Recommendation from the Scientific Committee on Occupational Exposure Limits for ethylene oxide

SCOEL/SUM/160
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Employment, Social Affairs and Inclusion

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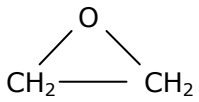
8-hour TWA:	Not assigned (*see "Recommendation")*
STEL (15-min):	Not assigned
Notation:	"Skin"
BLV:	Not assigned (*see "Recommendation")*
SCOEL carcinogen group:	B (*genotoxic carcinogen, for which a threshold is not sufficiently supported)*
Carcinogenic risk assessment:	*See Table 1 in "Recommendation"*

Substance identification

Chemical name:	Ethylene oxide
Synonyms:	ethene oxide, oxirane, 1,2-epoxyethane, dimethylene oxide
Structural formula:	
CAS No.:	75-21-8
EC No.:	200-849-9
Molecular formula:	C_2H_4O
Molecular weight:	44.05 g/mol
Melting point:	-112 °C
Boiling point:	10.5 °C (1.013 hPa)
Conversion factor 20 °C, 101.3 kPa:	1 ppm = 1.83 mg/m^3; 1 mg/m^3 = 0.55 ppm

EU Classification:

Press. Gas		
Flam. Gas 1	H220	Extremely flammable gas
Carc. 1B	H350	May cause cancer
Muta. 1B	H340	May cause genetic defects
Acute Tox. 3 *	H331	Toxic if inhaled
Eye Irrit. 2	H319	Causes serious eye irritation
STOT SE 3	H335	May cause respiratory irritation
Skin Irrit. 2	H315	Causes skin irritation

Criteria documents used: WHO (1985), Deutsche Forschungsgemeinschaft (1993, 1999), IARC (1994, 2008), Thier and Bolt (2000), EPA (2006). This was further supplemented by a literature search conducted by SCOEL.

1. Occurrence/use and occupational exposure

Ethylene oxide is a colourless flammable gas at room temperature with sweet odour. This chemically simple epoxide is the chief precursor to ethylene glycol and other high-volume chemicals. It is also used as a fumigant, cooler, flame retardant and sterilising agent in mixtures with nitrogen and carbon dioxide. It is normally handled as a refrigerated liquid, as it is explosive under heating or confinement. It reacts violently with water. Most of the data on occupational exposure are related to the production of ethylene oxide and to its use in industrial and hospital sterilisation (for details, see IARC 1994, 2008).

2. Health significance

2.1. Toxicokinetics

The basic metabolism of ethylene oxide in humans and in experimental animals is shown in Figure 1.

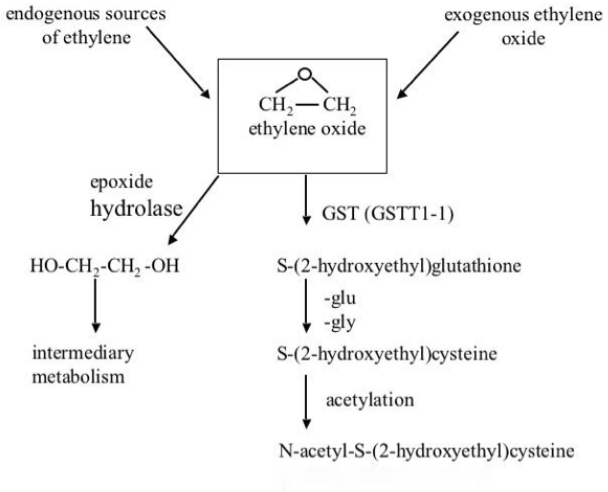


Figure 1. Metabolic pathways of ethylene oxide [adapted from Thier and Bolt (2000)].

2.1.1. Human data

There are no published toxicokinetic data regarding skin absorption in humans.

Ethylene oxide is readily taken up by the lungs. A study on workers exposed to ethylene oxide revealed an alveolar retention of 75–80 %, calculated from hourly determinations of ethylene oxide concentrations in environmental air ranging from 0.11 to 12.3 ppm and in alveolar air from 0.03 to 3.8 ppm (Brugnone *et al* 1985, 1986). At steady state, therefore, 20–25 % of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound, and 75–80 % is taken up by the body and metabolised. Blood samples taken from workers 4 h after the work shift and later gave venous blood/alveolar air coefficients of 12–17 and venous blood/ environmental air coefficients of 2.5–3.3. The difference from the value of 90 determined for the blood/air partition coefficient *in vitro* was explained by incomplete saturation of tissues and limitation of the metabolic rate by the lung uptake rate (see IARC 1994). The half-life of ethylene oxide in human blood has been calculated to be 3.5 hours (Pauwels and Veulemans 1998). Accordingly, no accumulation of ethylene oxide in humans over a working week is to be expected.

The detection of individual differences in susceptibility of humans has promoted

research into possible genetic factors which have an imprint on metabolism of ethylene oxide. Fuchs *et al* (1994) distinguished a "higher sensitive group" as opposed to a "less sensitive group", when studying single-strand breaks in blood cell DNA, by using the "alkaline elution" method, of persons occupationally exposed to ethylene oxide. Such individual differences in susceptibility to the genotoxicity of ethylene oxide are likely to be linked with differences in ethylene oxide metabolism, as explained below.

Hallier *et al* (1993) demonstrated that peripheral lymphocytes of persons lacking the glutathione transferase human *(h)GSTT1* gene (homozygote *hGSTT1*0*, addressed as "non-conjugators") were more susceptible towards the sister-chromatid-exchange (SCE) inducing effect of ethylene oxide than were lymphocytes of carriers of the *hGSTT1* gene ("conjugators"; Pemble *et al* 1994). "Conjugators" were again comprised of two subgroups, heterozygous "slow conjugators" and homozygous "high conjugators" (Wiebel *et al* 1999). The impact of the glutathione-dependent metabolic pathway for ethylene oxide in humans is visualised by urinary excretion of the respective mercapturic acid, *N*-acetyl-*S*-(2-hydroxyethyl)-*L*-cysteine (Barr and Ashley 1998). Also, there are differences in the background levels of the haemoglobin adduct 2-hydroxyethyl-valine between GSTT1-positive and GSTT1-negative individuals not occupationally exposed to ethylene oxide (Thier *et al* 1999).

Thier *et al* (1993, 1996) have studied the metabolism of ethylene oxide and of its homologue, propylene oxide, in *Salmonella typhimurium* TA1535 expressing or not expressing human or rat GSTT1 (hGSTT1+/- or rGSTT1+/-, respectively); the GSTT1-dependence of metabolism and genotoxicity of both epoxides was very clear-cut. Herrero *et al* (1997) have studied the role of human microsomal epoxide hydrolase (hmEH) expression in V79 Chinese hamster cells on genotoxicity (single strand breaks and alkali labile sites using the "alkaline elution" method) of epoxides. In contrast to styrene oxide where such DNA damage was reduced by presence of hmEH, there was no significant influence of hmEH expression on the genotoxic effect of ethylene oxide.

2.1.2. Animal data

Ethylene oxide reacts with nucleophilic groups such as carboxyl, amino, phenolic hydroxyl and sulphhydryl groups. The biocidal properties of the substance are ascribed to this reactivity. A biological half-life of 9 minutes was determined in mice which inhaled 1–35 ppm radioactively labelled ethylene oxide for 1 to 2 hours. Within 48 hours, 60-100 % of the inhaled radioactivity was excreted in the urine. During the first 4 hours after exposure, the radioactivity in liver and kidneys was markedly higher than that in other organs. After intravenous injection of ethylene oxide doses of 25 and 75 mg/kg body weight into dogs, the half-life of the substance in blood plasma was determined as 27.1 and 30.1 minutes, respectively. The plasma concentration sank within 5 hours to 2 % of the level determined immediately after the injection. Within 24 hours, 7–24 % of the ethylene oxide dose was excreted in the urine as ethylene glycol. Rats inhaled 100 ppm ^{14}C-labelled ethylene oxide for 6 hours; 18 hours after the end of exposure, 60 % of the recovered radioactivity was found in the urine, about 6 % in the faeces, and about 9 % as CO_2 and 1 % as unchanged ethylene oxide in the exhaled air. In the internal organs, the highest level of radioactivity was found in the liver, followed by the red blood cells, kidneys and adrenals. Pretreatment of the animals with ethylene oxide (100 ppm, 6 hours daily, 5 days weekly for 8 or 10 weeks) did not affect the metabolism (for details, see DFG 1993).

