ETHYLENE OXIDE

This substance was considered by previous Working Groups in February 1976 (IARC, 1976), June 1984 (IARC, 1985), March 1987 (IARC, 1987) and February 1994 (IARC, 1994). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

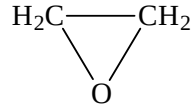
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

From IARC (1994) and IPCS-CEC (2001)
Chem. Abstr. Serv. Reg. No.: 75-21-8
Replaced CAS Reg. No.: 19034-08-3; 99932-75-9
Chem. Abstr. Name: Oxirane
IUPAC Systematic Name: Oxirane
RTECS No.: KX2450000
UN TDG No.: 1040
EC Index No.: 603-023-00-X
EINECS No.: 200-849-9
Synonyms: Dihydrooxirene; dimethylene oxide; EO ; 1,2-epoxyethane; epoxyethane; ethene oxide; EtO; ETO; oxacyclopropane; oxane; oxidoethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_4O Relative molecular mass: 44.06

1.1.3 *Chemical and physical properties of the pure substance*

From IARC (1994), Dever *et al.* (2004), Lide (2005), Rebsdat and Mayer (2005) and O'Neil (2006), unless otherwise specified

(a) *Description*: Colourless gas
(b) *Boiling-point*: 13.2 °C at 746 mm Hg [99.4 kPa]; 10.4–10.8 °C at 760 mm Hg [101.3 kPa]
(c) *Freezing-point*: –111 °C
(d) *Density* (*liquid*): 0.8824 at 10 °C/10 °C
(e) *Spectroscopy data*: Infrared [prism, 1109] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
(f) *Solubility*: Soluble in water, acetone, benzene, ethanol and diethyl ether
(g) *Vapour pressure*: 145.6 kPa at 20 °C (Hoechst Celanese Corp., 1992)
(h) *Relative vapour density (air = 1)*: 1.5 at 20 °C (IPCS-CEC, 2001)
(i) *Stability*: Reacts readily with acids; reactions proceed mainly via ring opening and are highly exothermic; explosive decomposition of vapour may occur at higher temperatures if dissipation of heat is inadequate.
(j) *Lower explosive limit*: 2.6–3.0% by volume in air
(k) *Octanol-water partition coefficient*: log P_{ow}, –0.30 (Sangster, 1989)
(l) *Flash-point*: Flammable gas (IPCS-CEC, 2001)
(m) *Inflammability limits in air*: 2.6–99.99% (V) (Shell Chemicals, 2005)
(n) *Autoignition temperature*: 428 °C (Shell Chemicals, 2005)
(o) *Dynamic viscosity*: 0.41 mPa at 0 °C (Shell Chemicals, 2005)
(p) *Conversion factor*: $mg/m^3 = 1.80 \times ppm$[1]

1.1.4 *Technical products and impurities*

Ethylene oxide for use as a fumigant and sterilizing agent used to be available in mixtures with nitrogen, carbon dioxide or dichlorodifluoromethane. Mixtures of 8.5–80% ethylene oxide/91.5–20% carbon dioxide (Allied Signal Chemicals, 1993) and 12% ethylene oxide in dichlorodifluoromethane were commonly used. As a result of concern about the role of chlorofluorocarbons in the depletion of stratospheric ozone and the phase-out of dichlorofluoromethane under the Montreal Protocol, the fluorocarbon materials now used to make blends of non-flammable ethylene oxide sterilants are hydrochlorofluorocarbons, hydrofluorocarbons and other flame-retardant diluent gases (Dever *et al*., 2004).

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 ºC) and pressure (101.3 kPa)

1.1.5 *Analysis*

Ethylene oxide in air can be determined by packed column gas chromatography (GC) with an electron capture detector (ECD) (NIOSH Method 1614), with an estimated limit of detection of 1 µg ethylene oxide per sample (National Institute for Occupational Safety and Health, 1987). A similar method is reported by the Occupational Safety and Health Administration in the USA (Tucker & Arnold, 1984; Cummins *et al*., 1987). In a similar method reported by the Canadian Research Institute for Health and Safety at Work (IRSST Method 81-2), the sample is absorbed on an active charcoal tube (SKC ST-226-36), desorbed by benzylic alcohol and analysed by GC/flame ionization detection (FID) (IRSST, 2005).

In another technique (NIOSH Method 3702), a portable gas chromatograph is used with a photoionization detector or photoacoustic detector (IRSST 39-A). The sample is either drawn directly into a syringe or collected as a bag sample; it is then injected directly into the gas chromatograph for analysis. The estimated limit of detection of this method is 2.5 pg/mL injection (0.001 ppm [0.002 mg/m^3]) (National Institute for Occupational Safety and Health, 1998).

Passive methods use derivatization techniques that convert ethylene oxide to 2-bromoethanol followed by GC/ECD analysis or collect ethylene oxide in acidic solution (in which it is converted to ethylene glycol) or on a selective membrane followed by colorimetric analysis (Kring *et al*., 1984; Puskar & Hecker, 1989; Puskar *et al*., 1990, 1991; Szopinski *et al*., 1991).

Methods for the analysis and quantification of ethylene oxide in emissions from production plants and commercial sterilizers by GC/FID have been reviewed (Steger, 1989; Margeson *et al*., 1990).

Ethylene oxide has been measured in alveolar air and blood (Brugnone *et al*., 1986). Several methods have been reported for the determination of *N*-(2-hydroxyethyl) adducts with cysteine, valine and histidine in haemoglobin: a radioimmunological technique, a modified Edman degradation procedure with GC/mass spectrometry (MS), a GC method with selective ion monitoring MS and a GC/ECD method (Farmer *et al*., 1986; Bailey *et al*., 1987; Bolt *et al*., 1988; Föst *et al*., 1991; Hagmar *et al*., 1991; Kautiainen & Törnqvist, 1991; Sarto *et al*., 1991; van Sittert *et al*., 1993; Schettgen *et al*., 2002).

Methods have been reported for the detection of residues of ethylene oxide used as a sterilant: headspace GC (Marlowe *et al*., 1987) and GC (Wojcik-O'Neill & Ello, 1991) for the analysis of medical devices; capillary GC for the analysis of drugs and plastics (Danielson *et al*., 1990); and headspace GC for the analysis of packaging materials and ethylene oxide in ethoxylated surfactants and demulsifiers (Dahlgran & Shingleton, 1987). Methods have also been developed for the determination of ethylene oxide residues in processed food products. In one such method, ethylene oxide is converted to ethylene iodohydrin and analysed by GC/ECD (Jensen, 1988).

1.2 Production and use

1.2.1 *Production*

Production of ethylene oxide began in 1914 by the chlorohydrin process, the main method used until 1937, in which ethylene chlorohydrin is converted to ethylene oxide by reaction with calcium oxide. The production of ethylene chlorohydrin resulted in the formation of two main organochlorine by-products, 1,2-dichloroethane and bis(2-chloroethyl)ether (see IARC, 1999a). Ethylene chlorohydrin was produced in either the same or a separate unit and was pumped over to the ethylene oxide production sector. The chlorohydrin process for the production of ethylene oxide was inefficient, because most of the chlorine that was used was lost as calcium chloride. Since 1931, that process has gradually been replaced by the direct vapour-phase oxidation process, in which ethylene is oxidized to ethylene oxide with air or oxygen and a silver catalyst at 10–30 atm (1–3 MPa) and 200–300 ºC (Dever *et al*., 2004; Anon., 2005).

In 2002, ethylene oxide was produced in more than 30 countries in Asia, Australia, Europe, the Middle East, North America and South America with a production capacity per year of 16.3 million tonnes (Lacson, 2003). Worldwide consumption of ethylene oxide was 14.7 million tonnes in 2002 (Dever *et al*., 2004) and 18 million tonnes in 2006 (Devanney, 2007). Table 1 shows the number of producers by region as well as the production levels of ethylene oxide in 2004; approximately 17 million tonnes of ethylene oxide were produced worldwide. Production in Canada increased from 625 000 tonnes in 1996 (WHO, 2003) to 1 084 000 tonnes in 2004.

1.2.2 *Use*

Ethylene oxide is an important raw material used in the manufacture of chemical derivatives that are the basis for major consumer goods in virtually all industrialized countries. Figure 1 gives an overview of global industry demand for ethylene oxide by application. More than half of the ethylene oxide produced worldwide is used in the manufacture of monoethylene glycol (Occupational Safety and Health Administration, 2005; Devanney, 2007). The percentage of total ethylene oxide that is used domestically to manufacture ethylene glycols varies widely between regions: North America (66%), western Europe (43%), Japan (68%) and the Middle East (99%) (Lacson, 2003).

Other derivatives of ethylene oxide include: diethylene glycol, which is used in the production of polyurethanes, polyesters, softeners (cork, glue, casein and paper), plasticizers and solvents and in gas drying; triethylene glycol, which is used in the manufacture of lacquers, solvents, plasticizers and humectants (moisture-retaining agents) and in gas drying; poly(ethylene) glycols, which are reacted with other materials and used in cosmetics, ointments, pharmaceutical preparations, lubricants (finishing of textiles, ceramics), solvents (paints and drugs) and plasticizers (adhesives and printing inks); ethylene glycol ethers, which are frequently a component of brake fluids, detergents and solvents (paints and lacquers) and are used to treat natural and refinery gas; ethanolamines,

which are used in textile finishing, cosmetics, soaps, detergents and natural gas purification; and ethoxylation products of fatty alcohols, fatty amines, alkyl phenols, cellulose and poly(propylene) glycol, which are used in the production of detergents and surfactants (non-ionic), biodegradable detergents, emulsifiers and dispersants (Occupational Safety and Health Administration, 2005; Devanney, 2007).

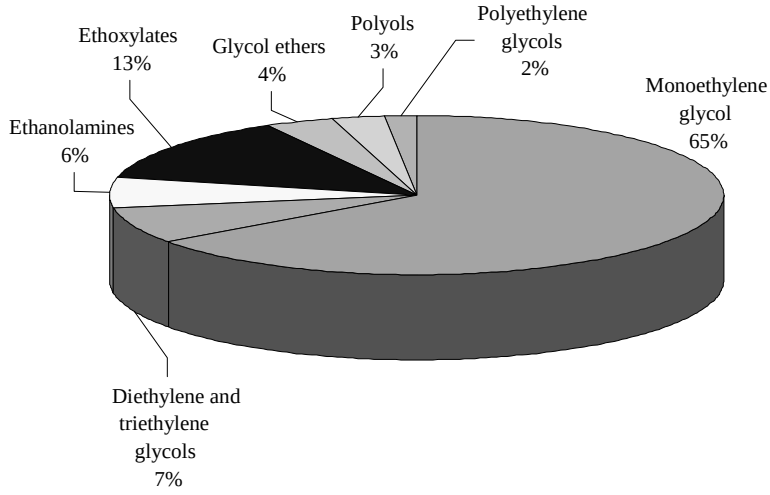
A very small proportion (0.05%) of the annual production of ethylene oxide is used directly in the gaseous form as a sterilizing agent, fumigant and insecticide, either alone or in non-explosive mixtures with nitrogen, carbon dioxide or dichlorofluoromethane (Dever *et al*., 2004).

Table 1. Production of ethylene oxide by region in 2004

Region	No. of producers	Production (thousand tonnes)
North America		
USA	10	4009
Canada	3	1084
Mexico	3	350
South America		
Brazil	2	312
Venezuela	1	82
Europe		
Belgium	2	770
France	1	215
Germany	4	995
Netherlands	2	460
Spain	1	100
Turkey	1	115
United Kingdom	1	300
Eastern Europe	NR	950
Middle East		
Iran	2	201
Kuwait	1	350
Saudi Arabia	2	1781
Asia/Pacific	>15	
China, mainland	NR	1354
China (Province of Taiwan)	4	820
India	> 2	488
Indonesia	1	175
Japan	4	949
Malaysia	1	385
Republic of Korea	3	740
Singapore	1	80

From Anon. (2004)
NR, not reported

Figure 1. Industrial products made from ethylene oxide
(globally, 17 million tonnes per annum)



From Devanney (2007)

Ethylene oxide is also used as a fumigant and sterilant for microbial organisms in a variety of applications. An estimated 9–10 million pounds [4–5 million tonnes] of ethylene oxide were used in 2002 to sterilize drugs, hospital equipment, disposable and reusable medical items, packaging materials, foods, books, museum artefacts, scientific equipment, clothing, furs, railcars, aircraft, beehives and other items (Lacson, 2003).

1.3 Occurrence

1.3.1 *Natural occurrence*

Ethylene oxide occurs endogenously as a metabolite of ethylene in certain plants and micro-organisms (see Section 4.1). Ethylene oxide can be generated from water-logged soil, manure and sewage sludge, but emissions are expected to be negligible (WHO, 2003).

1.3.2 *Occupational exposure*

Most of the data on occupational exposure are related to the production of ethylene oxide and its use in industrial and hospital sterilization.

Data were not available on exposures that are incurred outside North America and Europe, where almost half of the global amount of ethylene oxide is produced (Table 1).

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health in the USA between 1981 and 1983 indicated that 270 000 employees in the USA were potentially exposed to ethylene oxide at work (NOES, 1993). This implies that, with an estimated labour force (aged 16 years and over) of 106 million (US Census, 1980), fewer than 0.3% of employees was exposed. Of this number, 22% was estimated to be exposed to ethylene oxide and 78% to materials that contain ethylene oxide. Workers in hospitals and in the chemical and allied products industry (manufacture of plastics, synthetic materials and drugs) accounted for half of that number.

More recent data on employment in the industrial sectors that use ethylene oxide have been reported by the Occupational Safety and Health Administration (2005). Approximated employment figures were: ethylene oxide production workers, 1100; ethoxylators (use of ethylene oxide to make derivatives), 4000; and sterilant or fumigant use in hospitals, 40 000. In addition, approximately 2700 workers were employed in commercial sterilization by medical and pharmaceutical product manufacturers, in commercial sterilization by spice manufacturers, as contract sterilizers and in other sterilization and fumigation facilities.

The CAREX exposure information system (see General Remarks) includes estimates of the numbers of exposed workers in the 15 member states of the European Union in 1990–93. The estimates were based on expert judgments and did not involve exposure measurements. According to CAREX, 47 000 workers were exposed to ethylene oxide, which is under 0.1% of the 139 million employed who are covered by CAREX (Kauppinen *et al.*, 2000). The major industries in which exposure occurred were medical, dental and other health and veterinary services (22 000 exposed) and the manufacture of industrial chemicals and other chemical products (7000 exposed).

