about 4 months). During long-term exposure (occupational exposure, cigarette smoking), the concentration of adducts builds up to reach a steady state level which is 63 times the average daily adduct increment. Within certain limits, hemoglobin adducts can be used as a "molecular dosimeter" providing an integrated measure of exposure during the 4 months prior to blood sampling [87,88]. Adducts with N-terminal valine, in particular, have proven to be useful as biomarkers of exposure to EtO and PO. Because of the bifunctionality of ECH, its adducts with N-terminal valine are not chemically stable [89]. Hindsø Landin et al. [65] measured 2,3-dihydroxypropylvaline in ECH-exposed workers. However, this adduct is not specific to ECH, and the high background level in control subjects limited its use as a biomarker of ECH. A suitable method for quantification of adducts of this compound with hemoglobin needs to be developed.

Boogaard et al. [90] have determined the relationship between the exposure dose and hemoglobin adduct formation in maintenance workers exposed to EtO and PO in a chemical plant. Personal air monitoring was applied to the operators during the entire shift on every working day during a shutdown period for 3 weeks of maintenance work. Blood samples were collected before and immediately after this period. Highly significant relationships were found between the increment of adducts and the total exposure to EtO or PO. The steady state adduct levels at exposure concentrations of 1 ppm EtO and 1 ppm PO during work hours are estimated to be 4600 and 910 pmol HA-Val/g globin, respectively (calculated from equations given in [90]). Angerer et al. [91] estimated that occupational exposure to EtO at 1 ppm would result in about 4000 pmol HE-Val/g globin (Table 3). The values for EtO are consistent with the theoretically expected adduct increment 10–15 pmol HE-Val/g globin per ppmh (see footnote b of Table 3) [92].

Hemoglobin adducts of EtO and PO are found at low background levels in human populations without known exposure to these epoxides. In the case of adducts of EtO, this background can be explained in terms of exposure to ethylene in urban air, ethylene produced endogenously and tobacco smoking [6]. A relationship between the level of EtO adducts and the number of cigarettes smoked per day was shown in several studies [93–95] and a correlation between the levels of adducts from EtO and PO was demonstrated by Törnqvist and Ehrenberg [96]. EtO adduct levels of about 20 pmol/g globin have been reported in non-smokers and the increments in smokers are about 100 and 2 pmol/g globin per 10 cigarettes per day for adducts from EtO and PO, respectively.

Tavares et al. [97] analyzed EtO–hemoglobin adducts in smoking and non-smoking mothers and their newborns. Hemoglobin adducts were detected in all babies' blood samples demonstrating a transfer of either the epoxide or its precursor (ethylene) through the placenta. There was a significant correlation between newborns and mothers adduct levels. The babies adduct levels were consistently somewhat lower than the maternal levels in paired samples.

Hemoglobin adducts were used to improve the assessments of exposure to EtO in the cancer epidemiological study by Hagmar et al. [29] and in some of the cytogenetic studies listed in Table 1. Table 4 presents estimates of exposure levels during the months prior to the blood sampling based on: (a) air monitoring; and (b) hemoglobin adduct levels. For the latter calculations, we have used the relationship between steady state adduct level and exposure level established by Boogaard et al. [90] and Angerer et al. [91] (about 4300 pmol HE-Val/g globin for exposure to 1 ppm EtO 8 h per day, 5 days per week; see Table 3). In a few studies, there is a good agreement between the two estimates of exposure [29,60]. However, in a majority of the studies [51,52,61,62], the monitoring of air appears to overestimate the exposure to the workers. In some of the studies, the air concentrations of EtO during the 4 months prior to blood sampling (as calculated from adduct levels) is very low and the adduct increments from the occupational exposure are comparable to those caused by smoking [50,52,61,62].

6. Effects of polymorphism

EtO is a substrate of the polymorphic enzyme GSTT1. In Europe, up to 30% of the population are GSTT1-negative "non-conjugators". GSTT1-positive

individuals can be classified as heterozygous "conjugators" or homozygous "high-conjugators". In vitro studies have demonstrated that GSTT1 polymorphism influences the reaction of EtO with blood proteins and the rate of induction of SCE. Blood samples from non-conjugators respond with higher protein adduct levels [98,99] and increased SCE frequency [100] compared to samples from conjugators.