In mice treated intraperitoneally with ^{14}C-ethylene oxide, alkylation of guanine at the *N*-7 position was detected in the DNA of liver, spleen and testis. DNA-alkylation was also demonstrated in spermatozoa of mice exposed to ethylene oxide (for details, see DFG 1993).

Toxicokinetic models for ethylene oxide have been developed and gradually improved (Filser and Bolt 1984, Filser *et al* 1992, Krishnan *et al* 1992, Fennell 1996, Brown *et al* 1996, 1998, Csanády *et al* 2000). At very high inhalation exposures (100 ppm and above) of experimental animals, non-linearities in metabolic elimination of ethylene oxide are observed which are likely to be connected with depletions of glutathione as observed *in vivo* (Brown *et al* 1998) and in cellular systems *in vitro* (Mlejnek and Kolman 1999).

There are no published toxicokinetic data on skin absorption of ethylene oxide in experimental animals.

2.1.3. Biological monitoring

Due to its intrinsic chemical reactivity (comparative study: Kirkovsky *et al* 1998) ethylene oxide alkylates a variety of different sites of biological macromolecules, *i.a.* proteins at the electron-rich functional groups of the amino acids cysteine, histidine and valine. With respect to routine procedures, the ethylene oxide adduct of choice for biomonitoring of exposed persons is the hydroxyethylated *N*-terminal valine (HOEtVal) in haemoglobin. This procedure of biological monitoring has been successfully used in the European industry since the 1980s (Boogaard 2002). The use of dipeptide standards replacing the alkylated globins as standard has led to methodological improvements. The commercial availability of these standards, their stability and accurately known adduct contents make them the standards of choice (Pauwels and Veulemans 1998, Boogaard 2002). The sensitivity of the method, for instance, is reflected by a positive relationship of HOEtVal to tobacco smoke exposure quantified by means of questionnaire, by urinary cotinine, or the number of cigarettes smoked (Bono *et al* 1999).

Numerical correlations of human ethylene oxide exposure in ambient workplace air with HOEtVal adduct levels have been established by Angerer *et al* (1998) and by Boogaard *et al* (1999), based on field studies. According to these two studies, a constant workplace exposure to 1 ppm ethylene oxide results in a steady-state level of 4.0 and of 6.8 nmol HOEtVal per g globin, respectively (Boogaard 2002).

Müller *et al* (1998) and Thier *et al* (1999) have studied the influence of the genetic status of the polymorphic glutathione transferases *hGSTT1* and *hGSTM1* on the background hydroxyethylation of *N*-terminal valine in haemoglobin. Both studies were in accordance with (endogenous) ethylene oxide being a substrate of hGSTT1 isoenzyme, and homozygote *hGSTT1*0* individuals displayed background HOEtVal levels that were about 1/3 higher than those of persons with the intact *hGSTT1* gene or enzyme. Another parameter that influences the background level of HOEtVal in haemoglobin is the smoking status, with levels of smokers being about 50 % higher than those of non-smokers (Bono *et al* 1999, Thier *et al* 1999). In a series of recent occupational field studies the background HOEtVal level (in non-smoking persons not exposed to ethylene) was about 20 pmol/g globin (Boogaard *et al* 1999).

Taking the entire body of data together, there is a solid base for biological monitoring of exposed workers, using HOEtVal haemoglobin adduct monitoring. The comprehensive review of Boogaard (2002) has compiled the argumentations and elements for derivation of a biological monitoring standard (see also the Recommendation section).

Based on animal experimental data (Gérin and Tardif 1986) an alternative parameter for biological monitoring could be the urinary excretion of the mercapturic acid [*N*-acetyl-*S*-(2-hydroxyethyl)cysteine]. However, the published database in humans appears not to be sufficient for an evaluation.

The measurement of hydroxyethylmercapturic acid (HEMA) in urine can also be used to monitor occupational exposure to ethylene oxide (Popp *et al* 1994, Eckert *et al* 2011, Haufroid *et al* 2007).

2.2. Acute toxicity

2.2.1. Human data

There are numerous reports of casuistics of acute inhalation toxicity of ethylene oxide, mostly involving occupational intoxications. However, there are usually no details of the ethylene oxide concentrations or doses involved. Depending on the exposure conditions, the first symptoms appeared either during exposure or within a few minutes to several hours after the end of exposure. The main symptoms were headaches, nausea and generally persistent periodic vomiting. Dyspnoea, irritation of the eyes and upper respiratory mucosa, heart damage, excitation, stupor, vertigo and loss of consciousness were also observed. Clinical-pathological investigations revealed spontaneous nystagmus, impaired hearing, bilirubinuria, ventricular bigeminy, bradycardia, increased ventricular activation time. Permanent health impairment as a result of an acute ethylene oxide intoxication has not been described (for details, see DFG 1993). However, when materials sterilised with ethylene oxide were used for an extracorporeal bypass, the high residue levels caused haemolysis, increased levels of free haemoglobin and renal complications in the patients. Deaths of three children from toxic shock after open heart operations were ascribed to the ethylene oxide residues in the PVC parts of the heart-lung machines which had been sterilised with this substance (DFG 1999).

2.2.2. Animal data

The 4-hour LC_{50} for the mouse was determined as 835 ppm (95 % confidence interval 623–1 040 ppm), for the rat as 1 460 ppm (620–2 550 ppm), and for the dog as 960 ppm (95 % confidence interval not specified). In the rats, the symptoms were lacrimation, nasal discharge, diarrhoea and gasping for breath; in the mice the same symptoms were seen but without diarrhoea. Exposure to 2 830 ppm for 4 hours was lethal for dogs within the next 5 hours; the symptoms included lacrimation, salivation, vomiting, diarrhoea, spasms and dyspnoea (DFG 1993).

2.3. Irritation and corrosivity

Within a few hours after exposure of human skin to gaseous ethylene oxide, development of *dermatitis bullosa* with erythema and blisters was observed. The first signs of intoxication resulting from exclusively dermal uptake of gaseous ethylene oxide were often systemic effects such as vomiting and headaches. Likewise, after exposure of a large skin area to a 1 % aqueous solution of ethylene oxide for about 2 hours followed by a latent period of up to a few hours, the first symptoms were nausea, vomiting and headaches and then, after 12-24 hours, local effects on the skin such as severe blistering. This points both to a relevant systemic absorption through the skin and to local skin toxicity. When a series of aqueous solutions with ethylene oxide concentrations between 1 and 90 % were tested on human skin under experimental conditions, the 50 % solution produced the most severe skin reaction. Repeated dermal application resulted in allergic symptoms characterised by itching, erythema and oedema in three of the eight test persons. The skin symptoms observed after occupational intoxication caused by gaseous ethylene oxide were also described as acquired hypersensitivity of the skin in the form of *dermatitis bullosa allergica* although no allergy tests were carried out (DFG 1999).

In the medical sector, ethylene oxide residues released from sterilised anaesthetic equipment such as narcosis masks, rubber gloves, endotracheal tubes and tracheotomy tubes led to sometimes severe local irritation of skin and mucous membranes, resulting in stenosis.

According to occupational experience, the irritation potential of gaseous ethylene oxide at the portal of entry into the organism appears to be low at current workplace concentrations (DFG 1999).