The Finnish Register of occupational exposure to carcinogens reported that 126 workers were notified as having been exposed to ethylene oxide in 2004. This is below 0.01% of the 2.4 million people employed in Finland. Most of the workers exposed were employed in medical occupations. The Register does not include any information on exposure levels, and is based on annual notifications of employers; its completeness is unknown (Saalo *et al.*, 2006).

(a) Production of ethylene oxide and its derivatives

Area samples taken in the 1960s throughout a plant in the USA where ethylene oxide was produced by direct oxidation of ethylene showed concentrations of 0–55 ppm [0–

100 mg/m^3]. On the basis of these results, the general long-term exposure of operators to ethylene oxide was estimated to be 5–10 ppm [9–18 mg/m^3] (Joyner, 1964).

Area and personal samples were taken by the National Institute for Occupational Safety and Health during 1977 and 1978 in five plants in the USA where ethylene oxide and its derivatives were produced. In most of the 95 personal samples taken, which were representative of whole shifts, the concentration of ethylene oxide was below the detection limit (which varied from 0.1 to 8 mg/m^3); a few samples contained between 1 and 148 mg/m^3. Similarly, in most area samples, the concentration was below the detection limit or was in the range < 1–1.5 ppm [2–3 mg/m^3], apart from exceptional situations such as leaks (Lovegren & Koketsu, 1977a,b,c; Oser *et al*., 1978a,b, 1979). The fact that full-shift concentrations in these plants were usually well below the standards at that time (50 ppm [90 mg/m^3]) has been attributed to three main factors: the use of completely closed systems for the storage, transfer and production of ethylene oxide; the implementation of measures to prevent fire; and operation outdoors which resulted in dilution by natural air (Morgan *et al*., 1981).

Estimates of exposure to ethylene oxide were made for a Swedish company where ethylene oxide and its derivatives were produced by the chlorohydrin process. Average exposure was estimated to be less than 25 mg/m^3 during the period 1941–47 and 10–50 mg/m^3 during the 1950s and early 1960s, with occasional peaks above the odour threshold of 1300 mg/m^3. After manufacture of ethylene oxide was stopped in this company in 1963, exposure to 1–10 mg/m^3 (with occasional higher values) continued to occur because of its use in the manufacture of other compounds (Högstedt *et al*., 1979a).

At a plant in Germany where ethylene oxide was manufactured, 2-h area samples taken in 1978–79 contained less than 5 ppm [9 mg/m^3] under normal working conditions. Concentrations rose to 1900 ppm [3400 mg/m^3] for several minutes in exceptional cases during plant breakdown (Thiess *et al*., 1981).

The typical average daily exposures of workers in a 1979 survey of plants in the USA where ethylene oxide was manufactured and used were 0.3–4 ppm [0.5–7.3 mg/m^3]; worst-case peak exposures of maintenance workers were up to 9600 ppm [17 300 mg/m^3] for less than 1 min (Flores, 1983).

In one chemical manufacturing complex in the USA, yearly time-weighted average (TWA) exposures to ethylene oxide in 1977–80 were reported to have been below 1 ppm [1.8 mg/m^3] in all jobs except loading, during which technicians were exposed to up to 1.7 ppm [3 mg/m^3] yearly and 5.7 ppm [10 mg/m^3] individually. Peak exposures were usually < 20 ppm [< 36 mg/m^3], except in loading, during which concentrations of up to 235 ppm [420 mg/m^3] were reported (Currier *et al*., 1984).

In an ethylene oxide manufacturing plant in the Netherlands, geometric mean concentrations in 8-h personal samples were calculated to be < 0.01 ppm [< 0.02 mg/m^3] in 1974, 1978 and 1980 and 0.12 ppm [0.2 mg/m^3] in 1981; individual values ranged from not detected (< 0.05 ppm [< 0.1 mg/m^3]) to 8 ppm [14 mg/m^3] (van Sittert *et al*., 1985).

At another plant in Sweden where ethylene oxide was produced by oxygenation of ethylene, the 8-h TWA exposure to ethylene oxide was 9–15 mg/m^3 [5–8 ppm] in 1963–

76 and 2–4 mg/m^3 [1–2 ppm] in 1977–82 during the production of ethylene oxide and ethylene glycol, 6 mg/m^3 [3 ppm] in 1963–76 and 2 mg/m^3 [1 ppm] in 1977–82 during the processing of ethylene oxide and 2–6 mg/m^3 [1–3 ppm] in 1963–76 and 1–3 mg/m^3 [0.6–1.7 ppm] in 1977–82 during maintenance and technical service work. Certain workers in each category were reported to have had higher exposures (up to 600–1800 mg/m^3 [333–1000 ppm]) during periods of minutes (Högstedt *et al*., 1986).

In former Czechoslovakia, the 8-h TWA concentrations of ethylene oxide measured in 1982–84 in the working environment of an ethylene oxide production plant were 0–8.25 mg/m^3 [4.6 ppm] (Karelová *et al*., 1987).

Under the sponsorship of the Chemical Manufacturers' Association, company data were collected on current exposures to ethylene oxide of workers in 11 ethylene oxide production units and 24 ethoxylation units in the USA in 1987 (Table 2). Respirators were reported to be used in specific operations, such as rail car loading and unloading, maintenance and product sampling, during which engineering controls are not feasible (Heiden Associates, 1988a).

Table 2. Exposure of workers to ethylene oxide by type of unit and job category in the chemical manufacturing industry in the USA, 1987

Unit and job category	No. of samples	8-h TWA (mg/m^3)		No. of samples	Short-term (10–150 min) exposure (mg/m^3)	
		Mean[a]	Range		Mean[a]	Range
Ethylene oxide production						
Production workers	402	0.7	0.11–3.2	171	7.7	1.62–19.8
Maintenance workers	439	1.3	0.14–5.6	59	19.6	0.20–35.3
Supervisors	123	0.2	0.04–0.18	3	1.3	1.3–1.4
Distribution workers	218	2.9	0.36–6.8	111	11.7	3.6–17.6
Laboratory workers	189	0.7	0.12–4.3	39	1.4	0.4–2.2
Other workers	97	0.2	0.05–0.72			
Ethoxylation						
Production workers	640	0.4	0.12–1.26	172	2.0	0.02–9.9
Maintenance workers	191	1.1	0.02–4.7	56	13.3	0.11–54.9
Supervisors	54	0.4	0.05–0.72	5	8.6	0.9–23.8
Distribution workers	105	0.7	0.20–2.7	100	3.4	0.9–21.6
Laboratory workers	52	0.4	0.02–0.9	19	5.0	0.4–11.0
Other workers	24	0.4	0.18–0.54			

Adapted from Heiden Associates (1988a)
TWA, time-weighted average
[a] Weighted by number of workers exposed

Gardner *et al*. (1989) reported that monitoring since 1977 in four British plants where ethylene oxide and derivatives were produced indicated average exposures to less than

5 ppm [9 mg/m^3] in almost all jobs and to < 1 ppm [1.8 mg/m^3] in many jobs; occasional peaks up to several hundred parts per million occurred as a result of operating difficulties. In earlier years, peak exposures above the odour threshold of 700 ppm [1260 mg/m^3] were reported.

In industries in which ethylene oxide and its derivatives are manufactured, exposure to a large variety of chemicals other than ethylene oxide may occur, depending on the types of process and job. These include unsaturated aliphatic hydrocarbons (e.g. ethylene, propylene; see IARC, 1994), other epoxides (e.g. propylene oxide; see IARC, 1994), chlorohydrins (e.g. epichlorohydrin; see IARC, 1999a; and ethylene chlorohydrin), chlorinated aliphatic hydrocarbons (e.g. dichloromethane, dichloroethane; see IARC, 1999a), glycols and ethers (e.g. ethylene glycol, glycol ethers, bis(2-chloroethyl)ether; see IARC, 1999a, 2006), aldehydes (e.g. formaldehyde; see IARC, 2006), amines (e.g. aniline; see IARC, 1987), aromatic hydrocarbons (e.g. benzene, styrene; see IARC, 1987), alkyl sulfates and other compounds (Shore *et al*., 1993).

(b) Use of ethylene oxide for industrial sterilization

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of products such as medical equipment and products (e.g. surgical products, single-use medical devices), disposable health care products, pharmaceutical and veterinary products, spices and animal feed.

In an extensive survey of the industry in the USA conducted by the National Institute for Occupational Safety and Health, exposure to ethylene oxide was estimated on the basis of data collected in 1976–85 by 21 of 36 companies, most of which were involved in the sterilization of medical supplies and spices. Individual 8-h TWA concentrations of samples collected by active sampling on charcoal tubes in the personal breathing zones of workers were included in a model in which regression analysis was used to link exposure concentrations to seven significant variables: year of operation, volume of sterilizer or treatment vessel, period since the product was sterilized, product type, aeration procedure, presence of a rear exhaust valve in the sterilizer and exposure category (sterilizer, chamber area, maintenance, production, warehouse, clean room, quarantine and laboratory) (Greife *et al*., 1988; Stayner *et al*., 1993; Hornung *et al*., 1994). When the model was applied in a cohort study to the job histories of exposed workers in 13 of the companies surveyed, the estimated historical average concentrations ranged from 0.05 to 77.2 ppm [0.1–139 mg/m^3], with a mean of 5.5 ppm [9.9 mg/m^3] and a median of 3.2 ppm [5.8 mg/m^3] (Stayner *et al*., 1993). Wong and Trent (1993) used the industrial hygiene data from the same companies and estimated that sterilizer operators were exposed to an 8-h TWA concentration of 16 ppm [29 mg/m^3] before 1978 and of 4–5 ppm [7–9 mg/m^3] after 1978, while production workers were exposed to about 5 ppm [9 mg/m^3] before 1978 and 2 ppm [3.6 mg/m^3] after that year.

In a Swedish factory where hospital equipment was sterilized, area samples taken in 1977 in the storage area showed concentrations of ethylene oxide that ranged from 2 to 70 ppm [3.6–126 mg/m^3]; the 8-h TWA concentration in the breathing zone of workers in

the same area was 20 ppm [36 mg/m^3] (Högstedt *et al*., 1979b). In 1978, full-shift personal sampling indicated that sterilizing room operators had an exposure concentration of 2.4 ppm [4.3 mg/m^3]; area sampling indicated an exposure of 1.3 ppm [2.3 mg/m^3]. Personal sampling showed a concentration of 0.1 ppm [0.2 mg/m^3] in the packing room, and area sampling showed a concentration of 0.8 ppm [1.4 mg/m^3] in the stockroom (Högstedt *et al*., 1983). In another Swedish study, sterilization workers and a laboratory technician in the production of disposable medical equipment were reported to have been exposed to bursts of ethylene oxide at concentrations of 5–10 ppm [9–18 mg/m^3] for a total of 1 h per working day, while packers were exposed at an average of 0.5–1 ppm [1–2 mg/m^3] for the entire week (Pero *et al*., 1981). Sterilization workers, packers and truck drivers at another Swedish factory, where single-use medical equipment was produced, were reported to be exposed to an 8-h TWA concentration of 0.5–1 ppm [1–2 mg/m^3] (Pero *et al*., 1982). In two Swedish disposable medical equipment plants, sterilizers and packers were the most heavily exposed, but levels decreased steadily from 35–40 ppm [about 70 mg/m^3] in 1970 to < 0.2–0.75 ppm [< 1.5 mg/m^3] in 1985; the average levels of exposure of store workers and development engineers decreased from 5–20 ppm [9–36 mg/m^3] to < 0.2 ppm [< 0.4 mg/m^3] in the same period, while those of people in other job categories (repairmen, laboratory technicians, controllers and foremen) decreased from 1–4 ppm [2–7 mg/m^3] to < 0.2 ppm [< 0.4 mg/m^3] (Hagmar *et al*., 1991).

Engineering controls and new work practices designed to lower the exposure of workers to ethylene oxide were generally adopted in the USA in 1978 and 1979 (Steenland *et al*., 1991). Stolley *et al*. (1984) estimated that the 8-h TWA concentrations of sterilizer operators in three facilities in the USA before 1980 had been 0.5, 5–10 and 5–20 ppm [1, 9–18 and 9–36 mg/m^3], while data collected in the two plants that were still operating in 1980–82 indicated concentrations of less than 1 ppm [2 mg/m^3].

In Belgium, 12 workers involved in industrial sterilization in three plants were exposed to 8-h TWA concentrations of 0.1–9.3 ppm [0.2–16.7 mg/m^3], with averages in each plant of 1.7, 3.7 and 4.5 ppm [3.1, 6.7 and 8.1 mg/m^3] (Wolfs *et al*., 1983).

In a plant in eastern Germany where disposable medical equipment was sterilized, workers were found to have been exposed to an average concentration of about 60 mg/m^3 [27.1 ppm] in 1985 and about 30 mg/m^3 [13.6 ppm] from 1989 onwards (Tates *et al*., 1991).

Under the sponsorship of the Health Industry Manufacturers' Association, company data were collected in 1987 on current exposures to ethylene oxide of workers in 71 facilities in the USA where medical devices and diagnostic products were sterilized. The workers included sterilizer operators, maintenance workers, supervisors, warehouse workers, laboratory workers and quality control personnel. Respirators were reported to be used in specific operations, such as unloading the sterilizer, maintenance, quality control sampling, emergencies, loading aeration, and changing ethylene oxide bottles, cylinders and tanks. Concentrations were measured outside the respirators. The routine 8-h TWA concentration that occurred 2 or more days per week was > 1 ppm [> 1.8 mg/m^3] for 12.6% of workers, 0.5–1 ppm [0.9–1.8 mg/m^3] for 13.9%, 0.3–0.5 ppm

[0.5–0.9 mg/m^3] for 26.7% and < 0.3 ppm [< 0.5 mg/m^3] for 46.8%. Short-term sampling (for 5–120 min; average, 28 min; except in one factory where sampling was for 210 min for workers in other jobs) showed routine short-term exposures of > 10 ppm [> 18 mg/m^3] for 10.7% of workers, 5–10 ppm [9–18 mg/m^3] for 17.1% and < 5 ppm [< 9 mg/m^3] for 72.2%. Non-routine short-term exposure that occurred 1 day per week or near areas where there was exposure was > 10 ppm [> 18 mg/m^3] for 5.1% of workers, 5–10 ppm [9–18 mg/m^3] for 2.6% and < 5 ppm [< 9 mg/m^3] for 92.3% (Heiden Associates, 1988b).