The impact of polymorphism in vivo on the levels of hemoglobin adducts in the erythrocytes and SCE in lymphocytes of sterilizer operators that used EtO and non-exposed hospital workers was examined by Yong et al. [101]. The data were consistent with an increased formation of hemoglobin adducts in individuals with homozygous deletion of the *GSTT1* gene as compared with those with at least one copy of the gene. An unexpected finding in this study was a lower frequency of SCE in the GSTT1-negative individuals as compared to positive individuals ($P = 0.04$). Fennell et al. [102] measured adduct levels in cigarette smokers and estimated that the lack of a functional GSTT1 increased the internal dose of EtO derived from cigarette smoke by 50–70%.

Evidence of individual differences in susceptibility has also been presented by Fuchs et al. [58] who studied SSB in DNA of peripheral mononuclear blood cells from workers exposed to EtO. The non-smoking workers could be classified into two sub-populations. For both of these populations, a clear dose–response relationship was established. The "sensitive" group (67% of the individuals) demonstrated about five times higher SSB levels than the rest of the non-smokers. The authors speculated that these differences could be explained by polymorphism in detoxifying enzymes, as well as by individual variations in the DNA repair efficiency.

7. Interaction between smoking and epoxide exposure

Tobacco smoking is an important confounding factor in studies of biological effects in occupationally exposed populations. As already mentioned, tobacco smoke contains ethylene and propylene, which are precursors of EtO and PO. Thus, smoking contributes to exposure to these specific compounds. In addition, the smoke contains an array of other chemicals that may interact with an occupational exposure at several levels, such as activation of enzymes involved in the detoxification process or induction of DNA repair enzymes.

Fuchs et al. [58] showed that the elution rate of DNA from non-smokers, working in rooms with EtO concentrations of 0.1–0.5 and 0.5–2 mg/m^3, were 53 and 119%, respectively, higher than for DNA from workers exposed below 0.1 mg/m^3. For smokers, a similar tendency was observed but the response to the occupational exposure was smaller indicating an influence of smoking on the metabolism of EtO or on DNA repair. Oesch et al. [59] found reduced SSB levels in lymphocytes of EtO-exposed smokers compared to exposed non-smokers. The authors suggested that smoking might protect lymphocytes against additional genotoxic insults.

Mayer et al. [50] studied cytogenetic effects of EtO in smoking and non-smoking sterilization workers. Hemoglobin adducts were measured for an estimate of internal dose. When account was taken of the effect of smoking, and by combining former smokers and non-smokers, a significant increase in hemoglobin adducts in the sterilization workers compared with the controls was found. There was an indication of an interaction between EtO exposure and smoking ($P = 0.019$).

8. In vitro studies in mammalian cells

The choice of this topic is motivated by the contemporary trend in toxicology: more and more scientists choose alternative models instead of animal experiments. Use of mammalian cells in culture has many advantages—the mechanistic studies are facilitated in well-defined cell culture conditions; the results can be obtained much faster, compared with those in animal experiments; in vitro data are useful for risk assessment in humans exposed to carcinogenic chemicals. Both normal and immortalized cell lines can serve as model test systems for the study of the genotoxic effects.

The toxic effects of EtO, PO and ECH were studied mainly in cultured human cells, but also in cell lines of animal origin. Data obtained during 1990–2001 are summarized in Table 5 (earlier data concerning EtO were reviewed by Dellarco et al. [12]). Using human

Table 5
Effects of ethylene oxide, propylene oxide and epichlorohydrin in mammalian cells in vitro