2.4. Sensitisation

Ethylene oxide is a potent alkylating agent and reacts with hydroxyl, sulphhydryl, amino and carboxyl groups in human macromolecules. As a hapten, it becomes an active allergen after binding to human proteins. For ethylene oxide, especially allergies of immediate type are well documented. In addition, there are case reports describing contact dermatitis caused by reactions to ethylene oxide.

2.4.1. Human data

Anaphylactic reactions in dialysis patients with attacks of sneezing, retrosternal burning pains, laryngeal oedema, bronchial obstruction and hypersecretion, flushing and pruritus and sometimes even anaphylactic shock have been described by several authors (Bommer *et al* 1985, Röckel *et al* 1988, Rumpf *et al* 1985). Because of the large number of dialysis treatments carried out, these reactions are not unusual (4.3 per 100 000 dialyses) (Bommer *et al* 1985). As potential causes of these complications, IgE-mediated reactions to the disinfectants ethylene oxide and formaldehyde, to various isocyanates and various phthalates have been suggested. Direct complement activation by the cellulose filters used has also been discussed (Röckel *et al* 1989). However, various authors came independently to the conclusion that by far the main aetiopathic factor in the provocation of such reactions is allergy of immediate type to ethylene oxide. In such cases, the presence of IgE specific for conjugates of ethylene oxide with human serum albumin (HSA) has been demonstrated with the radioallergosorbent test (RAST) (Bommer *et al* 1985, Grammer *et al* 1984, Röckel *et al* 1989, Rumpf *et al* 1985).

An epicutaneous test with a 1 % aqueous solution of ethylene oxide revealed no evidence of hypersensitivity among 30 persons who had been exposed occupationally to the substance for several years. On the other hand, in tests for dermal toxicity of materials sterilised with ethylene oxide and containing various levels of ethylene oxide residues, hypersensitivity was demonstrated in one of the test persons (DFG 1999).

In a study of 83 dialysis patients, 16 dialysis unit personnel and 44 healthy control persons, IgE specific for conjugates of ethylene oxide with HSA was detected in 35 of the dialysis patients but in only 2 persons from the control group and in 2 dialysis unit personnel. Dialysis patients with IgE antibodies had allergic complications during dialysis more frequently than patients without antibodies. After use of materials which had not been sterilised with ethylene oxide for eight weeks, the antibodies in sensitised dialysis patients were present at much lower levels or were no longer detectable and the clinical symptoms had suddenly improved. Re-exposure to materials sterilised with ethylene oxide resulted in reappearance of the clinical symptoms (Bommer *et al* 1985).

Ethylene oxide has been considered to be a potential occupational sensitiser for nurses and other persons employed in the health services (Alberts 1993, Trotti and McCarthy 1989). However, the literature contains only very few reports of IgE-mediated reactions to ethylene oxide resulting from workplace exposure. Obstructive airway

diseases caused by occupational exposure to ethylene oxide have been described as non-immunological, chemical-irritative effects (Deschamps *et al* 1992).

As exemplified above (Section 2.3), aqueous solutions of ethylene oxide are highly irritating for human skin (maximum irritation with 50 % w/w solutions). There are several reports of contact dermatitis induced by ethylene oxide (WHO 1985). In some cases, the observed reactions were interpreted as cell-mediated allergy to ethylene oxide. In one female patient with bronchial asthma, two days after use of a respiratory mask which had been sterilised with ethylene oxide, dermatitis developed at the site of contact. Thorough differential diagnostic investigation led the authors to the conclusion that an allergic mechanism must be responsible for the reaction and that ethylene oxide was the allergen (Alomar *et al* 1981).

2.4.2. Animal data

In mice and rats treated by parenteral application of ethylene oxide-protein conjugates, the formation of specific IgE antibodies was demonstrated. By means of transfer tests, the specificity of the IgE antibodies could be demonstrated *in vivo* (Chapman *et al* 1986).

2.5. Repeated dose toxicity

2.5.1. Human data

Apart from the local effects described in Section 2.3, neurological symptoms were seen in 12 persons who had been exposed occupationally to ethylene oxide for about 6 months: headaches (6 persons), nausea (5), speech disorders and impairment of short-term memory (5), vertigo (3) and incoordination (2). Measurements carried out in the air of the room during one sterilisation cycle revealed a maximal ethylene oxide concentration of 36 ppm. This value was, however, not considered to be representative of the whole exposure period. In addition, in four persons with local and neurological symptoms the level of sister chromatid exchange in cultured peripheral lymphocytes was increased relative to the control values and showed no tendency to decrease even 18 weeks after the end of exposure (Garry *et al* 1979).

When 31 persons who had been exposed to ethylene oxide for several years were examined and compared with a control group, the following pathological findings were obtained: lymphocytosis, reduced Hb level, 1 case of leukaemia and 3 cases of anisocytosis (Ehrenberg and Hällström 1967). On the other hand, the examination of 37 workers in the chemical industry who had been exposed to 5–10 ppm ethylene oxide and of a control group revealed no evidence of substance-related impairment of health (Joyner 1964).

2.5.2. Animal data

In studies with cats, exposure for one to several hours daily to ethylene oxide concentrations, which were presumably much higher than stated (100 or 200 ppm), was lethal for the animals after about 22 days. Reduced food consumption and also apathy, paralysis of the hind limbs and an unsteady gait were the most conspicuous symptoms; autopsy revealed generalised hyperaemia of the internal organs and the brain, perivascular haemorrhage and liver and kidney damage (DFG 1993).

Exposure of rats, guinea pigs, rabbits, mice and monkeys in constant flow gassing chambers to an ethylene oxide concentration of 841 ppm, daily for 7 hours, caused severe irritation of the respiratory tract mucosa and was lethal after 1 to 8 exposures. Concentrations of 350–400 ppm (daily 6–7 hours, 7–123 exposures) caused delayed

growth, weight loss, diarrhoea and irritation of the respiratory tract mucosa; in addition, rats, rabbits and monkeys developed paralysis of the hind limbs with marked muscle atrophy. These symptoms regressed after the end of exposure. In these studies, the lung damage was most severe in mice; rats and guinea pigs were less affected and in rabbits and monkeys there were practically no signs of irritation. Exposure to 290 ppm, 6 hours daily for 6 weeks, led to tremor, vomiting, weakness in the hind legs and slight anaemia in 2 of 3 dogs. In mice and rats, there was no damage apart from haemosiderosis in the spleen; for dogs, pulmonary congestion and atrophy of the hind leg muscles with fatty degeneration were described (for details, see DFG 1993).

When 20 rats, 8 guinea pigs, 2 male and 2 female rabbits, 10 male mice and 2 female monkeys were exposed to 204 ppm (122–157 exposures in 176–226 days), growth was delayed in the rats and numerous rats and mice died; the guinea pigs, rabbits and monkeys survived. During the study, less active patellar reflexes, a positive Babinski's reflex and absence of withdrawal from superficial pain stimuli in the hind feet and skin of the legs and back were observed in the two monkeys; deep pain reflexes could be elicited in the feet, toe pads, etc.; paralysis and some evidence of muscle atrophy were seen in the rear extremities. Similar symptoms of paralysis developed in the rabbits as well. Blood count, blood urea determination and urine analysis before the end of the study produced no pathological findings in rats, guinea pigs, rabbits or monkeys. Increased lung, kidney and liver weights were found in rats at the end of the study and increased lung weights in guinea pigs. Histological examination revealed small testes with microscopic degeneration of some tubuli in male animals, slight cloudy swelling of the renal tubules, haemorrhage, congestion, emphysema and atelectasis of the lungs, especially in female rats (for details, see DFG 1993).

2.6. Genotoxicity

2.6.1. In vitro

In bacterial strains which may be mutated by base-pair substitution (*Salmonella typhimurium* TA1535, TA100), ethylene oxide was mutagenic without metabolic activation. Ethylene oxide has mutagenic activity in *Drosophila melanogaster*. In addition, the substance was shown to be mutagenic in an adenine-dependent strain of *Neurospora crassa*, in barley and rice. Ethylene oxide induces chromosomal aberrations and sister chromatid exchange and the substance yields positive results in the micronucleus test and the dominant lethal test. The genotoxic effects of ethylene oxide in mammalian cells exposed *in vitro* or *in vivo* have been summarised in detail by IARC (1994, 2008).