At a commercial sterilization operation in the USA, workers were exposed to 8-h TWA concentrations of 1–10 ppm [1.8–18 mg/m^3] in 1993–2001 and to 1.3–2.4 ppm [2.3–4.3 mg/m^3] in 2002, according to measurements carried out by the employer. The Occupational Safety and Health Administration monitored personal exposures in the same workplace and found 8-h TWA concentrations of 0.6–9.3 ppm [1.1–17 mg/m^3]. After improvement of working conditions, 8-h TWA concentrations of 0.2–1.2 ppm [0.4–2.2 mg/m^3] were found during follow-ups (Daniel *et al*., 2004).

Exposures in 10 factories in Taiwan (China) that used ethylene oxide as a sterilant in the manufacture of medical supplies were measured in 2005 (Chien *et al*., 2007). Sterilizer operators had an average short-term exposure level of 27.6 ppm [50 mg/m^3] during unloading of the sterilizer, and the mean 8-h TWA exposure was 7.4 ppm [13 mg/m^3]. High concentrations were measured particularly in the aeration area, near the sterilizer and in the warehouse. Increasing the number of post-sterilization purge cycles and improvements to ventilation in the aeration area and warehouse decreased the average short-term exposures to 55% of the earlier values.

Other substances to which workers involved in the sterilization of medical products may be exposed include gases that are present with ethylene oxide in the sterilizing mixture, such as chlorofluorocarbons and carbon dioxide (Heiden Associates, 1988b), and methyl formate in Sweden (Hagmar *et al*., 1991).

(c) Use of ethylene oxide in hospitals

Ethylene oxide is used widely in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come into contact with biological tissues. The National Institute for Occupational Safety and Health estimated that there were more than 10 000 sterilizers in use in health care facilities in the USA in 1977. Large sterilizers are found in central supply areas of most hospitals, and smaller sterilizers are found in clinics, operating rooms, tissue banks and research facilities (Glaser, 1979).

Exposure to ethylene oxide may result during any of the following operations and conditions: changing pressurized ethylene oxide gas cylinders; leaking valves, fittings and piping; leaking sterilizer door gaskets; opening the sterilizer door at the end of a cycle; improper ventilation at the sterilizer door; improperly ventilated or unventilated air gap between the discharge line and the sewer drain; removal of items from the sterilizer and transfer of the sterilized load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; and passing near

or working in the same room as sterilizers and aerators during operation (Mortimer & Kercher, 1989).

Levels of exposure to ethylene oxide in hospitals are summarized in Table 3.

The National Institute for Occupational Safety and Health conducted a series of studies between 1977 and 1990 to document the exposure to ethylene oxide of hospital sterilization staff in the USA. The main results are summarized in Table 4. The more recent studies from Japan and France (see Table 3) suggest that 8-h TWA concentrations are often < 1 mg/m^3 [0.6 ppm] in hospitals.

From 1984 to 2001, a total of 256 666 ethylene oxide samples were analysed by a major vendor of passive ethylene oxide monitoring devices in the USA. Most of the measurements (86%) were taken in hospitals. Workshift measurements were taken from 28 373 hospital workers in 2265 hospitals and short-term measurements from 18 894 workers in 1735 hospitals. The percentage of hospitals in which the 8-h TWA exposure limit of 1 ppm [1.8 mg/m^3] was exceeded once or more times in a year decreased from 21% in 1988 to 0.9% in 2001 (La Montagne *et al*., 2004).

In most studies, exposure to ethylene oxide appears to result mainly from peak emissions during operations such as opening the door of the sterilizer and unloading and transferring sterilized material. Proper engineering controls and work practices have been reported to result in full-shift exposure levels of less than 0.1 ppm [0.18 mg/m^3] and short-term exposure levels of less than 2 ppm [3.6 mg/m^3] (Mortimer & Kercher, 1989). In a survey of 125 hospitals in the USA, however, use of personal protective equipment was found to be limited to the wearing of various types of gloves while transferring sterilized items. No respirators were used (Elliott *et al*., 1988).

In a unit in Argentina that was equipped with old gas sterilizers with no mechanical ventilation, the 8-h TWA concentration of ethylene oxide was 60–69 ppm [108–124 mg/m^3] (Lerda & Rizzi, 1992).

Other substances to which sterilizer operators in hospitals may be exposed include other gases present in the sterilizing mixture such as chlorofluorocarbons (see IARC, 1999a; banned by the Montreal Protocol in 1989) and carbon dioxide (Wolfs *et al*., 1983; Deschamps *et al*., 1989). Some operating room personnel exposed to ethylene oxide may also be exposed to anaesthetic gases and X-rays (Sarto *et al*., 1984a; see IARC, 2000; Chessor *et al*., 2005), and some may have occasional exposure to low concentrations of formaldehyde (Gardner *et al*., 1989; see IARC, 2006).

(d) Other uses

In a wastewater treatment plant in the USA, ethylene oxide was used as a reaction chemical to modify starch in the starch processing area; in this area, full-shift personal breathing zone concentrations ranged from undetectable to 0.43 mg/m^3 [0.24 ppm] for operators and from undetectable to 2.5 mg/m^3 [1.4 ppm] for mechanics (McCammon *et al*., 1990).

Table 3. Concentrations of ethylene oxide observed in hospitals in various countries

Reference	Country	Year of sampling	No. of sites	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m^3)	
							Range	Mean
Hemminki *et al*. (1982)	Finland	1981	24	Sterilizer operators	8-h TWA	NR	0.2–0.0	
					Peaks	NR	≤ 450	
				Sterilizing chamber open	20 min	NR	9–18	
Mouilleseaux *et al*. (1983)	France	NR	4[a]	Loading, sterilizing, unloading, aerating; area sampling	Few minutes	270	0.9–414	
					6–8-h TWA	14	0.1–9	
Wolfs *et al*. (1983)	Belgium	NR	3	Sterilizer operators	8-h TWA	28	0.4–4.5	0.5–2.9
			1	Sterilizer operators; leaking equipment	8-h TWA	16	0.5–32.9	14.0
			1	Sterilizer operators; box sterilizer with capsules	8-h TWA	5	16.2–95.2	27.0
Hansen *et al*. (1984)	USA	NR	1			14	< 0.13–7.7	
						17	< 4.3–81	
						13	4–1430	
Sarto *et al*. (1984a,b)	Italy	NR	6	Old sterilizers				
				Opening sterilizer; area sampling	5 min	NR	23–288	113
				One sterilization cycle; personal sampling	Variable	NR	6.7–63.9	28.4
				Standard working day; personal sampling	8-h TWA	19 subjects	6.7–36	19.3
				Second-generation sterilizers				
				Opening sterilizer; area sampling	5 min	NR	9–47	15.5
				One sterilization cycle; personal sampling	Variable	NR	0.5–4.7	2.0
				Standard working day; personal sampling	8-h TWA	NR	0.4–0.9	0.63

Table 3 (contd)

Reference	Country	Year of sampling	No. of sites	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m^3)	
							Range	Mean
Brugnone *et al*. (1985)	Italy	NR	1	Sterilization workers	8-h TWA	10 subjects	1.90–4.71	
Karelová *et al*. (1987)	Former Czechosolovakia	1984		Sterilization workers; area sampling	8-h TWA	NR	0–4.8	
Sarto *et al*. (1987)	Italy	NR	1	Sterilizer workers Helpers	7–8-h TWA 7–8-h TWA	4 subjects 4 subjects	11.5–16.7 6.8–9.0	14.3 7.7
Deschamps *et al*. (1989)	France	1983–86	5	Opening sterilizer and handling sterilized material; personal samples	2.5–102 min	10	0.4–70	
Mayer *et al*. (1991)	USA	1985–86 1987 1988	1	Sterilizer operators; personal samples	8-h TWA	34 subjects NR 31	≤ 4.3 < 1.8 < 0.18	
Sarto *et al*. (1991)	Italy	NR	1	Sterilization workers Preparation workers	6.5-h TWA 6.5-h TWA	5 subjects 5 subjects	0.68[b] 0.045	
Schulte *et al*. (1992)	Mexico	NR	1	Sterilizer operators	8-h TWA	22 subjects	0–2.4	
Koda *et al*. (1999)	Japan	NR	2	Central supply division Working area (hospital A) Near sterilizer (hospital A) Working area (hospital B) Near sterilizer (hospital B)		 322 322 298 35	 0.2–1.1 0.2–1.1 0.5–1.4 2.0–2.3	 0.7 0.5 0.9 2.2

Table 3 (contd)

Reference	Country	Year of sampling	No. of sites	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m^3)	
							Range	Mean
Sobaszek *et al*. (1999)	France	1988–95	2	Sterilization sites				
				Unloading; area samples	8-h TWA	5	0.05–0.72	
				Unloading; personal samples	14–34 min	5	0.09–11.1	
				Bottle changing; personal samples	7–9 min	5	0.18–162	
Hori *et al*. (2002)	Japan	NR	6	Sterilization, one laundry				
				Area samples	8-h TWA	37	< 0.05–10.3	
				Personal samples	NR	37	< 0.05–0.49	
	USA	NR	9	Sterilizer operators	8-h TWA	51 subjects	0–0.54	

NR, not reported; TWA, time-weighted average

[a] One was a municipal sterilization and disinfection facility.

[b] All samples had the same concentration.

Table 4. Exposure of hospital sterilizer operators to ethylene oxide (personal samples) in studies conducted by the National Institute for Occupational Safety and Health, in the USA, 1977–90

Reference	Period of measurements	No. of hospitals	Operation or conditions	Duration of sampling	No. of samples	Concentration (mg/m^3)
Kercher & Mortimer (1987)	NR	1	Before installation of controls (1984)	Full-shift TWA	NR	[0.43] (average)
				Short-term (15–20 min)	NR	[3.4] (average)
				Short-term (1–2 min)	NR	[4.3] (average)
			After installation of controls (1985)	Full-shift TWA	NR	[< 0.1] (average)
				Short-term (15–20 min)	NR	[< 0.4] (average)
				Short-term (1–2 min)	NR	[1]
Boeniger (1988a)	1987	1	Decontamination room	8-h TWA	2	[0.58–0.77]
			Sterile room	8-h TWA	6	[0.02–1.37]
Boeniger (1988b)	1987	1	Full shift	4–7 h TWA	8	[0.04–0.40]
			Cracking sterilizer door open	30 sec	6	[< 0.05–7.7]
			Transferring load to aerator	30 sec	15	[0.23–18.9]
Elliott *et al*. (1988)	[1984–85]	12	Good engineering controls and good work practice	8-h TWA	4	ND
				Short-term (2–30 min)	3	ND
			Good engineering controls and poor work practice	8-h TWA	15	[ND–0.29]
				Short-term (2–30 min)	19	[ND–5.4]
			No engineering controls and good work practices	8-h TWA	14	[ND–0.83]
				Short-term (2–30 min)	4	[0.43–7.2]
			No engineering controls and poor work practices	8-h TWA	24	[ND–8.3]
				Short-term (2–30 min)	8	[0.43–186]
Mortimer & Kercher (1989)	1984–86	8		Full-shift TWA (6–8 h)	50	[ND–0.5]
				Short-term (1–30 min)	59	[ND–10.4]
Newman & Freund (1989)	1988	1		8-h TWA	8	[< 0.02]
Shults & Seitz (1992)	1991	1		6–8-h TWA	3	[< 0.02]

ND, not detected; NR, not reported; TWA, time-weighted average

1.3.3 *Environmental occurrence*

Most ethylene oxide is released into the atmosphere (WHO, 2003). Ethylene oxide degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals. The half-life of ethylene oxide in the atmosphere, assuming ambient concentrations of 5×10^5 hydroxy radicals/cm^3, is 211 days. Data suggest that neither rain nor absorption into aqueous aerosols remove ethylene oxide from the atmosphere (National Library of Medicine, 2005).

Releases of ethylene oxide (excluding sterilization) into the environment in Canada totalled 23 tonnes in 1996. The industry sectors that reported data were plastics and synthetics (0.24 tonnes), inorganic chemicals (6.1 tonnes), industrial organic chemicals (8.7 tonnes) and soap and cleaning compounds (8.0 tonnes) (WHO, 2003). An additional 3.0 tonnes per year are estimated to be released from the servicing of medical facilities that use ethylene oxide in sterilization processes and commercial sterilization operations (WHO, 2003). By 1997, the emissions had been reduced by 82% from the 1993 levels.

Emissions of ethylene oxide reported to the Environmental Protection Agency by industrial facilities in the USA declined from approximately 2900 tonnes in 1987 to 835 tonnes in 1991 and 135.3 tonnes in 2005 (National Library of Medicine, 2006). Ethylene oxide is one of the 33 hazardous urban air pollutants identified as those that pose the greatest threat to human health in the largest number of urban areas (Environmental Protection Agency, 2000).

In California, USA, concentrations of ethylene oxide in outdoor air were < 0.001–0.96 mg/m^3 (128 samples) in Los Angeles, 0.032–0.40 μg/m^3 [0.018–0.22 ppb] (36 samples) in northern California and 0.03–0.36 μg/m^3 [0.017–0.20 ppb] in a remote coastal location (Havlicek *et al*., 1992).

Three of 50 24-h air samples collected outside randomly selected residences in Alberta, Ontario and Nova Scotia in Canada contained 3.7–4.9 μg/m^3 ethylene oxide. Ethylene oxide was detected in only one sample (4 μg/m^3) taken inside these 50 residences. The limit of detection was 0.19 μg/m^3 (WHO, 2003).

1.3.4 *Other occurrence*

Food products, including herbs, spices, nuts, cocoa beans, cocoa, cocoa cake, raisins, dried vegetables and gums, were often treated with ethylene oxide in the 1980s. Of 204 food products from retail shops in Denmark that were examined for ethylene oxide residues in 1985, 96 samples were found to have concentrations of ethylene oxide that ranged from 0.05 to 1800 mg/kg. The food products surveyed included herbs and spices (14–580 mg/kg), dairy products (0.06–4.2 mg/kg), pickled fish (0.08–2.0 mg/kg), meat (0.05–20 mg/kg), cocoa products (0.06–0.98 mg/kg) and black and herb teas (3–5 mg/kg; one sample contained 1800 mg/kg). In a follow-up survey of 59 honey samples, no ethylene oxide residue was detected (Jensen, 1988).