Cell culture	Concentration of epoxide and exposure time	Observed effect	Reference
Ethylene oxide			
Human peripheral blood lymphocytes	5–20 mM; 6.5 h	A concentration-dependent increase of SCE frequency; 1.9 ± 0.5 (95% CI) SCE per cell per mMh[a]	[104]
Human peripheral mononuclear blood cells	0.5–10 mM; 2 h	A dose-dependent increase in SSB	[105]
Human diploid fibroblasts (VH-10)	2.5–10 mM; 1 h	A dose-dependent induction of mutations in the *HPRT* locus; 9.8 × 10^{-6} per mMh	[107,115]
		A dose-dependent induction of SSB and DSB	[108]
Chinese hamster cells (V79)	457–27,700 ppm; 30 min	A concentration-dependent increase of chromatid and chromosome aberrations. A significant increase of MN frequency, but only at high concentration (12,344 ppm)	[113]
Mouse embryo fibroblasts (C3H/10T1/2)	2.5–10 mM; 1 h	A dose-dependent induction of the neoplastic cell transformation	[120,121]
Propylene oxide			
Human peripheral lymphocytes	5–20 mM; 6.5 h	A concentration-dependent increase of SCE frequency; 1.7 ± 0.1 (95% CI) SCEs per cell per mMh	[104]
Human lymphocytes	0–2500 ppm; 72 h	A concentration-dependent increase of micronucleated binucleates. A concentration-dependent decrease of the mitotic ratio	[114]
Human diploid fibroblasts (VH-10)	2.5–20 mM; 1 h	A dose-dependent induction of SSB and DSB	[109,110]
Chinese hamster cells (V79)	1.25–10 mM; 2 h	A dose-dependent increase of SCE frequency	[111]
Mouse embryo fibroblasts (C3H/10T1/2)	2.5–20 mM; 1 h	Induction of neoplastic cell transformation	[122,123]
Syrian hamster embryo cells	2.5–20 mM; 1 h	Induction of neoplastic cell transformation	[122,123]
Epichlorohydrin			
Human lymphocytes from non-smokers and smokers	10^{-7} to 0.1 mM; 48 h	Significant increase of SCE frequency. No significant effect on CA when gaps were excluded. No significant effect on MN	[106]
Human diploid fibroblasts (VH-10)	0.5–2 mM; 1 h	A dose-dependent increase of SSB and DSB	[109,110]
Chinese hamster cells (V79)	0.125–1 mM; 2 h	A dose-dependent increase of SCE frequency	[111]
Mouse embryo fibroblasts (C3H/10T1/2)	0.25–1 mM; 1 h	Induction of neoplastic cell transformation only at the presence of tumor promoter TPA	[122,123]
Syrian hamster embryo cells	0.05–0.5 mM; 1 h	Induction of neoplastic cell transformation only at the highest concentration of 0.5 mM	[122,123]

[a] The dose is given as initial concentration × time of exposure [103].

cells as a model, in vitro studies were performed on peripheral blood cells from the donors [104–106], or on diploid fibroblasts [107–110,115,116].

EtO, PO and ECH are able to induce SCE in human cultured lymphocytes (Table 5). Agurell et al. [104] found the capacity of EtO and PO to induce SCE in human lymphocytes to be almost equal. This is in contradiction with earlier data from animal experiments [117] where PO was found to be practically unable to induce SCE. It was supposed [104] that in short-term tests (6.5 h of treatment) the mechanisms of SCE induction might be different from those operating in vivo. A concentration-dependent effect of ECH on SCE frequency was found in lymphocyte cultures of non-smoking and smoking donors [106]. Analysis of SCE frequencies induced by epoxides in Chinese hamster V79 cells [111] demonstrated an approximately 10 times higher SCE-inducing potency of ECH compared to PO.

There are only a few recent studies concerning the clastogenic effects of EtO, PO and ECH in cultured mammalian cells. In an early study by Poirier and Papadopoulo [112], an induction of CA after EtO treatment was shown in a human amniotic cell line. Later, the ability of EtO to induce CA was reported in Chinese hamster V79 cells [113]. Among the aberrations, chromatid and isochromatid breaks, fragments, minutes and exchanges were detected. Studies concerning MN are restricted; there are only two studies showing capacity of PO to induce MN in human binucleated lymphocytes [114] and in Chinese hamster V79 cells [113].