More recently, mechanisms of ethylene oxide mutagenicity were investigated by Tompkins *et al* (2009) in the pSP189 shuttle vector being replicated in human Ad293 cells. When plasmids containing, after contact with ethylene oxide, up to 29 000 7-hydroxyethylguanine (HOEtG) adducts per 10^8 nucleotides were evaluated, no other ethylene oxide-derived DNA adducts could be detected using sensitive LC-MS/MS methods, and no increases in mutations were found. Only when very high exposures to ethylene oxide were used, could levels of *N*1-hydroxyethyladenine, *O*6-hydroxyethylguanine and *N*3-hydroxyethyluracil be measured, and significant increases in mutations became apparent. The half-lives of the latter adducts were much shorter than that of HOEtG owing to active DNA repair processes. It was concluded that ethylene oxide represented a relatively weak mutagen (Tompkins *et al* 2009, for discussion see also Swenberg *et al* 2011).

2.6.2. In vivo – Human data

Many studies have been carried out to evaluate the effect of exposure to ethylene oxide on the incidences of chromosomal aberrations (including micronuclei) and sister chromatid exchange in peripheral blood lymphocytes of workers exposed occupationally to ethylene oxide. These include workers at hospital and factory sterilisation units and those working at ethylene oxide manufacturing and processing plants. The results, as summarised by IARC (1994), show that ethylene oxide induces chromosomal damage in exposed humans. In general, the degree of damage is correlated with the level and duration of exposure. The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is the formation of adducts, chromosomal aberrations or micronuclei. Alkali-labile sites and DNA single-strand breaks were not observed in lymphocytes of sterilisation workers, but the induction of DNA cross-linking was reported in one study.

The length of time that an individual is exposed to ethylene oxide may be an important factor in determining the relationship between genetic effects and exposure (Thiess *et al* 1981).

Four informative studies (Yager *et al* 1983, Stolley *et al* 1984, Galloway *et al* 1986, Tates *et al* 1991a) of genetic end-points in exposed workers were especially highlighted by IARC (1994), as follows.

Yager *et al* (1983) reported an increased incidence of sister chromatid exchange in peripheral blood lymphocytes of 14 hospital sterilisation workers exposed to 1 ppm ethylene oxide (8-h TWA) over that in 13 unexposed controls. In order to evaluate the relationship between exposure and sister chromatid exchange induction, workers were divided into a high-exposure group (five subjects) and a low-exposure group (nine subjects) on the basis of a six-month cumulative dose of ethylene oxide determined by measuring air concentrations during specified tasks and multiplying this value by the number of times each task was performed. The high-exposure group, which received an average cumulative dose of 501 mg ethylene oxide, showed a significant increase in sister chromatid exchange frequency over that in controls and in the low-exposure group (average cumulative dose, 13 mg ethylene oxide). Sister chromatid exchange frequency did not differ significantly between the low exposure group and the controls.

Tates *et al* (1991a) compared the frequencies of sister chromatid exchange in 9 hospital workers and 15 workers from factory sterilisation units occupationally exposed to ethylene oxide and in two respective control groups matched for age, sex and smoking habits (8 donors from administrative personnel working in the neighbourhood and 15 from the same factory). Exposure was measured by gas chromatography in the sterilisation rooms (20–25 ppm) and in front of the steriliser after opening (mean, 50 ppm) for the hospital workers and was monitored during four months (period covering the erythrocyte lifespan) for the factory workers. Additionally, HOEtVal concentrations were determined in two laboratories. Sister chromatid exchanges were analysed in independent cultures in two different laboratories. The mean frequency of sister chromatid exchange was significantly elevated by 20 % in the hospital workers and by almost 100 % in the factory workers; moreover, the frequency was clearly greater in daily than in occasionally exposed workers in the factory population.

Other investigators have also reported increased incidences of sister chromatid exchange in lymphocytes of workers exposed to ethylene oxide in hospital sterilisation units. The results from two studies showed that sister chromatid exchanges were not induced in workers who were exposed to less than 1 ppm ethylene oxide (see IARC 1994).

In a longitudinal study (Stolley *et al* 1984), 61 sterilisation workers from three work sites were evaluated for induction of sister chromatid exchange at 6, 12 and 24 months. At work site I there was low exposure (0.5 ppm TWA), at work site II there was moderate exposure (5–10 ppm) and at worksite III there was high exposure (5–20 ppm at the time of sampling; action had been taken six months previously to reduce the TWA from a range of 50 to 200 ppm). Workers at each site were further divided with regard to low and high potential for exposure on the basis of job classification and proximity to steriliser operations and controls. Controls were primarily randomly selected site personnel (53) considered to have no exposure; community controls (29) were also included. Initial exposures were confirmed by measurements of ethylene oxide in breathing zones. After the initial sampling, blood was taken at each of three sampling times at the work sites and at 6 and 18 months for community controls. The effects on sister chromatid exchange frequency of age, sex, smoking habits and cytogenetic scores were taken into account. The results showed no increase in sister chromatid exchange frequency for any exposure at work site I or for the workers with potentially low exposure at work site II. Pair-wise comparisons between groups at work site II indicated that the group with potentially high exposure had significantly higher mean frequencies of sister chromatid exchange than the group with potentially low exposure initially ($p = 0.003$), at 12 months ($p < 0.001$) and at 24 months ($p = 0.023$). Similarly, the differences in mean sister chromatid exchange frequency between the group with potentially high exposure and control groups were increased significantly initially and at 12 and 24 months ($p = 0.011$, $p < 0.001$ and $p = 0.018$, respectively). At work site III, the mean sister chromatid exchange frequency in the group with potentially low exposure differed significantly from those in the work site control group at the initial ($p = 0.024$) and six-month ($p = 0.008$) testings, but not subsequently. Subjects at work site III did not continue to receive exposure after the initial blood samples were taken. These results indicate that the induction of sister chromatid exchange in workers exposed to ethylene oxide is related to the concentration of ethylene oxide at the workplace and that it persists up to six months after cessation of exposure.

Galloway *et al* (1986) evaluated chromosomal aberration frequencies in lymphocytes from the same group studied by Stolley *et al* (1984). The results showed no increase in chromosomal aberration frequencies at work sites I or II in any of the samples. Frequencies were significantly elevated in two samples from the group with potentially high exposure at work site III in comparison with controls and with the group with potentially low exposure taken at 6 and 24 months. Significance was achieved for total structural aberrations only at 24 months ($p = 0.0.18$) and when data were pooled over time ($p = 0.003$). The 24-month samples from the group with potentially low exposure at work site III had significantly higher numbers of chromosomal aberrations than those from the community controls but not those from the site controls. The authors indicated that the work site III controls may have been exposed accidentally to low levels of ethylene oxide during a leak in 1980, which would account for the higher levels of aberrations than in the other work site controls. The effects of possible confounding variables on the control aberration frequencies were analysed: There was no detectable effect of sex; smoking and age had small but significant effects on the frequencies of chromatid aberrations and chromosomal exchanges, respectively. Exposures at levels of 5 ppm or above (8-h TWA) are thus required for ethylene oxide to induce chromosomal aberrations in peripheral blood lymphocytes. Similar results were reported from other studies (see IARC 1994).

Tates *et al* (1991a) reported a significant increase in the frequency of micronuclei in lymphocytes from factory workers exposed to ethylene oxide at concentrations ranging from 14 to 400 ppm; the 40-h TWA was estimated to be 5 ppm on the basis of measurements of Hb adducts. Other studies showed no significant increase in the

incidence of micronuclei in lymphocytes from workers exposed to ethylene oxide (IARC 1994).