A total of 200 samples of spices that are known to be consumed commonly without cooking (e.g. pepper, cinnamon/cassia, chilli, curry powder and paprika) were taken from

wholesalers and retailers in New Zealand in 1999. Only two samples of cinnamon contained detectable amounts (limit of detection, 2 mg/kg) of ethylene oxide (6 and 15 mg/kg). Ethylene oxide intake, based on average spice consumption in New Zealand, was estimated to be 0.21 μg per person per day (conservative estimate) (Fowles *et al*., 2001).

Ethylene oxide occurs as a contaminant of skin care products because current commercial preparations of polyglycol ethers may contain ethylene oxide monomer residues of up to 1 ppm (Filser *et al*., 1994). This is in line with a study in which skin care products were reported to contain 0.08–1.5 mg/L ethylene oxide (Kreuzer, 1992).

Ethylene oxide is formed during the combustion of fossil fuel, but the amount is expected to be negligible (WHO, 2003).

Mainstream tobacco smoke contains 7 μg/cigarette ethylene oxide (IARC, 2004).

Patients may be exposed during dialysis when the equipment has been sterilized with ethylene oxide (IPCS-CEC, 2001).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for ethylene oxide in a number of countries, regions or organizations are presented in Table 5.

A tolerance of 50 ppm (mg/kg) has been established in the USA for residues of ethylene oxide when used as a post-harvest fumigant in or on raw black walnut meats, copra and whole spices (Environmental Protection Agency, 1992a). Ethylene oxide, either alone or with carbon dioxide or dichlorodifluoromethane, is permitted in the USA as a fumigant for the control of micro-organisms and insect infestation in ground spices and other processed natural seasoning materials, except mixtures to which salt has been added. Residues of ethylene oxide in ground spices must not exceed the established tolerance of 50 ppm (mg/kg) in whole spices (Environmental Protection Agency, 1992b).

Table 5. Occupational exposure limits and guidelines for ethylene oxide

Country/region or organization	TWA (ppm)[a]	STEL (ppm)[a]	Carcinogenicity[b]	Notes
Australia	1		2	
Belgium	1		Ca	
Brazil	39			
Canada,				
British Columbia	0.1	1	1	ALARA; skin
Quebec	1		A2	Recirculation prohibited
China (mg/m^3)	2	5		STEL based on ultra limit coefficient
China, Hong Kong SAR	1		A2	

Table 5 (contd)

Country/region or organization	TWA (ppm)[a]	STEL (ppm)[a]	Carcinogenicity[b]	Notes
China (Province of Taiwan)	1	2		
Czech Republic (mg/m^3)	1	3		Skin
Finland	1			
France	1.8			
Germany	1 (TRK)		2 (MAK)	Skin
Ireland	5		Ca2	
Japan-JSOH	1		1	Skin sensitizer-2
Malaysia	1			
Mexico	1		A2	
Netherlands (mg/m^3)	0.84		Ca	
New Zealand	1		A2	
Norway	1		Ca	
Poland (mg/m^3)	1	3	Ca	
Romania	1			
South Africa-DOL CL	5			
Spain	1		Ca2	
Sweden	1	5	Ca	Skin
United Kingdom	5		R45	
USA				
ACGIH	1		A2	
NIOSH REL	0.1	5	Ca	Per day
OSHA PEL	1	5 (ceiling)	Ca	

From ACGIH® Worldwide (2005); SZW (2006); Chien *et al*. (2007)
ACGIH, American Conference of Governmental Industrial Hygienists; ALARA, as low as reasonably achievable; DOL CL, Department of Labour ceiling limits; JSOH, Japanese Society of Occupational Health; MAK, maximum allowed concentration; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; STEL, short-term exposure limit; TRK, technical guidance concentration; TWA, time-weighted average

[a] Unless otherwise specified

[b] 2 (Australia), probable human carcinogen; 2 (Germany), considered to be carcinogenic to humans; Ca (except Norway), carcinogen/substance is carcinogenic; Ca (Norway), potential cancer-causing agent; 1, substance which causes cancer in humans/carcinogenic to humans; A2, suspected human carcinogen/carcinogenicity suspected in humans; Ca2, suspected human carcinogen; R45, may cause cancer

2. Studies of Cancer in Humans

The main findings of epidemiological studies of ethylene oxide and cancer risk are summarized in Table 6.

2.1 Case reports

Högstedt *et al*. (1979b) reported three cases of haematopoietic neoplasms that had occurred between 1972 and 1977 in workers at a Swedish factory where ethylene oxide and methyl formate had been used since 1968 to sterilize hospital equipment. Attention had been drawn to the case cluster by the factory safety committee. One woman with chronic myeloid leukaemia and another with acute myelogenous leukaemia had worked in a storage hall where they were exposed for 8 h per day to an estimated 20 ± 10 (standard deviation [SD]) ppm [36 ± 18 mg/m^3] ethylene oxide. The third case was that of a man with primary Waldenström macroglobulinaemia who had been manager of the plant since 1965 and had been exposed to ethylene oxide for an estimated 3 h per week. [The Working Group noted that Waldenström macroglobulinaemia is classified in the WHO Classification of Diseases as lymphoplasmocytic lymphoma.]

Tompa *et al.* (1999) described a cluster of 16 cases of cancer (including eight women with breast cancer) over a 12-year period among 98 nurses who were exposed to ethylene oxide at a sterilizer unit in a hospital in Hungary. Airborne concentrations of ethylene oxide in the working area were reported to vary from 5 to 150 mg/m^3.

2.2 Cohort studies

2.2.1 *Europe*

Högstedt *et al*. (1979a, 1986) and Högstedt (1988) examined workers at a Swedish chemical plant where ethylene oxide had been produced by the chlorohydrin process. The cohort comprised men who had taken part in a medical survey in 1959–61 and included 89 operators with regular exposure to ethylene oxide, 78 maintenance staff with intermittent exposure and 66 unexposed men. All of the men had been exposed or employed for at least 1 year. Average exposures to ethylene oxide during 1941–47 were estimated to have been below 25 mg/m^3, but occasional peaks exceeded the odour threshold of 1300 mg/m^3. During the 1950s and through to 1963, an average concentration of 10–50 mg/m^3 was estimated. In 1963, production of ethylene oxide ceased, but the compound continued to be used in manufacturing processes, and random samples showed workplace concentrations of ethylene oxide in the range of 1–10 mg/m^3, with occasional higher values. Other exposures in the plant included chloroform (IARC, 1999b), chlorinated acetals, chloral (IARC, 1995), DDT (IARC, 1991), ethylene glycol, surfactants, cellulose ethers, ethylene (IARC, 1994), ethylene chlorohydrin, ethylene dichloride, bis(2-chlorethyl)ether (IARC, 1987, 1999a) and propylene oxide (IARC, 1994, 1995, 1997).

Table 6. Epidemiological studies of exposure to ethylene oxide and cancer at various sites

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
COHORT STUDIES							
Europe							
Lymphohaematopoeitic (LH)							
Högstedt *et al.* (1979a, 1986), Sweden	89 operators with regular exposure to ethylene oxide and 78 maintenance staff with intermittent exposure, employed for ≥ 1 year at a chemical plant, followed 1962–85		Leukaemia	Operators Maintenance staff	2 1	[10] [5]	Estimated average exposure before 1963, 5–25 ppm [9–45 mg/m^3]; one CML, one acute leukaemia, one CLL
Thiess *et al.* (1981), Germany	602 male employees in a company in western Germany who worked for at least 6 months in ethylene oxide production, followed to June 1980		Myeloid leukaemia Lymphatic sarcoma		1 1	6.67 NR	
Högstedt *et al.* (1986), Sweden	203 workers employed ≥ 1 year in production of sterilized supplies, followed 1978–82		LH (200–209)	All cohort members	2	[15]	Estimated average past exposure in storeroom, 20 ppm [36 mg/m^3]; one AML was part of a cluster which had originally prompted the study; one acute blastic leukaemia

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Högstedt *et al.* (1986), Sweden	355 chemical workers and maintenance and technical personnel employed at a chemical plant, followed 1964–81	Air sampling and interview with experienced staff	CML	All cohort members	1	11.6 deaths expected from all causes	TWA exposures, 1–8 ppm [1.8–14.4 mg/m^3] in 1963–76; 0.4–2.0 ppm [0.7–3.6 mg/m^3] in 1977–82
Högstedt (1988), Sweden	Follow-up of Högstedt *et al.* (1979a,b, 1986)					**SMR**	
			Leukaemia	All cohort	7	9.21 (NR)	
				Men	6	3.54 (1.3–7.7)	
			Blood and lymphatic malignancies	All cohort	9	4.59 (NR)	
				Men	4	6.11 (1.7–15.7)	
Gardner *et al.* (1989), United Kingdom (updated by Coggon *et al.*, 2004)	1471 workers employed in production or use of ethylene oxide at 4 chemical companies in 1956–85, followed to 31 December 2000	Environmental and personal monitoring since 1977	Leukaemia (204–208)	All cohort members	4	1.41 (0.39–3.62)	Measured TWA concentrations < 5 ppm [9 mg/m^3] in almost all jobs but with occasional peaks up to several hundred ppm; exposures probably higher in past
			Hodgkin lymphoma (201)		1	1.40 (0.04–7.82)	
			NHL (200)		4	1.38 (0.38–3.53)	
			Multiple myeloma (203)		3	2.03 (0.42–5.94)	
	1405 workers potentially exposed to ethylene oxide in sterilization units at 8 hospitals during 1964–86, followed to 31 December 2000		Leukaemia	All cohort members	1	0.55 (0.01–3.06)	
			Hodgkin lymphoma		1	2.98 (0.08–16.6)	
			NHL		3	1.59 (0.33–4.66)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Kiesselbach *et al.* (1990), Germany	2658 employees from 6 chemical companies exposed to ethylene oxide for ≥ 12 months during 1928–82, followed to 31 December 1982		LH Leukaemia	All cohort members	5 2	1.00 (0.32–2.3) 0.85 (0.10–3.1)	No data on exposure levels; risk estimates may have been seriously biased since most deaths in cohort were not ascertained from death certificates.
Hagmar *et al.* (1991, 1995), Sweden	2170 workers employed for ≥ 12 months during 1964–85 at 2 plants where medical equipment was sterilized with ethylene oxide, followed for cancer incidence to 1990		LH Leukaemia	All cohort members All cohort members ≥ 0.14 ppm–years with induction period of 10 years	6 2 2	1.8 (0.65–3.88) 2.4 (0.30–8.81) 7.1 (0.87–25.8)	
Bisanti *et al.* (1993), Italy	1971 male chemical workers licensed to handle ethylene oxide for ≥ 1 year during 1938–84, followed 1940–84		LH Lymphosarcoma and reticulosarcoma Leukaemia	All cohort members	6 4 2	2.5 (0.91–5.5) 6.8 (1.9–17) 1.9 (0.23–7.0)	The 2 leukaemia deaths occurred in men with < 5 years of exposure and < 10 years after first exposure.
Kardos *et al.* (2003), Hungary	299 women employed on a hospital ward using ethylene oxide sterilizer in 1976–93, followed 1987–99		Leukaemia	All cohort members	1	4.38 deaths expected from all causes	Deaths in the cohort ascertained from a different source from the reference rates

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Breast							
Gardner *et al.* (1989), United Kingdom (updated by Coggon *et al.*, 2004)	1011 women potentially exposed to ethylene oxide in sterilization units at 8 hospitals during 1964–86, followed to 31 December 2000		Breast	All cohort members Continual Unknown	11 5 6	0.84 (0.42–1.51) 0.70 (NR) 1.16 (NR)	
Hagmar *et al.* (1991, 1995), Sweden	2170 workers employed for ≥ 12 months in 1964–85 at 2 plants where medical equipment sterilized with ethylene oxide, followed for cancer incidence to 1990		Breast	All cohort members	5	0.46 (0.15–1.08)	
Kardos *et al.* (2003), Hungary	299 women employed on a hospital ward using ethylene oxide sterilizer in 1976–93, followed 1987–99		Breast	All cohort members	3	4.38 deaths expected from all causes	Deaths in the cohort ascertained from a different source from the reference rates; one or more breast cancer cases may have been part of a cluster that prompted the study.