The induction of DNA breakage by the three epoxides has been demonstrated in cultured human cells treated in vitro. A dose-dependent increase of DNA SSB after treatment with EtO or its putative metabolite, glycolaldehyde, was detected in human peripheral mononuclear blood cells [105]. It was also found that glycolaldehyde, but not EtO itself, is able to induce DNA–protein cross-links [105]. In human diploid fibroblasts, a dose-dependent induction of DNA SSB and double-strand breaks (DSB) was detected after treatment with EtO, as well as after treatment with PO or ECH [108]. The number of SSB measured by alkaline DNA unwinding was highest for ECH, compared with that induced by EtO or PO (211 versus 93 or 41 SSB/100 Mbp per mMh, respectively). The yield of DSB, measured by pulsed-field gel electrophoresis (PFGE), was 18 times higher for ECH and 15 times higher for EtO compared to PO (4.8 and 4.0, respectively, versus 0.27 DSB/100 Mbp per mMh). It was also demonstrated that human fibroblasts are able to repair, at least in part, DNA SSB and DSB induced by these epoxides [108,110]. For example, the use of PFGE demonstrated that about 50% of DSB induced by 7.5 mMh treatment with EtO were rejoined within 18–20 h. In the case of 10 mMh of PO, 96% of the DSB were rejoined 20 h after exposure. After treatment with ECH, about 65% (1 mMh) and 39% (2 mMh) were rejoined during the same time interval.

The mutagenic effect of EtO was studied in human fibroblasts [107,115,116], and the frequency of *HPRT* mutants was estimated by selection in 6-TG containing medium. A dose-dependent increase of mutant frequency was found within the dose range 2.5–10 mMh of EtO. *HPRT* mutations in 28 independent clones were characterized using Southern blot, polymerase chain reaction and DNA sequencing [115]. It was shown that EtO induces not only point mutations, such as base substitutions and splice mutations, but also large deletions where the whole *HPRT* gene or several exons were missing (about 50% of all mutations).

The induction of intrachromosomal recombination with EtO and PO has been studied using a reversion mutation assay in the *HPRT* gene in the spontaneous mutant clone SP5 derived from Chinese hamster V79 cells [118]. In this mutant, a duplication of exon 2 and its flanking regions was found inserted between the two *Eco*RI sites of intron 1. The removal of this insertion fragment could be detected by this assay. Thirty-four different carcinogenic chemicals were tested, among them EtO and PO. In contrast to some other monofunctional alkylating agents (e.g. ethyl methanesulfonate, *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine), these epoxides did not induce a detectable recombinogenic effect.

The effect of EtO, PO and ECH on cell-cycle progression has recently been studied in human fibroblasts by means of flow-cytometric analysis and by measurement of DNA synthesis [119]. It was shown that all studied epoxides affected the G_1/S progression; G_1 arrest was induced 6–18 h after the treatment with EtO, PO, or ECH. The mode of cell death in response to epoxide treatment was necrosis, rather than apoptosis

[119], as indicated by the lack of chromatin condensation and so-called "apoptotic bodies".

During the last decade new knowledge has also been collected concerning the ability of the epoxides to induce neoplastic cell transformation in mouse embryo fibroblasts (C3H/10T1/2 cells) and in Syrian hamster embryo cells (Table 5). It was shown that both EtO and PO induce cell transformation in the absence of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [120–123]. The frequency of neoplastic transformation was significantly increased above the background ($P < 0.001$). Moreover, the frequency of transformation was considerably increased in the presence of TPA [121,123].

The capacity of ECH to induce neoplastic cell transformation was also studied in C3H/10T1/2 and Syrian hamster embryo cells [122,123] (Table 5). In the absence of TPA, ECH was able to transform cells only at the highest dose (0.5 mMh) that could be tested due to the toxicity of the compound. However, significantly increased transformation frequencies, compared to the control values, were observed even at lower doses in the presence of TPA [123].