Associations between different genetic end-points were analysed in two studies. Galloway *et al* (1986) reported a weak overall association between the frequencies of chromosomal aberrations and sister chromatid exchange in 61 employees in three work sites and in 304 unexposed controls. The correlation was significant ($p < 0.001$) in potentially exposed groups but not in control groups, and, for any individual, one observation could not be used to predict the other. Tates *et al* (1991a) confirmed the correlation ($p < 0.001$) between chromosomal aberration and sister chromatid exchange frequencies in pooled data for 9 hospital and 15 factory workers. Additionally, sister chromatid exchange frequencies were shown to correlate better with HOEtVal levels than with chromosomal aberration frequencies which, in turn, correlated better with HOEtVal levels than with micronucleus formation frequency.

hprt Mutations were found in circulating lymphocytes of factory workers exposed to ethylene oxide in a study by Tates *et al* (1991b). The sensitivity of this end-point was considered to be lower than that of Hb adducts and cytogenetic end-points. In the discussion of their data, Tates *et al* (1991a) also pointed to earlier data of Hagmar *et al* (1988), showing that neither chromosomal aberrations nor micronuclei were significantly enhanced at exposure levels of 1–2 ppm. Another study of the micronucleus frequency in lymphocytes and in exfoliated cells of the nose and mouth from subjects exposed to very low levels of ethylene oxide (TWA of 0.02–0.3 ppm) showed no increase over the controls (Sarto *et al* 1991).

Taking these studies together, it is clear that occupational ethylene oxide exposure can lead to genotoxic damage in exposed humans. Chromosomal aberrations could be established at exposure levels of 5 ppm and above. At exposures of 1 ppm, no clear cytogenetic changes could be established so far.

2.6.3. In vivo – Animal data

In peripheral lymphocytes of monkeys exposed to ethylene oxide (100 or 50 ppm, 7 h/day, 5 days/week for 24 months, 12 animals per group), a significant increase in chromosomal aberrations and sister chromatid exchange was seen in both dose groups. In another inhalation study carried out with rats (100, 33 and 10 ppm, 6 h/day, 5 days/week for 24 months), no increase in chromosomal aberrations was seen in the exposed animals (for details, see DFG 1993).

Apurinic/apyrimidinic sites (AP) that result from chemical or glycosylase-mediated depurination of ethylene oxide-induced DNA adducts may be an additional mechanism leading to mutations and chromosomal aberrations. Therefore, Rusyn *et al* (2005) tested the hypothesis that ethylene oxide exposure results in the accumulation of AP sites and induces changes in expression of genes for base excision DNA repair. Male Fischer 344 rats were exposed to 100 ppm ethylene oxide by inhalation for 1, 3 or 20 days (6 h/day, 5 days a week). Animals were sacrificed 2 h after exposure for 1, 3 or 20 days, as well as 6, 24 and 72 h after a single-day exposure. Experiments were performed with tissues from brain, spleen and liver. Exposure to ethylene oxide resulted in time-dependent increases in *N*7-(2-hydroxyethyl)guanine in brain, spleen, and liver and *N*7-(2-hydroxyethyl)valine (HOEtVal) in globin. No increase in the number of aldehydic DNA lesions, an indicator of AP sites, was detected in any of the tissues between controls and ethylene oxide-exposed animals, regardless of the duration or strength of exposure. Ethylene oxide exposure led to a 3–7-fold decrease in expression of 3-methyladenine-DNA glycosylase (Mpg) in brain and spleen in rats exposed to ethylene oxide for 1 day. Expression of 8-oxoguanine DNA glycosylase, Mpg, AP endonuclease, polymerase beta and alkylguanine methyltransferase were

increased by 20–100 % in livers of rats exposed to ethylene oxide for 20 days. These data were interpreted to suggest that DNA damage induced by exposure to ethylene oxide is readily repaired without accumulation of AP sites (Rusyn *et al* 2005).

Ethylene oxide is not only effective at producing somatic cell mutations but also at inducing genetic damage in germ cells in whole mammals, although the mechanism by which it produces genetic lesions in germ cells is uncertain (Dellarco *et al* 1990).

2.7. Quantitative aspects of DNA adduct formation, related to genotoxicity

Ethylene oxide is produced endogenously via its precursor ethylene in the physiological metabolism of the body, due to oxidative breakdown of proteins and/or lipids (Kessler and Remmer 1990, Czanády *et al* 2000). Formation and persistence of the major DNA adduct, *N*7-(2-hydroxyethyl)guanine (HOEtG), have been studied for a number of years, and the methodologies for determination of DNA adducts of ethylene oxide have been continuously refined (for details, see IARC 1994, 2008, Thier and Bolt 2000).

Levels of HOEtG in target and in non-target tissues were found surprisingly similar (Walker *et al* 1990, 1992, Bolt *et al* 1997, Wu *et al* 1999), and it was concluded that the tissue/organ specificity for tumour induction by ethylene oxide should primarily be due to factors other than DNA-adduct formation (Walker *et al* 1990). This is reflected by the diverse picture of tumours induced by ethylene oxide in rodents which cannot be interpreted only on toxicokinetic and metabolic grounds, and thus also points to further tissue-specific factors. It is also in accordance with the assumption of a non-linearity in the exposure-carcinogenic response relationship, a pivotal issue in the most recent quantitative cancer potency estimate of Kirman *et al* (2004).

According to recent data, HOEtG is rapidly repaired from the DNA and does therefore not accumulate upon subacute or subchronic exposure (Rusyn *et al* 2005, Marsden *et al* 2007).

Taking all available evidence together and based on PBPK modelling, Csanády *et al* (2000) calculated a range of endogenous formation of ethylene oxide in humans between 0.4 and 11 nmol/h per kg body weight. According to Zhao and Hemminki (2002), the mean DNA adduct level in lymphocytes of (non-smoking) humans is 3.0–3.8 adducts per 10^7 nucleotides. In granulocytes of non-exposed persons (5 never smokers, values unadjusted) the mean ± SE was 10.8 ± 7.0 HOEtG adducts per 10^7 nucleotides (Yong *et al* 2007). These values are basically consistent with data of others (see IARC 1994, 2008). In rats (different organs examined) Marsden *et al* (2007) found a physiological background of 0.11–0.35 HOEtG adducts per 10^7 nucleotides in rat tissues, which is lower than that observed in humans (see above).

Based on the available experimental material, including comparisons between adduct data for unexposed and ethylene-exposed animals, the dose-response curves for biomarkers in ethylene oxide-exposed rats and mice and results of the rodent carcinogenicity studies, Walker *et al* (2000) concluded that under normal conditions too little ethylene oxide arises from possible ethylene exposure to produce a significant mutagenic or carcinogenic response.

DNA adduct-exposure relation at low levels

In earlier publications it was noted that the observed background HOEtG levels in rats would correspond to exogenous ethylene oxide exposures of about 1 ppm (Wu *et al* 1999, van Sittert *et al* 2000). However, it must be noted that the analytical techniques for adduct detection were gradually refined throughout the last 20 years.

Using the technically most advanced liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique for the quantitation of ethylene oxide-induced DNA adducts, Marsden *et al* (2007) established the above mentioned background adduct levels of HOEtG in tissues of non-exposed rats. The HOEtG adduct levels gradually increased with administration of a single or of three daily doses (i.p.) of 0.1 and 1.0 mg/kg body weight of ethylene oxide; at the lowest dose of 0.01 mg/kg there was no difference of total HOEtG adduct levels as compared to those detected in control animals. It was concluded that at this lowest dose any DNA adduct increase was negligible as compared to the endogenous damage already present. At higher doses, the adduct response was not significantly non-linear. From the repeated dose study it was concluded that the DNA damage did not accumulate with repeated ethylene oxide administration (Marsden *et al* 2007). In a subsequent study, the authors obtained evidence that administration of ethylene oxide at high doses increased the endogenous formation of ethylene oxide and associated adducts, probably triggered by oxidative stress (Marsden *et al* 2009).