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Stomach							
Thiess *et al.* (1981), Germany	602 employees exposed to alkylene oxides and other substances, employed in 1928–80	Environmental monitoring	Stomach	All cohort members	4	[1.49] (NR)	
Högstedt *et al.* (1979a, 1986); Högstedt (1988), Sweden	89 operators with regular exposure to ethylene oxide and 78 maintenance staff with intermittent exposure, employed for ≥ 1 year at a chemical plant, followed 1962–85		Stomach	All cohort members	5	**SMR** 9.03 (2.9–21.1)	
Högstedt (1988), Sweden	539 men employed for ≥ 1 year at a chemical plant followed 1960–85		Stomach	*Length of employment* 1–9 years ≥ 10 years All	 4 6 10	**SMR** 5.97 (NR) 6.08 (NR) 6.02 (2.9–11.1)	
Gardner *et al.* (1989), United Kingdom (updated by Coggon *et al.*, 2004)	1471 workers in the production or use of ethylene oxide at 4 chemical companies during 1956–85, followed to 31 December 2000	Environmental and personal monitoring since 1977	Stomach	All cohort members Definite Probable Unknown	5 4 1 0	**SMR** [0.62 (0.20–1.46)] 0.78 (NR) 0.57 (NR) 0 (NR)	Measured TWA concentrations < 5 ppm [9 mg/m^3] in almost all jobs but with occasional peaks up to several hundred ppm; exposures probably higher in the past

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Kiesselbach *et al*. (1990), Germany	2658 employees from 6 chemical companies exposed to ethylene oxide for ≥ 12 months in 1928–82, followed to 31 December 1982		Stomach	All cohort members	14	**SMR** 1.38 (0.75–2.31)	No data on exposure levels; risk estimates may have been seriously biased since most deaths in cohort were not ascertained from death certificates.
Hagmar *et al*. (1991, 1995), Sweden	2170 workers employed for ≥ 12 months during 1964–85 at 2 plants using medical equipment sterilized with ethylene oxide, followed for cancer incidence to 1990		Stomach	All cohort members Induction period of 10 years	0 0	**SIR** 0 (0–4.55) 0 (0–8.38)	
Ambroise *et al*. (2005), France	181 male workers employed as pest-control workers 1979–94, followed for mortality through to 2000		Stomach	All cohort members	1	**SMR** 3.18 (0.08–17.70)	No information available on individual level of exposures to pesticides, rodenticides or formaldehyde

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Brain							
Thiess *et al*. (1981), Germany	602 employees exposed to alkylene oxides and other substances, employed in 1928–80	Environmental monitoring	Malignant tumour of the brain	*Duration of exposure*			
				0.5–4 years	0	NR	
				5–9 years	0	NR	
				10–19 years	0	NR	
				≥ 20 years	1	[41.7] (NR)	
Hagmar *et al*. (1991, 1995), Sweden	2170 workers employed for ≥ 12 months in 1964–85 at 2 plants using medical equipment sterilized with ethylene oxide, followed for cancer incidence to 1990		Brain			**SIR**	
				All cohort members	4	1.69 (0.46–4.34)	
				All cohort members ≥ 0.14 ppm–years	3	3.80 (0.78–11.1)	
				Induction period of 10 years	3	2.80 (0.58–8.19)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Pancreas							
Hagmar *et al.* (1991, 1995), Sweden	2170 workers employed for ≥ 12 months during 1964–85 at 2 plants where medical equipment was sterilized with ethylene oxide, followed for cancer incidence to 1990		Pancreas			**SIR**	
				All cohort members	2	2.47 (0.30–8.92)	
				All cohort members ≥ 0.14 ppm–years	1	2.86 (0.07–15.9)	
				Induction period of 10 years	1	2.22 (0.06–12.4)	
Ambroise *et al.* (2005), France	181 male workers employed as pest-control workers 1979–94, followed for mortality through to 2000		Pancreas			**SMR**	No information available on individual level of exposures to pesticides, rodenticides or formaldehyde
				All cohort members	0	0 (0–10.77)	
USA							
Lymphohaematopoeitic (LH)							
Morgan *et al.* (1981), eastern Texas (reported in Shore *et al.*, 1993)	767 men employed in 1955–77 at a chemical plant for ≥ 5 years with potential exposure to ethylene oxide, followed 1955–85	Industrial hygiene survey in 1977	LH	All cohort members	3	10 (0.21–2.9)	Exposures in 1977 < 10 ppm [18 mg/m^3]; included 2 cases of Hodgkin disease
			Leukaemia		0	0.0 (0.0–3.4)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Steenland *et al*. (1991); Stayner *et al.* (1993); Steenland *et al*. (2004)	18 235 workers employed at 14 industrial plants that used ethylene oxide for sterilization since 1943 with ≥ 3 months exposure to ethylene oxide, followed to 1998					**SMR**	Adjusted for age, race (white/non-white), date of birth (within 5 years); in an internal case–control analysis (excluding 1 small plant), log cumulative exposure to ethylene oxide lagged by 15 years significantly related to mortality from LH cancers in men ($p = 0.02$), but not in women; duration of exposure, peak exposure and average exposure less predictive of mortality from LH cancer; similar pattern observed for lymphoid-cell tumours
			LH	All cohort members	79	1.00 (0.79–1.24)	
				Cumulative exposure in ppm–days			
				0–1199	18	0.77 (NR)	
				1200–3679	20	1.31 (NR)	
				3680–13 499	18	1.10 (NR)	
				≥ 13 500	18	0.94 (NR)	
			Lymphoid-cell	Men with 15- year lag (results from Cox regression)			
				Cumulative exposure in ppm–days			
				0		1.00	
				>0–1199		0.90 (0.16–5.24)	
				1200–3679		2.89 (0.65–12.86)	
				3680–13 499		2.74 (0.65–11.55)	
				≥ 13 500		3.76 (1.03–13.64)	
						p-trend = 0.13	
			Hodgkin lymphoma	All cohort members	6	1.24 (0.53–2.43)	
				Cumulative exposure in ppm–days			
				0–1199	0	0 (NR)	
				1200–3679	1	0.99 (NR)	
				3680-13 499	3	2.97 (NR)	
				≥ 13 500	2	2.20 (NR)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Steenland *et al*. (1991); Stayner *et al*. (1993); Steenland *et al*. (2004) (contd)			NHL	All cohort members	31	1.00 (0.72–1.35)	
				Cumulative exposure in ppm–days			
				0–1199	7	0.76 (NR)	
				1200–3679	8	1.34 (NR)	
				3680–13 499	6	0.85 (NR)	
				≥ 13 500	9	1.21 (NR)	
			Multiple myeloma	All cohort members	13	0.92 (0.54–0.87)	
				Cumulative exposure in ppm–days			
				0–1199	1	0.26 (NR)	
				1200–3679	5	1.89 (NR)	
				3680–13 499	3	0.92 (NR)	
				≥ 13 500	4	1.03 (NR)	
			Leukaemia	All cohort members	29	0.99 (0.71–1.36)	
				Cumulative exposure in ppm–days			
				0–1199	10	1.15 (NR)	
				1200–3679	6	1.06 (NR)	
				3680–13 499	6	0.93 (NR)	
				≥ 13 500	3	0.43 (NR)	
Benson & Teta (1993), West Virginia	278 men intermittently exposed to ethylene oxide in a chlorohydrin unit since 1949, followed to 1988		LH	All cohort members	8	2.94 (1.27–5.80)	Primarily exposed to ethylene chlorohydrin, ethylene dichloride and bischloroethyl ether

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Teta *et al.* (1993), West Virginia	1896 men potentially exposed to ethylene oxide since 1940 at 2 chemical plants but who never worked in chlorohydrin unit, followed to 1988		LH Lymphosarcoma and reticulosarcoma Leukaemia	All cohort members	7 2 5	0.6 (0.2–1.2) 1.0 (0.1–3.56) 1.1 (0.4–2.5)	
Norman *et al.* (1995), New York State	1132 workers employed in 1974–80 at a sterilizing plant that used ethylene oxide, followed for cancer incidence to 1957		Leukaemia	All cohort members	1	1.85 (p = 0.42)	
Olsen *et al.* (1997), Texas	1361 men employed for ≥ 1 year and potentially engaged for ≥ 1 month in ethylene or propylene chlorohydrin production since 1941 at 4 chemical plants, followed to 1992		LH	Ever in ethylene chlorohydrin production Ever in ethylene chlorohydrin production with allowance for 25-year induction period from first exposure	10 6	1.29 (0.62–2.38) 1.4 (0.52–3.12)	
Breast							
Norman *et al.* (1995), New York State	1132 workers employed during 1974–80 at a sterilizing plant that used ethylene oxide, followed for cancer incidence to 1957		Breast	All cohort members All cohort members	12 12	1.72* (0.99–3.00) 1.57** (0.90–2.75)	*Expected numbers from SEER rates for 1978–81 **Expected numbers from SEER rates for 1981–85

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Steenland *et al.* (2003)	7576 women worked for ≥ 1 year at 13 plants, followed for breast cancer incidence to 1998		Breast	All cohort members	319	0.87* (0.77–0.97)	*Recognized to be an underestimate because of incomplete ascertainment of cases
			Breast excluding carcinoma *in situ*	All cohort members	299	0.94 ([0.84–1.05])	
			Breast	*Exposures in ppm–days with 15-year lag*			
				0	81	1.00** (lagged out)	**Odds ratios calculated by Cox regression in a nested case–control analysis
				< 647	45	1.07 (0.72–1.59)	
				647–2026	46	1.00 (0.67–1.50)	
				2027–4919	46	1.24 (0.85–1.90)	
				4920–14 620	45	1.17 (0.78–1.78)	
				> 14 620	48	1.74 (1.16–2.65)	
				Exposures in ppm–days with 15-year lag			***Analysis restricted to subset of 5139 women with data on potential confounders from interviews; adjusted for parity, breast cancer in first-degree relative
				0	81	1.00*** (lagged out)	
				< 647	45	1.06 (0.66–1.71)	
				647–2026	46	0.99 (0.61–1.60)	
				2027–4919	46	1.24 (0.76–2.00)	
				4920–14 620	45	1.42 (0.88–2.29)	
				> 14 620	48	1.87 (1.12–3.10)	
Steenland *et al.* (2004)	18 235 workers at 14 industrial plants that used ethylene oxide for sterilization since 1943 with ≥ 3 months' exposure to ethylene oxide, followed to 1998		Breast	All cohort members	103	0.99 (0.84–1.17)	At least one cancer occurred in a man.
				All female cohort members	NR	0.99 (0.81–1.20)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Stomach							
Steenland *et al*. (1991); Stayner *et al.* (1993); Steenland *et al*. (2004)	18 254 workers at 14 industrial plants that used ethylene oxide for sterilization since 1943 with ≥ 3 months exposure to ethylene oxide, followed to 1998		Stomach			**SMR**	.
				All cohort members (Steenland *et al*., 2004)	25	1.07 (0.74-1.49)	
				Cumulative exposure in ppm–days			
				< 1200	5	1.74 (0.57–4.07)	
				1200–8500	4	1.24 (0.29–2.60)	
				> 8500	1	0.23 (0.11–1.32)	
				Total	10	0.90 (0.43–1.66)	
				(Stayner *et al.*, 1993)		*p* trend = 0.04	
Benson & Teta (1993), West Virginia	278 men intermittently exposed to ethylene oxide in a chlorohydrin unit since 1949, followed to 1988		Stomach	All cohort members	1	[0.70] (0.2–3.92)	Primarily exposed to ethylene chlorohydrin, ethylene dichloride and bischloroethyl ether
Teta *et al.* (1993), West Virginia	1896 men potentially exposed to ethylene oxide since 1940 at 2 chemical plants but who never worked in chlorohydrin unit, followed to 1988		Stomach			**SMR**	
				All cohort members	8	1.60 (0.69–3.15)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Norman *et al.* (1995), New York State	1132 workers employed during 1974–80 at a sterilizing plant using ethylene oxide, followed for cancer incidence to 1957		Stomach	All cohort members	0	–	
Olsen *et al.* (1997), Texas	1361 men employed for ≥ 1 year and potentially engaged for ≥ 1 month in ethylene or propylene chlorohydrin production since 1941 at 4 chemical plants, followed to 1992		Stomach			**SMR**	
				Ever in ethylene chlorohydrin production	2	65 (8–234)	
				Ever in ethylene chlorohydrin production with allowance for 25-year induction period from first exposure	2	[1.17 (0.14–4.23)]	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Brain							
Steenland *et al*. (1991); Stayner *et al.* (1993); Steenland *et al*. (2004)	18 254 workers employed at 14 industrial plants using ethylene oxide for sterilization since 1943 with ≥ 3 months exposure to ethylene oxide, followed to 1998		Brain			**SMR**	.
				All cohort members (Steenland *et al.*, 2004)	14	0.59 (0.36–0.91)	
				Cumulative exposure in ppm–days			
				< 1200	0	0.0	
				1200–8500	4	0.99 (0.27–2.53)	
				> 8500	2	0.59 (0.07–2.12)	
				Total	6	0.54 (0.20–1.18)	
				(Stayner *et al.*, 1993)		*p*-trend = 0.43	
Benson & Teta (1993), West Virginia	278 men intermittently exposed to ethylene oxide in a chlorohydrin unit since 1949, followed to 1988		Brain and other nervous system	All cohort members	1	[1.17] (0.3–6.56)	Primarily exposed to ethylene chlorohydrin, ethylene dichloride and bischloroethyl ether
Teta *et al.* (1993), West Virginia	1896 men potentially exposed to ethylene oxide since 1940 at 2 chemical plants who never worked in chlorohydrin unit, followed to 1988		Brain and other nervous system			**SMR**	
				All cohort members		1.50 (0.55–3.27)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Norman *et al.* (1995), New York State	1132 workers employed during 1974–80 at a sterilizing plant using ethylene oxide, followed for cancer incidence to 1957		Brain	All cohort members	0	–	
Olsen *et al.* (1997), Texas	1361 men employed for ≥ 1 year and potentially engaged for ≥ 1 month in ethylene or propylene chlorohydrin production since 1941 at 4 chemical plants, followed to 1992		Brain and other nervous system (191–192)			**SMR**	
				Ever in ethylene chlorohydrin production	3	1.23 (0.25–3.58)	
				Ever in ethylene chlorohydrin production with allowance for 25-year induction period from first exposure	3	[2.73 (0.56–7.97)]	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Pancreas							
Steenland *et al.* (1991); Stayner *et al.* (1993); Steenland *et al.* (2004)	18 254 workers employed at 14 industrial plants using ethylene oxide for sterilization since 1943 with ≥ 3 months exposure to ethylene oxide, followed to 1998		Pancreas			**SMR**	.
				All cohort members (Steenland *et al.*, 2004)	38	0.92 (0.69–1.21)	
				Cumulative exposure in ppm–days			
				< 1200	3	0.69 (0.14–2.03)	
				1200–8500	10	1.70 (0.81–3.12)	
				> 8500	3	0.50 (0.10–1.47)	
				Total (Stayner *et al.*, 1993)	16	0.98 (0.57–1.61) *p*-trend = 0.38	
Benson & Teta (1993), West Virginia	278 men intermittently exposed to ethylene oxide in a chlorohydrin unit since 1949, followed to 1988		Pancreas	All cohort members	8	4.92 (1.58–11.40)	Primarily exposed to ethylene chlorohydrin, ethylene dichloride and bischloroethyl ether
Teta *et al.* (1993), West Virginia	1896 men potentially exposed to ethylene oxide since 1940 at 2 chemical plants, who never worked in chlorohydrin unit, followed to 1988		Pancreas			**SMR**	
				All cohort members	4	0.61 (0.17–1.56)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
Norman *et al.* (1995), New York State	1132 workers employed in 1974–80 at a sterilizing plant using ethylene oxide, followed for cancer incidence to 1957		Pancreas	All cohort members	2	3.92 (p = 0.09)	
Olsen *et al.* (1997), Texas	1361 men employed for ≥ 1 year and potentially engaged for ≥ 1 month in ethylene or propylene chlorohydrin production since 1941 at 4 chemical plants, followed to 1992		Pancreas			**SMR**	
				Ever in ethylene chlorohydrin production	1	0.25 (0.1–1.40)	
				Ever in ethylene chlorohydrin production with allowance for 25-year induction period from first exposure	1	0.40 (0.1–2.26)	

Table 6 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustments and comments
CASE–CONTROL STUDY							
Swaen *et al.* (1996), Belgium	210 employees of a chemical manufacturer between 1966 and 1992		Hodgkin lymphoma	All cohort members	3 exposed cases	**Odds ratio** 8.5 (1.4–39.9)	
META-ANALYSIS							
Teta *et al.* (1999), Germany, Italy, Sweden, United Kingdom, USA	Nearly 33 000 workers					**Meta-SMR**	
			Leukaemia		35	1.08 (0.61–1.93)	
			NHL		33	1.34 (0.96–1.89)	
			Stomach		59	1.23 (0.71–2.13)	
			Brain		25	0.96 (0.49–1.91)	
			Pancreas		37	0.95 (0.69–1.31)	

AML, acute myelogenous leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myelogenous leukaemia; ICD, International Classification of Diseases; NHL, non-Hodgkin lymphoma; NR, not reported; SEER, Surveillance, Epidemiology and End Results; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TWA, time-weighted average

The cohort was followed from 1962 to 1985 through national registries. Among the ethylene oxide operators, 34 deaths from all causes occurred (25.0 expected), including 14 cancer deaths (6.1 expected) of which five were due to stomach cancer (0.6 expected) and two to leukaemia (0.2 expected; one chronic myelogenous and one acute leukaemia not further specified). No overall excess mortality from cancer was observed among the maintenance staff who had intermittent exposure or among the unexposed workers; however, four of the maintenance men had died from stomach cancer (0.6 expected) and one from chronic lymphocytic leukaemia (0.2 expected).