In vitro studies may contribute to the assessment of the genotoxicity of the studied epoxides. Data on their relative effectiveness are of great value for risk estimations. For example, in a cell transformation assay in mouse embryo fibroblasts, the transformation frequency per mMh of the epoxides (in the presence of a tumor promoter) decreased in the order ECH > EtO > PO (8:4:1) [123]. The capacity of the three epoxides to induce DNA breakage was compared in human fibroblasts using different assays. Similarly to cell transformation assay, the effectiveness decreased in the order ECH > EtO > PO and the relative effectiveness was 5:2:1 and 17:14:1 for SSB and DSB, respectively [110]. The higher toxicity of ECH may, in part, be explained by its higher chemical reactivity [10]. In addition, the ability of ECH to act as a bifunctional compound may play a significant role.

9. Relative risk assessments/the rad-equivalence approach

According to the approach suggested by Ehrenberg [124], the rad-equivalence of a chemical dose may be applied tentatively for an estimation of the risk to man posed by exposure to a genotoxic agent. This approach is based on: (a) dosimetry in humans; (b) the relative mutagenic effectiveness of the chemical compared to sparsely ionizing radiation; and (c) the cancer risk coefficient for sparsely ionizing radiation.

EtO, PO and ECH belong to the so-called "radiomimetic" genotoxic chemicals that induce similar biological end-points as ionizing radiation. These include gene mutation, DNA strand breaks and neoplastic cell transformation. Therefore, it is possible to compare the effect of the epoxide and of ionizing radiation in different in vitro and in vivo assays and calculate the rad-equivalent (rad/mMh or, according to contemporarily used units, Gy/mMh). Recently, the effects (cell killing, mutagenicity in the *HPRT* locus and DNA DSB induction) of EtO and ionizing radiation have been studied in human diploid fibroblasts [116]. The mutagenic effectiveness of EtO was compared with that of ionizing radiation. The rad-equivalence of EtO was calculated to be approximately 40 rad (0.4 Gy)/mMh. The rad-equivalence of EtO in the cell transformation assay in C3H/10T1/2 cells [123] was 75 ± 26 rad/mMh. The transforming effectiveness of EtO was compared with that of ionizing radiation in the presence of TPA, and the rad-equivalence of EtO was calculated to be approximately 90 rad (0.9 Gy)/mMh.

van Sittert et al. [125] determined the mutagenic effectiveness of EtO in rats and found that the effect of 1 mMh of EtO is equal to that of 19 rad (range 10–36 rad) of an acute X-ray exposure. The authors calculated the target dose in humans from 1 year of occupational exposure to 1 ppm EtO as 0.26 mMh, which would be equivalent to 4.8 rad. The occupational exposure limit of 1 ppm EtO would thus correspond to the current annual effective dose limit (50 mSv = 5 rad) for radiation workers. The United Nations Scientific Committee on the Effects of Atomic Radiation [126] estimates that the lifetime cancer risk (cancer death) after exposure to 1 Sv of ionizing radiation is about 9×10^{-3} for leukemia (4.5×10^{-3} using a dose-and-dose-rate-effectiveness factor of 2). This implies that the risk prediction for leukemia would be about 1×10^{-2} for an occupational exposure to 1 ppm (assuming 45 years of exposure).

10. Quantitative interpretation of epidemiological and cytogenetic studies

The epidemiological data collected on EtO suggests that the carcinogenic potency of this compound is low in humans. Teta et al. [20] used three studies [28,31,33], which were considered to have the required size, individual exposure estimates and follow-up, for an "added lifetime risk" prediction under environmental and occupational exposure scenarios. The added risk prediction for leukemia was 2.2×10^{-4}, or lower, for occupational exposure to 1 ppm (predicted risk by age 70 given 45 years of exposure). This risk estimate is considerably lower than prior animal-based estimates by the US Environmental Protection Agency (US EPA) (2.6×10^{-2}) [127] and the Occupational Safety and Health Administration (OSHA) (2.1×10^{-3} to 3.3×10^{-3}) [128] and the recent assessment by van Sittert et al. [125]. The risk estimations by US EPA and OSHA are based on the same rat database, but different mathematical models were used [33]. The variation in the different estimates is two orders of magnitude demonstrating the difficulties involved in risk extrapolation to humans.