Using gas chromatography-electron capture mass spectrometry (GC-EC-MS) of Kao and Giese (2005), Yong *et al* (2007) studied a group of steriliser operators exposed to less than 1 ppm ethylene oxide and non-exposed controls from 10 hospitals. From an earlier study (Schulte *et al* 1992) it was anticipated that the levels of ethylene oxide induced haemoglobin adducts were associated with ethylene oxide exposures in these groups. The study of Yong *et al* (2007) examined HOEtG adduct levels in granulocytes, in relation to actual (8-h TWA) exposures and to cumulative exposures (ppm-hour) during a 4-month period before sample collection. There was a considerable inter-individual variability in HOEtG adduct levels, with a range of 1.6-241.3 adducts/10^7 nucleotides, the most prominent confounder being cigarette smoking. Differences between the exposure groups (non-exposed; lower exposure: < ppm-h cumulative or 0.03 ± 0.05 ppm 8-h TWA; higher exposure: >32 ppm-h cumulative or 0.36 ± 0.31 ppm 8-h TWA), however, were not statistically significant. The unadjusted HOEtG levels per 10^7 nucleotides for never smokers within these groups were as follows: for non-exposed (n = 5) 10.8 ± 7.0, for the lower exposure group (n = 21) 18.2 ± 6.6, and for the higher exposure group (n = 8) 11.1 ± 4.4. Thus, it may be concluded that human workplace exposures within the range that was studied (higher dose: 0.36 ± 0.31 ppm 8-h TWA) do not lead to significant elevation of the HOEtG adduct levels in granulocytes over the endogenous background.

2.8. Carcinogenicity

2.8.1. Human data

The carcinogenicity data for ethylene oxide have been compiled and discussed in detail by IARC (1994, 2008). Reference can therefore be made to the recent evaluation by IARC (2008).

In summary, epidemiological evidence of the risk for human cancer was derived by IARC principally from the follow-up of 14 cohorts of exposed workers either in chemical plants where ethylene oxide was produced or converted into derivatives or in facilities where it was used as sterilant. Data from 10 of the cohorts were collated in a meta-analysis that was published in 1999, but this did not include updates of 2 cohorts that were published after that time. Many of the cohort members employed at chemical factories were also exposed to other chemicals. According to IARC (2008), the most informative epidemiological investigation was a study by the US National Institute for Occupational Safety and Health (NIOSH) of more than 18 000 employees at 14 industrial facilities in the USA where ethylene oxide was used to sterilise medical supplies or food spices, or to test sterilising equipment. This investigation benefited

not only from greater statistical power than other studies (as a consequence of its large size), but also from the lower potential for confounding by concomitant exposure to other chemicals and from incorporation of detailed quantitative assessment of individual ethylene oxide exposures. For these reasons, IARC (2008) gave the greatest weight to this study when assessing the overall balance of epidemiological evidence.

In its assessment, IARC (2008) focused on lymphatic and haematopoietic cancers and cancers of the breast, stomach, pancreas and brain, because these sites had been suggested by one or more epidemiological studies, or because such tumour sites were reported in rodent bioassays. Evaluation of possible risks for lymphatic and haematopoietic cancer was hampered by temporal changes and inconsistencies in the histopathological classification of diagnoses. The interpretation of results for these malignancies was constrained by the diagnostic groupings that had been used by researchers when the studies were conducted and possible errors in the specification of tumours on death certificates. On this basis, IARC (2008) found "some epidemiological evidence for associations between ethylene oxide and lymphatic /haematopoietic cancers, and specifically lymphoid tumours, such as non-Hodgkin's lymphoma, multiple myeloma and chronic lymphocytic leukaemia".

It was noted that in the most recent follow-up of the US NIOSH cohort no overall excess of deaths from non-Hodgkin's lymphoma or multiple myeloma was observed in comparison with national death rates. However, in an internal analysis, mortality from lymphoid tumours was associated with measures of cumulative exposure to ethylene oxide among men. Other studies did not point consistently to an increase for non-Hodgkin's lymphoma or multiple myeloma in comparisons with external reference populations, although moderate elevations of risk were reported in some investigations. Early reports of an excess risk for all types of leukaemia combined were not confirmed by later studies. In the latest analysis of the US NIOSH cohort, mortality for all types of leukaemia combined was close to that expected from national rates. Weak evidence for an exposure-response relationship between cumulative exposure to ethylene oxide and leukaemia was observed in a previous analysis of this cohort. Results from other cohort studies did not point clearly or consistently to an increased risk of leukaemia. Due to the limited number of cases of Hodgkin's lymphoma in published studies it was not feasible to draw meaningful conclusions.

According to IARC (2008), four of the studies provided useful information on the association between exposure to ethylene oxide and breast cancer. The US NIOSH study and a cohort of hospital sterilisation workers in the UK examined mortality from breast cancer and found no overall excess risk. Three studies examined the incidence of breast cancer: the US NIOSH study and a cohort from Sweden found no overall excess for breast cancer, while another cohort study from NY State, US, found a borderline significant excess risk of about 60 %. Cancer incidence was recognised by IARC (2008) to be underestimated in the US NIOSH study, which was thus negatively biased for the investigation of overall cancer incidence. A study conducted within the US NIOSH cohort was designed to investigate the association between exposure to ethylene oxide and the risk for breast cancer in greater detail. Internal analyses in this study found increased relative risks for breast cancer in the higher categories of cumulative exposure to ethylene oxide and a significant exposure-response relationship, both of which persisted in analyses that controlled for parity and history of breast cancer in a first-degree relative. The risk for the highest category of cumulative exposure was almost doubled.

While early epidemiological studies had suggested increased risks for stomach and pancreatic cancer in workers exposed to ethylene oxide, such findings were not supported by more recent and larger studies, nor did the balance of epidemiological evidence point to an increased risk for brain cancer in exposed humans (IARC 2008).

For details of these studies, reference is made to the recent comprehensive compilation by IARC (2008).

2.8.2. Animal data

Early studies revealed no carcinogenic effects after long-term treatment of animals with ethylene oxide. However, inadequate documentation or inappropriate experimental protocols make these reports ambiguous and unsuitable for the assessment of the carcinogenic activity of this substance (DFG 1993).

For a period of 2 years, Bär and Griepentrog (1969) fed a group of 50 young rats (own laboratory strain) a standard diet, which had been exposed to ethylene oxide gas. The ethylene oxide levels in the diet, which was exposed to the gas in weekly batches, were between 500 and 1 400 ppm on day 1 and between 53 and 400 ppm after 6 days. In this study, no increase in tumour incidence relative to the control group and no other treatment-related pathological findings were seen in the exposed animals. The experiment does not seem to have been designed as a carcinogenicity study. There was no positive control group treated with a known carcinogen so that the sensitivity of the laboratory strain for chemical carcinogenesis cannot be assessed. The incidence of spontaneous tumours in the untreated control group (4 tumours) is conspicuously low.

Subcutaneous injection of ethylene oxide dissolved in arachis oil (concentration and single doses not specified) did not lead to induction of local tumours in a group of 12 rats after a total dose of 1 000 mg/kg body weight and a treatment period of 94 days (Walpole 1958). The observation period was not specified, nor the origin of the rat strain used; the number of animals is too small to determine an unknown carcinogenic potential.

Epicutaneous treatment of 30 female Swiss-Millerton mice, three times weekly, with a 10 % solution of ethylene oxide in acetone at an estimated dose of 10 mg per application did not lead to local tumour induction (median survival time 493 days). The effective tissue dose was not specified in this report and cannot be estimated because of the high volatility of the acetone solvent and the test substance. The results are not appropriate for the assessment of the carcinogenic potential of ethylene oxide (van Duuren 1965).

In more recent studies, ethylene oxide applied in various forms to various animal species has repeatedly been shown to be carcinogenic (DFG 1993).

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice (A/J) that is highly susceptible to the development of this neoplasm, inhalation of ethylene oxide (0, 70 or 200 ppm, 6 h/day, 5 days/week for 6 months) produced a dose-related increase in numbers of pulmonary adenomas (Adkins *et al* 1986; for details, see also IARC 2008).

Groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 50 or 100 ppm ethylene oxide (6 h/d, 5 days/week for 102 weeks). Incidences of alveolar/bronchiolar carcinomas in male mice were 6/50 (control), 10/50 (low dose) and 16/50 (high dose), and the combined incidences (adenomas and carcinomas) were 11/50, 19/50 and 26/50, respectively. In females, the respective numbers of carcinomas were 0/49, 1/48 and 7/49, and of adenomas and carcinomas combined 2/49, 5/48 and 22/49 (Picut *et al* 2003; for details, see also IARC 2008).

Three different doses of ethylene oxide (single doses per animal of 1.0, 0.3 or 0.1 mg ethylene oxide dissolved in 0.1 ml tricaprylin) were administered by subcutaneous injection to groups of 100 female NMRI mice, once weekly for 95 weeks. The average

total doses per mouse were 64.4, 22.7 and 7.3 mg. In the treated groups, the incidence of animals with local tumours (mostly fibrosarcomas) was dose-dependent (11, 8 and 5 animals with tumours) and significantly higher than the incidence in the control group treated with tricaprylin (Dunkelberg 1979, 1981).

In another study (Dunkelberg 1982), ethylene oxide dissolved in oil was administered in doses of 30 or 7.5 mg/kg body weight, twice weekly by stomach tube to groups of 50 female Sprague-Dawley rats. The average total doses administered in the study, which was continued for almost 3 years, were 5 112 and 1 186 mg/kg body weight, respectively. Stomach tumours (mostly squamous cell carcinomas of the forestomach) were detected in 62 % and 16 % of the animals. In some animals carcinomas *in situ* were found. In the control group given oil alone, there were no pathological stomach lesions.

In an inhalation study, groups of 120 male and 120 female Fischer 344 rats were exposed to ethylene oxide concentrations of 100, 33 or 10 ppm, 6 hours daily, 5 days per week for about 2 years (Snellings *et al* 1984a,b). Animals were taken from the study at 6-month intervals for clinical chemical and histological studies. After 24 months, there was a significant increase in mononuclear cell leukaemia in the females of the 100-ppm group. In the females of the 33-ppm group, the incidence of this kind of tumour was also higher than in the control group. In the males of the 100-ppm group, the number of animals with peritoneal mesotheliomas was significantly increased relative to the control values. There was also a significant increase in the incidence of primary brain tumours in the males of the 100-ppm group; the incidence of these tumours in the males of the 33-ppm group and the females of the 100- and 33-ppm groups was higher than in the control group.

In another inhalation study with male Fischer 344 rats, groups of 80 animals were exposed to ethylene oxide concentrations of 50 or 100 ppm, 7 hours daily, 5 days per week for 104 weeks. The incidences of mononuclear cell leukaemia, brain tumours (gliomas) and peritoneal mesotheliomas were increased relative to the control values (Lynch *et al* 1984).

2.8.3. Published cancer risk assessments

In a British cancer mortality study, conducted on 2 876 ethylene oxide exposed persons, Coggon *et al* (2004) found no significant associations of any tumour category with ethylene oxide exposures that had occurred in the UK in recent decades (at exposures < 5 ppm TWA).

Van Sittert *et al* (2000), applying the "radiation-dose equivalent" approach of Ehrenberg *et al* (1983), concluded that exposure to 1 ppm ethylene oxide per year corresponded to a radiation dose of about 5 rad, and that this low risk of occupational exposure would be consistent with the outcome of epidemiological investigations. Considering the genotoxic effects of ethylene oxide in animals and humans, the DNA binding of other related carcinogens, the natural DNA adduct background and the genotoxicity of low energy transfer radiation they postulated that long-term occupational exposure to airborne ethylene oxide at or below 1 ppm did not "produce an unacceptable increased risk in man".

The assessment of IARC (1994) of a possible association between ethylene oxide exposure and lympho-/haematopoietic cancer mortality was the starting point of a more refined human risk assessment by Kirman *et al* (2004). Based on the meta-analysis of Teta *et al* (1999), the authors selected two epidemiology studies of sufficient size and length of follow-up, which were also considered to provide adequate exposure information to quantify an exposure-response relationship for ethylene

oxide. These were (i) the leukaemia/lymphoid tumour mortality data from the Union Carbide (UCC) cohort of Teta *et al* (1993), and (ii) the leukaemia/lymphoid tumour data from the US NIOSH study (Stayner *et al* 1993). These data were used for a quantitative cancer potency estimate. It was argued that the weight of evidence supported the use of a nonlinear assessment, and a unit risk value of 4.5×10^{-8} was derived for ethylene oxide, with a range between 1.4×10^{-8} and 1.4×10^{-7} reflecting the given uncertainty associated with a theoretical linear term at low concentrations.

In a consecutive assessment, the authors used epidemiological data on individual workers in the NIOSH (Steenland *et al* 2004) and updated UCC (Swaen *et al* 2009) occupational studies to characterise potential excess cancer risks of environmental exposure to ethylene oxide. In addition to refined analyses of the separate cohorts, the power was increased by analysing the combined cohorts. It was noted that in previous SMR analyses of the separate studies and the present analyses of the updated and pooled studies of over 19 000 workers, none of the SMRs for any combination of the 12 cancer endpoints and six sub-cohorts analysed were statistically significantly greater than one including leukaemia, lymphohaematopoietic tissue, lymphoid tumours, non-Hodgkin's lymphoma (NHL), and breast cancer. In the present study, no evidence of a positive cumulative exposure-response relationship was found. Fitted Cox proportional hazards models with cumulative ethylene oxide exposure did not have statistically significant positive slopes. This lack of dose-response was corroborated by categorical analyses. Considering these limitations, Cox model estimates of the concentrations corresponding to a 1×10^{-6} extra environmental cancer risk were made for possible environmental regulatory use. There were all greater than approximately 1 ppb (0.001 ppm). The weakness of the existing database and the resulting uncertainties were indicated (Valdez-Flores *et al* 2010).

On request by SCOEL, the authors translated their results to concentrations relevant to occupational settings. For this purpose, the estimated occupational ethylene oxide exposure concentrations corresponding to specified extra risks were calculated for lymphoid mortality as the most appropriate endpoint, despite the existing lack of a statistically significant exposure-response relationship. Estimated concentrations were for occupational exposures [40 years of occupational inhalation exposure to ethylene oxide from age 20 to age 60 years]. The estimated occupational inhalation exposure concentrations (ppm) corresponding to specified extra risks of lymphoid mortality to age 70 years in a population of male and female ethylene oxide workers were based on Cox proportional hazards models of the most recent updated epidemiology cohort mortality studies of ethylene oxide workers (Valdez-Flores *et al* 2010) and a standard life-table calculation. An occupational exposure at an inhalation concentration of 2.77 ppm ethylene oxide was estimated to result in an extra risk of lymphoid mortality of 4 in 10 000 (0.0004) in the combined worker population of men and women from the two studies. The corresponding estimated concentration decreased to 2.27 ppm when based on only the men in the updated cohorts combined. The authors pointed out that the difference in these estimates reflected the difference between combining all of the available data or focusing on only the men and excluding the women who did not show an increase in lymphoid mortality with ethylene oxide inhalation exposure. The results of sensitivity analyses using other mortality endpoints (all lymphohaematopoietic tissue cancers, leukaemia) were considered to support the choice of lymphoid tumour mortality for estimation of an ethylene oxide-induced extra risk (Valdez-Flores *et al* 2011).