Two hundred and three workers employed for at least 1 year at the Swedish factory described in Section 2.1 were subsequently followed up for mortality through national census, death and emigration registries (Högstedt *et al*., 1986). During 1978–82, five deaths occurred (4.9 expected), four of which were from cancer (1.6 expected). Two of the deaths were from lymphatic and haematopoietic malignancies (0.13 expected), but one of these decedents (who had acute myelogenous leukaemia) had been part of the original case cluster that had prompted the study. The other died from acute blastic leukaemia.

The above reports also described a third cohort of Swedish workers who were exposed to ethylene oxide in a plant where the compound was produced by direct oxidation of ethylene (Högstedt *et al*., 1986; Högstedt, 1988). The cohort comprised 128 workers who were employed in the production of ethylene oxide or ethylene glycol and had had almost pure exposure to ethylene oxide; 69 workers who were employed in the processing of ethylene oxide and propylene oxide to non-ionic surfactants and polyols and whose principal exposure was to ethylene oxide and propylene oxide but who had also been exposed to various amines, sodium nitrate (IARC, 2009), formaldehyde (IARC, 2006) and 1,2-butene oxide; and 158 maintenance and technical personnel who had had multiple exposures that included ethylene oxide. Analyses of air samples and interviews with experienced staff indicated 8-h TWA exposures to ethylene oxide of 1–8 ppm [1.8–14.4 mg/m^3] during 1963–76, which fell to 0.4–2.0 ppm [0.7–3.6 mg/m^3] during 1977–82. Expected numbers of cancers and deaths were calculated from 5-year age-, sex- and calendar year-specific rates for the national population. During follow-up from 1964 to 1981 using national registries, eight deaths were observed in the entire cohort compared with 11.6 expected; one man in the maintenance and repair group died from chronic myelogenous leukaemia. During extended follow-up to 1985, a fatal case of reticular-cell sarcoma was recorded among the production workers [expected number not given]. [The Working Group noted that the cohort was not defined precisely.]

Högstedt (1988) also presented findings on cancer incidence (ascertained through the national cancer registry) for the three cohort studies described above. After exclusion of the three cases in the initial cluster at the sterilizing plant, seven leukaemias were observed (0.8 expected from national rates [standardized incidence ratio (SIR), 9.2]). For blood and lymphatic malignancies, nine deaths were observed (two expected; standardized mortality ratio [SMR], 4.59). Confidence intervals (CIs) were reported only for the SMRs of men.

Thiess *et al*. (1981) examined the mortality of 602 active and former male employees at a company in western Germany who had worked for at least 6 months in an area of alkylene oxide production. Until 1965, ethylene oxide had been made from ethylene chlorohydrin, but thereafter it was produced by direct oxidation of ethylene. Propylene oxide had been made since 1959 by a propylene chlorohydrin process. Industrial hygiene measurements in 1978 showed that the average concentration of ethylene oxide was < 4 ppm [7.2 mg/m^3], but no earlier measurement was available. Discussions with long-standing employees indicated that exposures before that time would have been higher. Other potential exposures included propylene oxide, butylene oxide, dioxane, epichlorohydrin, dichloropropane, ethylene chlorohydrin, propylene chlorohydrin, aniline, piperazine, cyclohexylamine, cyclohexane, formaldehyde, isobutyraldehyde, ethyleneimine, hydrocyanic acid, hydrogen sulfide, aluminium chloride, benzene, phenol, cyanuric acid, acrylic acid and acetylene alcohols (IARC, 1987, 1994, 1999a, 2006). The first worker was employed in 1928, and follow-up was from that year until 30 June 1980. The expected numbers of deaths in the cohort were calculated for each 5-year age group, using mortality rates for the populations of Ludwigshafen and Rhinehessia-Palatinate during 1970–75 and of Germany during 1971–74 as a reference. In addition, an internal comparison group of 1662 persons who were employed in a styrene production facility on the same site was used. During follow-up, 56 deaths were recorded in the exposed cohort, whereas the expected numbers were 71.5 (Ludwigshafen), 73.4 (Rhinehessia-Palatinate), 76.6 (Germany) and 57.9 (styrene cohort). Fourteen of the deaths were due to cancer, whereas 16.6 were expected from national statistics [SMR, 0.84]. The deaths from cancer included one case of myeloid leukaemia (< 0.15 expected) and one case of lymphatic sarcoma. [The Working Group noted that no indication of the completeness with which the cohort was identified was given, and that the methods of follow-up were not stated. It is not clear how losses to follow-up were handled in the analysis.]

Gardner *et al*. (1989) studied 2876 workers at four British chemical companies where ethylene oxide or its derivatives had been manufactured and in eight hospitals where ethylene oxide had been used as a sterilant. In one company, ethylene oxide had been produced by the chlorohydrin process during 1950–60 and by direct oxidation of ethylene from 1959 onwards; in the second company, the chlorohydrin process had been used during 1955–70 and direct oxidation was used thereafter; in the third company, ethylene oxide had been produced during 1960–81 only by direct oxidation; and in the fourth company, ethylene oxide had been used in the manufacture of derivatives since 1959. The eight hospitals had started using ethylene oxide between 1962 and 1972. The cohort comprised all workers at each factory and hospital who had had probable exposure to ethylene oxide during specified periods for which employment records were complete. Sixteen subjects had to be excluded because such information was not available in full. Jobs held by cohort members at the factories were classified as having involved definite, probable or possible exposure to ethylene oxide. At the hospitals, jobs were classed as involving continual, intermittent or possible exposure. Environmental and personal moni-

toring since 1977 had shown a TWA concentration of < 5 ppm [9 mg/m^3] in almost all jobs, but with occasional peaks of exposure up to several hundred parts per million as a result of operating difficulties in the chemical plants and during loading and unloading of sterilizers in the hospitals. Exposures were thought to have been higher in earlier years, and peak exposures above the odour threshold of 700 ppm [1260 mg/m^3] were reported both by the chemical manufacturers and at the hospitals. Cohort members at the manufacturing plants were potentially exposed to many other chemicals, including chlorohydrin, propylene oxide, styrene and benzene; some of the hospital workers had occasionally been exposed to formaldehyde and carbon tetrachloride. The cohort was followed for mortality through the National Health Service Central Register, with supplementary information from social security records for some subjects. In the most recently published analysis of this study (Coggon *et al.*, 2004), the results of which subsumed earlier findings, the cohort was followed for mortality to 2000. Follow-up for 51 untraced subjects was cut off at the last known date of employment. A further 206 subjects had emigrated or were otherwise lost to follow-up, and were considered to be at risk up to the date when they were last known to be alive. Observed mortality was compared with that expected from national death rates by sex, age and calendar period. No significant elevations of mortality were observed for any category of cancer either in the cohort as a whole, or separately in the chemical manufacturers and hospital workers. Among the 1471 chemical workers (all but one of whom were men), 366 deaths (366.9 expected) occurred from all causes, including 120 (108.6 expected; SMR, 1.11; 95% CI, 0.92–1.32) from all cancers combined. Increased non-significant risks were found for leukaemia, lymphoma and multiple myeloma. Lymphatic and haematopoietic cancer was more common in chemical workers who were classed as having had definite exposure to ethylene oxide (nine deaths versus 4.9 expected; SMR, 1.84), but there was no excess in the hospital workers who were classed as continually exposed (one death versus 2.6 expected).

Most of the above cohort was included in a larger study of employees from six chemical companies in western Germany (Kiesselbach *et al.*, 1990). The 2658 cohort members had been exposed to ethylene oxide for at least 12 months before 31 December 1982. The year of first exposure ranged from 1928 to 1981, but most had first been exposed after 1950. Other possible exposures included benzene (IARC, 1987), 4-aminobiphenyl and 2-naphthylamine (IARC, 1987), but no information was given on the extent of exposure to these substances. Subjects who had left employment were traced through local registries and, in the case of foreigners who had returned home, by letter or by asking fellow countrymen who were still working in the plant. Of the cohort members, 97.6% were traced successfully to 31 December 1982. For those who had died, the cause of death was ascertained from death certificates (27.6% of all deaths), lay statements, the physician who last treated the patient or hospital reports. Mortality was compared with that expected from 5-year age-, sex- and calendar period-specific rates in the national population; no statistics were available for periods before 1951, and the rates for 1951 were used. In total, 268 deaths were observed, whereas 307.6 were expected. There were

68 deaths from cancer (69.9 expected; SMR, 0.97; 95% CI, 0.76–1.24), including three from oesophageal cancer (1.5 expected), 14 from stomach cancer (10.2 expected; SMR, 1.38; 95% CI, 0.75–2.31) and five from lymphatic and haematopoietic cancer (5.0 expected; SMR, 1.00; 95% CI, 0.32–2.34). Two deaths were ascribed to leukaemia (2.4 expected). When expected numbers were calculated on the basis of rates in the states in which each plant was situated, the findings were very similar. Based on calculations in which the first 10 years of exposure for each subject were ignored, mortality ratios were similar to those in the main analysis. It was possible to classify exposure to ethylene oxide for 67.2% of subjects as 'weak', 'medium' or 'high'. The excess mortality from stomach cancer was greatest in those with weak or medium exposure and with less than 15 years of total exposure. When foreign workers were excluded from the analysis, there was no change in the observed number of deaths and mortality ratios were only slightly increased. [The Working Group noted that the full eligibility criteria for inclusion in the cohort were not reported, no data were given on probable levels of exposure to ethylene oxide or on the nature of the processes in which subjects worked, and risk estimates may have been seriously biased since certificates were available for only about one-quarter of deaths in the cohort.]

Hagmar *et al*. (1991) studied employees at two Swedish plants that produced disposable medical equipment that was sterilized with ethylene oxide. In plant A, a 50:50 mixture of ethylene oxide and methyl formate had been used since 1970. In 1973, personal sampling for two packers indicated an exposure to ethylene oxide of 24 ppm [43 mg/m^3]. After 1981, monitoring was carried out annually over 1–3 days for sterilizers and packers and showed a continuous decrease in exposure such that, after 1985, only sterilizers were exposed to concentrations greater than 0.2 ppm [0.4 mg/m^3] (the limit of detection of the method used). In plant B, a 50:50 mixture of ethylene oxide and methyl formate was used from 1964 but was replaced by an ethylene oxide:carbon dioxide mixture in 1978. In 1975, personal monitoring indicated exposures of 4–5 ppm [7–9 mg/m^3] ethylene oxide for four packers. After 1985, the 8-h TWA concentration was < 0.2 ppm [0.4 mg/m^3] for all employees except sterilizers and store workers. The authors estimated that sterilizers were exposed to up to 75 ppm [135 mg/m^3] in the earliest years of operation at this plant. On the basis of estimates of exposure in different job categories and time periods, the authors calculated individual cumulative exposures for 97% of subjects at plant A and 89% at plant B. The cohort comprised 594 men and 557 women who had been employed at plant A for at least 12 months between 1970 and 1985 and who were still working after 1 June 1975, and 267 men and 752 women who had been employed at plant B for at least 12 months between 1964 and 1985 and were still working after 1 January 1972. These subjects were followed through to 1986 for mortality and from 1972 to 1985 for cancer registration. None was lost to follow-up. Expected mortality was calculated on the basis of calendar year-, sex- and 5-year age-specific rates (cut-off at age 80 years) for the county in which the plants were located, and expected cancer incidence was calculated from corresponding registration rates in the same area. Fifteen deaths were observed (25.7 expected),

including eight from cancer (9.0 expected), two from gastrointestinal cancer (2.1 expected) and one from haematopoietic and lymphatic cancer (1.0 expected). The observed/expected numbers of incident cancers were 21/26.8 for cancers at any site, 0/0.5 for stomach cancer, 1/1.6 for brain cancer, 2/1.3 for lymphoma and myeloma and one case of polycythaemia vera with 0.7 cases of leukaemia, polycythaemia vera and myelofibrosis expected. Among subjects with more than 1 ppm–year of cumulative exposure to ethylene oxide, two cases of cancer (3.3 expected) and no lymphatic or haematopoietic cancer (0.2 expected) were observed. Follow-up for cancer incidence was subsequently extended to 1990, again with no losses to follow-up (Hagmar *et al*., 1995). In total, 40 cases of cancer were recorded with 46.28 expected. No significant excesses in mortality were observed for any individual site of cancer, either with or without allowance for a 10-year induction period from first exposure. With no allowance for an induction period, the numbers of observed/expected cases were 5/10.8 (SIR, 0.5; 95% CI, 0.15–1.08) for cancer of the breast, 6/3.37 (SIR, 1.78; 95% CI, 0.65–3.88) for lymphatic and haematopoietic cancer and 2/0.82 (SIR, 2.44; 95% CI, 0.30–8.81) for leukaemia. Among 930 subjects with cumulative exposures of at least 0.14 ppm–years, after allowance for a minimum induction period of 10 years, two cases of leukaemia were observed compared with 0.28 expected (SIR, 7.1; 95% CI, 0.87–25.8).