A large number of studies concern cytogenetic effects (including SSB) in occupationally exposed workers. In some of the studies, a clear dose–response relationship was observed. For example, a clear relationship between EtO exposure and response was observed for SSB in the study by Fuchs et al. [58] and for SCE in the study by Lerda and Rizzi [53]. According to Tates et al. [51], the relative sensitivity of various cytogenetic end-points used for detection of EtO exposure decreases in the order HFC > SCE > CA > MN > *HPRT* mutation. Exposure levels around or higher than the recommended limits are needed to give a detectable response above the background in cytogenetic assays of conventional scope. Moreover, the quantitative interpretation of the cytogenetic data is difficult. Not only are confounding exposures to genotoxic agents in tobacco smoke or in the work environment a major difficulty, but also the limited data on the biological persistence of the effects. Among numerous other factors that may affect the result, particularly in studies of small groups of individuals, are differences in metabolism. Such differences were demonstrated, for example, in studies of SSB [58] and SCE [101,102].

One of the major problems in cancer epidemiological studies, as well as cytogenetic studies, appears to be the poor records of exposure. In several of the biomonitoring studies listed in Table 4, the air monitoring data failed to predict the exposure dose to individual workers as estimated by adduct measurements. The exposure is often variable and occurs intermittently and data on concentrations of chemicals in air refer rather to potential exposure than to actual dose received by the exposed individual. This problem could, at least in part, be solved by determination of hemoglobin adducts. As compared to cytogenetic assays, hemoglobin adduct measurements are extremely sensitive—occupational air concentrations down to 0.01 ppm EtO would give measurable adduct levels. Compared to DNA adducts, hemoglobin adducts have the great advantage that their stability in vivo is known. This facilitates the calculation of in vivo doses from hemoglobin adduct levels.

11. Concluding remarks

According to the classification by IARC [1,2], EtO is a "known human carcinogen (Class 1)", whereas PO is classified as "possibly carcinogenic to humans (Class 2B)", and ECH as "probably carcinogenic to humans (Class 2A)". This classification reflects the strength of evidence for carcinogenicity in humans rather than the magnitude of the effect. During the last decade, there have been changes in the regulatory guidelines towards a greater flexibility and an increased reliance on human data. In 1994, EtO was upgraded from Class 2A to Class 1. The classification was mainly based on carcinogenicity data in rodents and supported by human cytogenetic monitoring studies in humans [129]. ECH was upgraded from Class 2B to Class 2A in 1999.

In the interim time, several large-scale epidemiological studies, including about 33,000 workers with potential exposure to EtO, have been completed. In spite of this large database, a carcinogenic effect of EtO in humans has not been convincingly proven. Concerning the two other epoxides, cancer epidemiology data are lacking for PO or unconvincing for ECH.

The difficulties in risk assessment are many. One of them is the long latency time for cancer development, sometimes up to 30 years. Another problem is

the concomitant exposure to other genotoxic chemicals in workplaces and in other environments. Data on previous exposure to the epoxides are often incomplete or missing, and confounding factors, such as exposure to other chemicals, smoking, diet and lifestyle, are not well documented.

Within the past decade new knowledge about reactions of the epoxides with DNA and hemoglobin was collected. Methods to study individual differences in metabolism and to measure DNA and hemoglobin adducts as biomarkers of exposure were developed. In human cytogenetic studies, new methods, such as fluorescence in situ hybridization to detect persistent chromosomal changes, are now under development. The use of these methods as adjuncts in epidemiological studies will provide an improved basis for risk estimations. Last, but not least, in vitro studies in various model cell systems have become an important component of risk assessment.

The discrepancy between the cancer risk estimates for EtO, derived from human epidemiological studies and calculated using the rad-equivalence approach, respectively, is discouraging. We cannot exclude that humans are less sensitive to the carcinogenic activity of EtO than predicted by the rad-equivalence approach.

The recommended hygienic standards in Sweden, based on 8 h TWA, are set to 0.5, 1 and 5 ppm for ECH, EtO and PO, respectively. These standard values correspond to the relative effectiveness of the three epoxides in different in vitro test systems discussed earlier in this review. For the time being, in the absence of reliable human data, we can accept these hygienic standards. Obviously, it is important in the future to consolidate the procedures for estimation of risk to humans.

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