By contrast to these risk assessments, which were primarily based on data from human investigations in the peer-reviewed literature, a recent risk assessment issued by the German Ausschuß für Gefahrstoffe (AGS 2011) has exclusively used experimental tumour data. Taking a BMD_{10} for lung tumours in mice of 19.44 ppm as "point of departure", consistently higher total human cancer risks (of 4:1 000 at 2.36

mg/m^3, with linear extrapolation to lower exposure orders of magnitude) were derived. However, at the same time AGS also stated that the quantitative relevance of these mouse lung tumours for humans was unclear, as this target for ethylene oxide had not been confirmed for humans epidemiologically or for rats experimentally. It was also stated by AGS that the main rat tumours, peritoneal mesotheliomas and MNC leukaemias, had obviously little relevance for humans, and that the carcinogenic potency of ethylene oxide, related to distinct target tissues, appeared to be low in humans.

Because of this weakness in basing a quantitative cancer risk assessment for ethylene oxide on experimental tumour data, SCOEL considers an assessment based on human studies as more appropriate for the situation at human workplaces (see *Recommendation section*).

2.9. Reproductive toxicity

2.9.1. Human data

An increase in the incidence of spontaneous abortion from 5.6 % (control group) to 16.7 % for women exposed occupationally to ethylene oxide during pregnancy (1 068 pregnant women) was revealed in a study by questionnaire (Hemminki *et al* 1982); in groups of pregnant women exposed to formaldehyde or glutaraldehyde, the adjusted incidences were not different from those in the control group.

2.9.2. Animal data

Teratogenic effects in the skeletal system of rat foetuses were seen when pregnant dams were given ethylene oxide doses of 150 mg/kg by intravenous injection on days 6, 7 and 8 of gestation (La Borde and Kimmel 1980). The 75-mg/kg dose did not have such effects. In preliminary studies, an intravenous dose of 200 mg/kg was shown to be lethal.

Exposure of male and female rats to ethylene oxide concentrations of 100, 33 or 10 ppm, 6 hours daily, 5 days per week for 12 weeks before mating, and of the females until day 19 of gestation and then during lactation resulted in preimplantation and postimplantation losses only in the 100-ppm group. Survival of the F_I generation was unaffected (Snellings *et al* 1982a). Similarly, exposure of pregnant rats to 100, 33 or 10 ppm for 6 hours daily from day 6 to day 15 of gestation did not have any teratogenic effects (Snellings *et al* 1982b).

3. Recommendations

The relevant endpoint for discussion of limiting occupational exposures to ethylene oxide is its carcinogenicity.

Ethylene oxide is a weak alkylating agent that is directly mutagenic and carcinogenic. After external exposure it is distributed within the entire organism, and in quantitative terms DNA alkylation is relatively uniform in the body. This affects in a similar way tumour target and non-target tissues. The carcinogenicity is clearly evident from animal experiments. In rats (Fischer 344), ethylene oxide has induced brain tumours, mononuclear cell leukaemias and peritoneal mesotheliomas, in mice (B6C3F1) lung adenomas and carcinomas. In long-term experiments, significant carcinogenic effects were seen upon repeated daily inhalation of 33 ppm and 100 ppm ethylene oxide. The carcinogenicity of ethylene oxide is reasonably connected with its DNA alkylating and resulting genotoxic properties. IARC (2008) has assessed ethylene oxide to be

carcinogenic for humans ("Group 1"), rating the epidemiological evidence itself as being limited, but considering further elements of mechanism/mode of action and "other relevant data".

Peritoneal mesotheliomas represent a quantitatively major malignancy induced in rats by chemical carcinogens (e.g. acrylamide). In humans, such tumours can be induced by asbestos, but not by such chemicals. There are no epidemiological indications, whatsoever, of such a target site for ethylene oxide-induced carcinogenesis in humans. There are, however, indications of haematopoietic/lymphatic cancer in humans that must be taken seriously, as evaluated by IARC (2008). No consistent evidence in humans could be found for brain, lung and mammary tumours. The available data on human haematopoietic/lymphatic cancer were therefore used as a starting point of published quantitative risk assessments (Kirman *et al* 2004, Valdez-Flores *et al* 2010, 2011).

A unique feature for ethylene oxide is that low levels of this chemical are produced endogenously by both the human and animal organism, and ethylene oxide represents therefore a physiological body constituent. In experimental animals, repeated exposures to 10 ppm resulted in statistically elevated DNA adducts, the main adduct being *N*7-(2-hydroxethyl)guanine (HOEtG). In earlier publications, the physiological background of HOEtG in rats was estimated to correspond to repeated exogenous exposures at 1 ppm ethylene oxide, but during the last 20 years there have been gradual improvements of the methods of adduct detection and quantitation (see Section 2.7). The most recent experimental study of Marsden *et al* (2007; see Section 2.7), using LC-MS/MS, arrived at the conclusion that at a single or repeated dose (i.p.) to rats of 0.01 mg/kg of ethylene oxide any DNA adduct increase was negligible, compared to the endogenous damage already present. The extent of DNA damage was linear with the dose applied, and the damage did not accumulate with repeated ethylene oxide administration. The transpolation of these data into a human risk assessment is a matter of current research, and more work into the underlying mechanisms will be needed to arrive at valid risk conclusions (Swenberg *et al* 2011). One problem is that experimental ethylene oxide exposures of rodents lead to a relatively uniform distribution of HOEtG adducts in the DNA of both target and non-target organs for carcinogenicity, another problem is that promutagenic adducts other than HOEtG are detected only after very high experimental exposures.

Human field data of Yong *et al* (2007) indicated that human workplace exposures within the studied range of 0.36 ± 0.31 ppm 8-h TWA did not lead to significant elevation of the HOEtG adduct levels in granulocyte DNA over the endogenous background (see Section 2.7). This could suggest that the genotoxic risk of ethylene exposures at lower levels ought to be practically negligible. At higher concentrations, ethylene oxide exposures have led to genotoxic damage in occupationally exposed humans. Cytogenetic signs of genotoxicity were visible at exposure levels of 5 ppm and above. At exposures of 1 ppm, no genotoxic changes could be directly established in exposed humans so far (see Section 2.6.2).

Although a non-linear dose-response (genotoxicity) relationship can reasonably be assumed based on arguments of mode of action (Kirman *et al* 2004), a definite no-effect level based on dose-response data cannot be defined. In this situation, SCOEL provisionally categorises ethylene oxide into "Group B" as a genotoxic carcinogen, for which a threshold is not sufficiently supported (Bolt and Huici-Montagud 2008).

This situation calls for a quantitative cancer risk assessment. Because of the argumentation given above (Section 2.8.3) this should preferably be based on epidemiological data on haematological malignancies. The most recent assessment based on studies in approximately 20 000 workers of the combined NIOSH and

UCC/Dow cohorts by Valdez-Flores *et al* (2011) was considered by SCOEL as most reliable at present. The data (for males and females combined) are compiled in Table 1.

There is a solid data base for biological monitoring, based on HOEtVal haemoglobin adduct monitoring. Boogaard (2002) has compiled the argumentations and elements for establishing biological limit values (BLV). A derivation was performed by Boogaard *et al* (1999) for exposure conditions of an 8-h TWA of 0.5 ppm, corresponding to a HOEtVal level of 3.2 nmol per g globin. In consequence, a TWA of 0.1 ppm would correspond to a biological haemoglobin adduct value of 0.64 nmol (640 pmol) HOEtVal/g globin. Other extrapolated figures are included in Table 1. The values given in Table 1 can still be distinguished from the general background value of about 20 pmol HOEtVal/g globin.

A skin notation is warranted, as clear signs of systemic toxicity were reported after local application of ethylene oxide (Section 2.3).

Table 1. Occupational exposure concentrations (TWA; exposure for working lifetime) and haemoglobin adduct values corresponding to specified extra risks of lymphoid cancer mortality for males and females combined, according to the evaluation of Valdez-Flores *et al* (2011).

Extra risk	Corresponding TWA (ppm)	Corresponding Hb adducts (nmol HOEtVal/g globin)
4×10^{-3}	21.35	136.6
1×10^{-3}	6.58	42.1
4×10^{-4}	2.77	17.7
1×10^{-4}	0.712	4.56
4×10^{-5}	0.286	1.83
1×10^{-5}	0.072	0.46

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