Bisanti *et al*. (1993) studied a cohort that comprised all 1971 male chemical workers in the Lombardy and Piedmont regions of Italy who had held a licence to handle ethylene oxide for at least 1 year during 1938–84; 637 had held licences for ethylene oxide only and 1334 for ethylene oxide and other toxic gases. Some workers may have been exposed to ethylene oxide before they obtained a licence. The cohort was followed from 1 January 1940 to 31 May 1984, and vital status was ascertained at the census office at the place of residence of each subject. Sixteen subjects (0.8%) who were lost to follow-up were considered to be still alive. Expected numbers of deaths were calculated from 5-year age-, sex- and calendar period-specific rates for the regional (Lombardy) population. Seventy-six deaths were recorded (98.8 expected), including 43 from cancer (33.0 expected). The SMRs were 1.22 (95% CI, 0.40–2.87) for stomach cancer, 2.54 (95% CI, 0.52–7.44) for cancer of the pancreas, 1.61 (95% CI, 0.04–8.95) for cancer of the kidney, 6.82 (95% CI, 1.86–17.45) for lymphosarcoma and reticulosarcoma and 1.93 (95% CI, 0.23–6.99) for leukaemia. The two deaths from leukaemia occurred among men who had had less than 5 years of exposure and after a latency of less than 10 years since first exposure to ethylene oxide. Among the men who had held licences only for ethylene oxide, 27 deaths (30.1 expected) occurred (SMR, 0.87; 95% CI, 0.57–1.27), 15 of which were from cancer (10.5 expected; SMR, 1.42; 95% CI, 0.79–2.34), including one from stomach cancer (1.3 expected; SMR, 0.76; 95% CI, 0.02–4.26), three from lymphosarcoma and reticulosarcoma (0.2 expected; SMR, 16.93; 95% CI, 3.49–49.53) and two from leukaemia (0.3 expected; SMR, 6.50; 95% CI, 0.79–23.49). Results obtained from national mortality rates as the basis for expected numbers were similar. [The Working Group noted that no data were available on levels of exposure to ethylene oxide or on exposure to other chemicals.]

Following the observation of a cluster of breast cancer cases at a hospital in Hungary (Tompa *et al*., 1999; see Section 2.1), Kardos *et al*. (2003) systematically followed 299 women who were employed on a ward where an ethylene oxide sterilizer was used during 1976–93. The cohort was followed from 1987 to 1999, and deaths among cohort members were ascertained from various databases, with personal contact to confirm the vital status of those who did not appear in any of these records. A total of 11 deaths from cancer were identified (three breast, two ovary, two lung and one each of large bowel, uterus, leukaemia and peritoneum) compared with 4.38 expected from national age- and calendar period-specific rates (SMR, 2.51; 95% CI, 1.25–4.49). Expected numbers based on local mortality rates were similar. [The Working Group noted that deaths in the cohort were ascertained from a different source from the reference rates. Also, it was unclear whether any of the cases from the original cluster were included in the study.]

Prompted by a perceived excess of cancer among pest-control officers of a large French city, Ambroise *et al*. (2005) conducted a historical cohort study of 181 men who had worked in the pest control department during 1979–84. The cohort was followed for mortality up to 2000 through registry offices of birthplaces and records held by the Institut National de la Statistique et des Etudes Economiques; causes of death were obtained by matching with a national file of death certificates or from records held by the personnel department (three cases). For three subjects who died abroad, vital status was established by interview with colleagues. Ethylene oxide had been used to sterilize hospital equipment, but no exposure measurements were reported. Individual exposures to ethylene oxide were assigned by application of a job–exposure matrix to occupational histories abstracted from administrative records. At least 140 subjects were classed as having worked with ethylene oxide. In the cohort as a whole, 39 deaths from all causes occurred, including 21 from cancer (9.36 expected from regional sex- and age-specific rates by calendar year). However, no statistically significant excess of mortality was observed for any specific site of cancer and no consistent trend of cancer mortality was observed in relation to estimated cumulative exposures to ethylene oxide. [The Working Group noted that less weight can be given to the overall excess of cancer in this study, since the investigation was prompted by a perceived excess of tumours.]

2.2.2 *USA*

Morgan *et al*. (1981) reported a retrospective cohort study of 767 men who had been employed between 1955 and 1977 at a chemical plant in eastern Texas where ethylene oxide was produced. All of the men had worked at the factory for at least 5 years and were ‘potentially exposed’ to the compound. Potential exposure to ethylene oxide was determined by personnel at the company on the basis of work histories. In an industrial hygiene survey in 1977, all samples taken in the production area contained less than 10 ppm [18 mg/m^3] ethylene oxide. Vital status was ascertained for more than 95% of the cohort members from a combination of plant records, ‘personal knowledge’ and telephone follow-up. Altogether, 46 deaths were recorded, whereas 80 were expected on the

basis of US vital statistics. Death certificates were obtained for 42 of the 46 deceased subjects. Eleven deaths were from cancer (15.2 expected), and non-significant excesses were found for mortality from cancers of the pancreas (three versus 0.8 expected) and brain and central nervous system (two versus 0.7 expected) and from Hodgkin disease (two versus 0.4 expected); no deaths from leukaemia occurred. [The Working Group noted that details on the nature of the manufacturing process, the extent to which exposure readings were representative of earlier conditions in the plant and potential confounding exposures were lacking.] The results of an extended follow-up of this cohort to 1985 (follow-up rate, 99.7%) were reported by Shore *et al*. (1993) as part of a meta-analysis of cohort studies on ethylene oxide. Three deaths from brain cancer (1.1 expected), three from lymphatic and haematopoietic cancer (3.0 expected), none from leukaemia (1.1 expected) and none from stomach cancer [expected number not given] were observed.

A series of studies was carried out on a cohort of 2174 male employees at two chemical plants in West Virginia where ethylene oxide had been produced and used (Greenberg *et al*., 1990; Benson & Teta, 1993; Teta *et al*., 1993). It was produced by the chlorohydrin process during 1925–57 and by direct oxidation from 1937 to 1971. After 1971, the plants continued to use ethylene oxide that had been produced elsewhere. The cohort comprised men who had been employed at the plants during 1940–78 and assigned at any time before 1979 to a chemical production department in which ethylene oxide was judged to have been manufactured or used at the time of the assignment. The first large-scale environmental monitoring project at the plant began in 1976. The 8-h TWA concentration of ethylene oxide in departments where it was used was generally less than 1 ppm [1.8 mg/m^3] but ranged up to 66 ppm [120 mg/m^3]. The authors estimated that the 8-h TWA concentration in ethylene oxide production by direct oxidation in the 1960s ranged from 3 to 20 ppm [5.4–36 mg/m^3] and that exposures during production by the chlorohydrin process were probably higher. Departments were classified as having high, medium or low exposure concentrations according to the operations carried out, and the classification was validated by reference to reported incidents of acute exposure. The cohort was followed to the end of 1988, and vital status was ascertained for more than 98% of subjects. Death certificates were obtained for 99% of decedents, and expected numbers of deaths were calculated on the basis of national 5-year age- and calendar period-specific rates in white men.

A total of 278 men had worked in a chlorohydrin unit that primarily produced ethylene chlorohydrin, with ethylene dichloride and bischloroethyl ether as by-products (Benson & Teta, 1993). For part of the time, propylene chlorohydrin had also been produced. Ethylene oxide was handled only sporadically and in small volumes. Of these men, 147 had died while 140.8 deaths were expected. The deaths included 40 from cancer (30.8 expected; SMR, 1.30; 95% CI, 0.9–1.8), eight from lymphatic and haematopoietic cancer (2.7 expected; SMR, 2.9; 95% CI, 1.3–5.8) and eight from pancreatic cancer (1.6 expected; SMR, 4.9; 95% CI, 1.6–11.4). In a comparison with workers from other plants in the same locality, the risks for cancer of all types, for lymphatic and haematopoietic

cancer, leukaemia and pancreatic cancer increased with duration of assignment to the chlorohydrin unit.

Among the 1896 men who had never been assigned to the chlorohydrin unit, 431 deaths occurred whereas 547.7 were expected (Teta *et al*., 1993). The numbers of observed/expected deaths were 110/128.1 (SMR, 0.86; 95% CI, 0.7–1.0) for cancer at any site, 8/5.0 (SMR, 1.6; 95% CI, 0.7–3.2) for stomach cancer, 4/6.6 (SMR, 0.6; 95% CI, 0.2–1.6) for pancreatic cancer, 6/4.0 (SMR, 1.5; 95% CI, 0.6–3.3) for cancers of the brain and nervous system, 7/11.8 (SMR, 0.6; 95% CI, 0.2–1.2) for lymphatic and haematopoietic cancer, 2/2.0 for lymphosarcoma and reticulosarcoma (International Classification of Diseases [ICD]-9 200), 5/4.7 (SMR, 1.1; 95% CI, 0.4–2.5) for leukaemia and aleukaemia and 0/1.2 for Hodgkin disease. No significant excess of mortality was observed for any cause of death. No excesses of mortality from leukaemia or stomach cancer were observed among men who had spent 2 or more years in high-exposure departments. Comparison with death rates of workers from plants in the same location who had never been assigned to ethylene oxide production or use showed no significant trend with duration of assignment for all cancers, leukaemia or pancreatic, brain or stomach cancers; however, a two- to threefold increase in the risk for leukaemia (based on three cases) was observed among workers with more than 10 years of assignment to ethylene oxide departments. This study confirmed and amplified the findings of an earlier case–control study at the same plants (Ott *et al*., 1989).

Steenland *et al*. (1991) followed 18 254 employees at 14 industrial plants where ethylene oxide had been used to sterilize medical supplies or spices or in the testing of sterilizing equipment. The plants were selected because they held adequate records on personnel and exposure and their workers had accumulated at least 400 person–years at risk before 1978. Only workers with at least 3 months of exposure to ethylene oxide were included in the cohort. Forty-five per cent of the cohort were men, 79% were white, 1222 were sterilizer operators and 15 750 were employed before 1978. Analysis of 627 personal 8-h TWA samples indicated that average exposure during 1976–85 was 4.3 ppm [7.7 mg/m^3] for sterilizer operators; on the basis of 1888 personal samples, the average level for other exposed workers was 2.0 ppm [3.6 mg/m^3]. Many companies began to install engineering controls in 1978, and exposures before that year were thought to have been higher. There was no evidence of potentially confounding exposure to other occupational carcinogens. The cohort was followed up to 1987 through the national death index and records of the Social Security Administration, the Internal Revenue Service and the US Postal Service; 95.5% were traced successfully. The expected numbers of deaths were calculated from rates in the US population, stratified according to age, race, sex and calendar year. In total, 1177 cohort members had died (1454.3 expected), including 40 for whom no death certificate was available. There were 343 deaths from cancer (380.3 expected; SMR, 0.9; 95% CI, 0.8–1.0). The observed/expected numbers of deaths were 36/33.8 (SMR, 1.07; 95% CI, 0.7–1.5) for all lymphatic and haematopoietic cancer, including 8/5.3 (SMR, 1.5; 95% CI, 0.7–3.0) for lymphosarcoma-reticulosarcoma [ICD-9 200], 4/3.5 (SMR, 1.1; 95% CI, 0.3–2.9) for Hodgkin lymphoma, 13/13.5 (SMR,

0.97; 95% CI, 0.5–1.7) for leukaemia, 8/6.7 (SMR, 1.2; 95% CI, 0.6–2.4) for non-Hodgkin lymphoma [ICD-9 202] and 3/5.1 (SMR, 0.6; 95% CI, 0.1–1.7) for myeloma; 6/11.6 (SMR, 0.5; 95% CI, 0.2–1.1) for cancer of the brain and nervous system; 11/11.6 (SMR, 0.95; 95% CI, 0.5–1.7) for cancer of the stomach; 16/16.9 (SMR, 0.95; 95% CI, 0.5–1.5) for cancer of the pancreas; 8/7.7 (SMR, 1.0; 95% CI, 0.4–2.1) for cancer of the oesophagus; and 13/7.2 (SMR, 1.8; 95% CI, 0.96–3.1) for cancer of the kidney. Mortality ratios for subjects who were first exposed before 1978 were virtually identical to those for the full cohort. No significant trend in mortality was observed in relation to duration of exposure, but the mortality ratios for leukaemia (1.79 based on five deaths) and non-Hodgkin lymphoma (1.92 based on five deaths) were higher after allowance for a latency of more than 20 years. Among the sterilizer operators, mortality ratios (and observed numbers of deaths) were 2.78 (two) for leukaemia and 6.68 (two) for lympho-sarcoma/reticulosarcoma; no death from stomach cancer was observed.

In a further analysis of the same study (Stayner *et al*., 1993), an exposure–response analysis was conducted using previously derived quantitative estimates of individual exposure to ethylene oxide (Greife *et al.*, 1988). Analysis was limited to 13 of the facilities studied, since information on exposures at the other facility was inadequate. Mortality from lymphatic and haematopoietic cancer was greatest in the highest category of cumulative exposure to ethylene oxide (> 8500 ppm–days) (13 deaths; SMR, 1.24; 95% CI, 0.66–2.13), but the trend across three categories of cumulative exposure was weak (χ^2, 0.97; p = 0.32). A similar pattern was observed for non-Hodgkin lymphoma, but not for leukaemia. In addition, a Cox proportional hazard model was used to examine risk in relation to cumulative exposure (ppm–days), average exposure (ppm), maximal exposure (ppm) and duration of exposure (days) to ethylene oxide. A significant positive trend in risk with increasing cumulative exposure to ethylene oxide was observed for all neoplasms of the lymphatic and haematopoietic tissues [p = 0.03, two-tailed]. Moreover, this trend was strengthened [p = 0.004] when the analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukaemia, ICD-9 204; non-Hodgkin lymphoma, ICD-9 200, 202). The exposure–response relationship between cumulative exposure to ethylene oxide and leukaemia was positive but non-significant [p =0.15]. The regression coefficients for neoplasms of the lymphatic and haematopoietic tissues for duration of, average and maximal exposure were either weakly positive or negative. In this analysis, no significant increase was found for cancers of the stomach, pancreas, brain or kidney. [The Working Group gave greater weight to the internal exposure–response analyses using Cox regression than to those based on SMRs, since the latter are more vulnerable to bias.]

Wong and Trent (1993) reported a separate analysis of mortality in approximately the same population (Steenland & Stayner, 1993), with similar results. The cohort comprised 18 728 employees, and follow-up was to the end of 1988. [The Working Group noted that this report adds little useful information to that provided by Steenland *et al*. (1991).]

Norman *et al*. (1995) studied cancer incidence among 1132 workers (82% women) who were employed during 1974–80 at a plant in New York State that used ethylene

oxide to sterilize medical equipment and supplies. The cohort included both regular employees (45%) and others who had worked only on a temporary basis and who were considered to have lower potential exposures. The investigation was prompted by the demonstration of elevated levels of sister chromatid exchange in the workforce. Leaks of ethylene oxide had been documented on several occasions. From three 2-h samples collected in 1980, the 8-h TWA exposures of sterilizer operators were estimated to be 50–200 ppm [90–360 mg/m^3]. Hygiene at the plant was subsequently improved, and later 8-h TWA exposures were thought to be 5–20 ppm [9–36 mg/m^3]. The cohort was followed for cancer incidence up to 1987, using data from various sources: health examinations (mostly conducted during 1982–85), telephone interviews (up to 1987), mailed surveys in 1987 and 1990, annual searches of the New York State Cancer Registry during 1985–89 and a search of the National Death Index in 1998. Expected numbers of cancers were derived from age- and sex-specific rates in the Surveillance, Epidemiology and End Result (SEER) programme for 1978–81. A small proportion of subjects (about 2%) had to be excluded from the analysis of cancer incidence because information was insufficient. A total of 28 cancers were identified (24 from the cancer registry and four from other sources), including 12 breast cancers (6.96 expected; SIR, 1.72; 95% CI, 0.99–3.00). When the expected number of breast cancers was calculated from SEER rates for 1981–85, it was slightly higher (7.64), and the excess fell just short of statistical significance (SIR, 1.57; 95% CI, 0.90–2.75). Among regular employees, nine cases of breast cancer occurred compared with 5.28 expected from SEER rates for 1981–85 (SIR, 1.70; 95% CI, 0.89–3.23). When analysis was restricted to workers who had completed at least one health examination or follow-up survey (approximately 79% of the total), the number of cases of breast cancer observed was again 12, but the expected number (from SEER rates for 1978–81) was reduced to 4.98. The time between first exposure and diagnosis of breast cancer was ≤ 11 years for each of the 12 observed breast cancer cases, and for one case was only 12 months. No statistically significant excess of mortality was observed for cancers at any other sites.

Lucas and Teta (1996) subsequently drew attention to the potential for early detection bias, among others, in this cohort, because the participants were under active health surveillance and the earlier investigation of cytogenetic abnormalities may have raised awareness of cancer risks. The authors acknowledged this concern, but noted that none of the cases of breast cancer was discovered by screening carried out at the health examinations that formed part of the study (Norman *et al.*, 1996).

Olsen *et al.* (1997) analysed mortality from pancreatic and lymphatic and haematopoietic cancer at four chemical plants where ethylene oxide had been produced by the chlorohydrin process. Production of ethylene oxide had occurred during 1941–67 at one plant, 1951–71 and 1971–80 at the second, 1959–70 at the third and 1936–50 at the fourth. At other times, and sometimes in parallel with ethylene oxide, the plants had also produced propylene oxide by the chlorohydrin process. Workers engaged in the production of ethylene chlorohydrin and its conversion to ethylene oxide had potential exposure to ethylene oxide, but no data were reported on levels of exposure. The cohort

comprised men who had been employed at the relevant facilities for at least 1 year and who had worked for at least 1 month in a job that had probably been in ethylene or propylene chlorohydrin production at some time since these processes began. Vital status was followed through to 1992 from date of entry into the cohort or (for those who entered the cohort before 1940) from 1940. This was achieved by record linkage with the Social Security Administration and the National Death Index, and causes of death were obtained for those cohort members who had died. In the main analysis, cause-specific mortality was compared with that expected from death rates in the US national population stratified by age, sex, race (white) and calendar year. Among the 1361 men eligible for study, 300 deaths occcurred in total, including 281 in the subset who had worked at some time in the ethylene chlorohydrin process. Within this subcohort, 70 deaths from cancer overall (73.8 expected) were observed, including one from pancreatic cancer (3.7 expected) and 10 from lymphatic and haematopoietic cancer (7.1 expected; SMR, 1.29; 95% CI, 0.62–2.38). With allowance for a 25-year induction period from first exposure, six lymphatic and haematopoietic cancers (4.2 expected; SMR, 1.44; 95% CI, 0.52–3.12) were observed. In internal analyses that used a control group of other male employees from the same company at two of the three sites where the study plants were located, there was a weak trend of increasing mortality from lymphatic and haematopoietic cancer in relation to duration of employment in any chlorohydrin production (ethylene or propylene), but this was not statistically significant. No data were presented on specific malignancies other than pancreatic and lymphatic and haematopoietic cancers.

In a report related to the study of Steenland *et al*. (1991), Steenland *et al*. (2003) examined the incidence of breast cancer in a subset of 7576 women who had worked for 1 year or longer at 13 of the 14 plants. The other plant was excluded because of its small size (only 19 women who were employed for 1 year). A postal questionnaire (supplemented by a telephone interview for non-responders) was used to collect information from cohort members (or, if they had died, from their next of kin) on history of breast cancer and various known and suspected risk factors for the disease. For plants that were still using ethylene oxide in the mid-1980s when the cohort had originally been assembled, individual work histories were updated with the assumption that women continued to work in the same job with the same level of exposure to ethylene oxide through to the date when they were last employed at the plant (in practice, this had little impact on estimates of cumulative exposures since, by the mid-1980s, exposure intensities were very low). Mortality follow-up for the cohort was extended to 1998 by the same methods that had been used previously. The incidence of breast cancer (also through to 1998) was established from a combination of questionnaire reports, death records and cancer registrations; the latter were available in nine of the 11 states in which the plants were located. Life-table analysis was used to compare overall incidence of breast cancer in the cohort with that in the general population of women covered by the (SEER) programme, with adjustment for age, calendar period and race (white/non-white). A total of 319 incident cases of breast cancer were identified including 20 with carcinoma *in situ* and 124 who had died by the end of 1998. This gave an SIR of 0.87 (95% CI, 0.77–0.97)

for the cohort as a whole, which was recognized to be an underestimate because ascertainment of cases was incomplete for women who were not interviewed and for those who did not live in states that had cancer registries. When cases of carcinoma *in situ* were excluded, the SIR increased slightly to 0.94. With a 15-year lag, there was a significant trend of higher SIRs with higher cumulative exposures ($p = 0.002$), but this was less marked ($p = 0.16$) in an unlagged analysis. In addition to the external comparison, a nested case–control design with Cox regression was used to assess internal exposure–response relationships. In these analyses, risk sets matched on race were constructed for each case by randomly selecting 100 controls from the pool of all women who had survived without breast cancer to at least the same age as the index case. In an analysis that included all 319 cases and in which exposures were lagged by 15 years, the odds ratio for the highest fifth of cumulative exposure relative to no exposure was 1.74 (95% CI, 1.16–2.65). In a similar analysis that was restricted to the subset of 5139 women who were interviewed (233 cases) and adjusted for parity and history of breast cancer in a first degree relative (the two potential risk factors that were found to be important predictors of breast cancer), the corresponding odds ratio was 1.87 (95% CI, 1.12–3.10).

[The Working Group noted that, for cancers that have a high survival rate in the general population, such as breast cancer, studies based on incidence rather than mortality may be more sensitive in the detection of an elevated risk associated with an occupational exposure. In contrast, several methodological difficulties complicate the interpretation of studies on the incidence of breast cancer in occupational cohorts, including potential differences in reproductive histories associated with employment and the possibility of differential rates by occupational exposure. The Steenland *et al.* (2003) study was able to address some but not all of these potential limitations and was judged by the Working Group to provide the most pertinent evidence on the potential association of breast cancer with exposure to ethylene oxide.]

An updated analysis of mortality from cancer in the cohort of employees at 14 industrial plants was reported by Steenland *et al*. (2004) and included 18 235 subjects who were followed up to 1998. Work histories for individuals employed at plants that were still using ethylene oxide at the time the cohort had originally been assembled were extended to their last date of employment at the relevant plant, with an assumption that they did not change their job or exposure to ethylene oxide during the additional period of employment. Life-table analyses were conducted with the national population of the USA as a reference. In total, 2852 deaths from all causes (SMR, 0.90; 95% CI, 0.88–0.93) were observed, including 860 from cancer (SMR, 0.98; 95% CI, 0.92–1.03). The only category of malignancy for which mortality was significantly elevated was cancer of the bone (SMR, 2.82; 95% CI, 1.23–2.56). However, this finding was based on only six observed deaths, and there was no indication of an increase in risk with increasing cumulative exposure. Overall, mortality from cancer of the stomach (25 deaths; SMR, 0.98; 95% CI, 0.74–1.49), cancer of the breast (103 deaths; SMR, 0.99; 95% CI, 0.84–1.17), lymphatic and haematopoietic cancer (79 deaths; SMR, 1.00; 95% CI, 0.79–1.24), non-Hodgkin lymphoma (31 deaths; SMR, 1.00; 95% CI, 0.72–1.35), Hodgkin disease (six deaths;

SMR, 1.24; 95% CI, 0.53–2.43), myeloma (13 deaths; SMR, 0.92; 95% CI, 0.54–0.87) and leukaemia (28 deaths; SMR, 0.99; 95% CI, 0.71–1.36) was unremarkable. In an internal analysis (excluding one small plant for which exposure data were not available), cases of lymphatic and haematopoietic and breast cancer were matched for race, sex and date of birth with controls (100 per case) who had survived without these cancers to at least the age of the index case. When log cumulative exposures to ethylene oxide were lagged by 15 years, a statistically significant positive trend ($p = 0.02$) was observed for mortality from lymphatic and haematopoietic cancer in men but not in women. However, duration of, peak, average or cumulative exposure did not predict mortality from lymphatic and haematopoietic cancer. A similar pattern was observed for lymphoid-cell tumours specifically (including non-Hodgkin lymphoma, myeloma and lymphocytic leukaemia). With a lag of 20 years, mortality from breast cancer was highest in women who had the highest quarter of exposures (odds ratio, 3.13; 95% CI, 1.42–6.92 relative to no exposure).

2.3 Case–control study

Swaen *et al*. (1996) carried out a case–control study within the workforce of a chemical manufacturing plant in Belgium to investigate an increased incidence of Hodgkin lymphoma that had been noted by the medical director at the facility. Ten cases, diagnosed during 1968–91, were compared with a total of 200 individually matched controls. The controls had been employed at the plant for at least 3 consecutive months and were actively employed at the time that their matched case was diagnosed (a person could serve as a control for more than one case). The job histories of cases and controls were abstracted from personnel records and reviewed by a company industrial hygienist (blinded to health status), who assessed their potential exposure to a range of chemicals. For 24.3% of subjects who had inadequate occupational histories, additional data were then sought (unblinded to health status) from medical records. Three cases were classed as exposed to ethylene oxide (odds ratio, 8.5; 95% CI, 1.4–39.9), but one of these cases had been reclassified as a large-cell anaplastic carcinoma when pathology samples were reviewed.

2.4 Meta-analysis

Teta *et al*. (1999) updated the earlier meta-analysis of Shore *et al*. (1993) with inclusion of data from the study reported by Olsen *et al*. (1997) and the updated follow-up of Swedish sterilant workers reported by Hagmar *et al*. (1995). Thus, a total of 10 individual cohorts were studied. Altogether, 876 cases of cancer were recorded compared with 928 expected, giving a meta-SMR standardized for age, sex and calendar year of 0.94 (95% CI, 0.85–1.05). Observed/expected numbers for specific cancers were: pancreas, 37/39 (meta-SMR, 0.95; 95% CI, 0.69–1.31); brain, 25/26 (meta-SMR, 0.96; 95% CI, 0.49–1.91); stomach, 59/48 (meta-SMR, 1.23; 95% CI, 0.71–2.13); leukaemia,

35/32 (meta-SMR, 1.08; 95% CI, 0.61–1.93); and non-Hodgkin lymphoma, 33/25 (meta-SMR, 1.34; 95% CI, 0.96–1.89). None of the cancers analysed showed significant trends in risk with increasing duration or intensity of exposure; however, the risk for brain cancer increased with time since first exposure ($p < 0.05$ based on four studies).

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

Carcinogenicity bioassays of inhalation exposure to ethylene oxide in mice and rats are summarized in Tables 7 and 8, respectively.

3.1.1 *Mouse*

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice that is highly susceptible to the development of this neoplasm, groups of 30 female strain A/J mice, 8–10 weeks of age, were exposed by inhalation to 0, 70 or 200 ppm [0, 128 or 366 mg/m^3] ethylene oxide (at least 99.7% pure) for 6 h per day on 5 days per week for up to 6 months in two independent experiments; in the second experiment, the 70-ppm group was omitted. Two groups of 30 female mice were exposed to room air and served as negative controls, and two groups of 20 female mice received a single intraperitoneal injection of 1000 mg/kg bw urethane and served as positive controls for both experiments. At the end of the 6th month, the survivors were killed and examined for pulmonary adenomas. In the first experiment, survival was 30/30 (0 ppm), 28/30 (70 ppm), 29/30 (200 ppm) and 19/20 (urethane); that in the second experiment was 29/30 (0 ppm), 28/30 (200 ppm) and 19/20 (urethane). The numbers of animals with pulmonary adenomas among survivors (and tumour multiplicity) in the first experiment were: untreated controls, 8/30 (0.46 ± 0.38 [± SD] adenomas/mouse); low-dose, 16/28 (0.86 ± 0.45); high-dose, 25/29 (2.14 ± 0.49); and urethane-treated, 19/19 (20.1 ± 1.77); those in the second experiment were: untreated controls, 8/29 (0.22 ± 0.38); ethylene oxide-treated, 12/28 (0.73 ± 0.98); and urethane-treated, 19/19 (23.5 ± 6.49). The tumour multiplicity increased significantly in each experiment ($p < 0.05$, Duncan's new multiple-range test); in the first experiment, it also increased significantly in a dose-dependent manner [$p < 0.001$, Cochran-Armitage trend test] (Adkins *et al*., 1986).

Groups of 50 male and 50 female B6C3F$_1$ mice, 8 weeks of age, were exposed by inhalation to 0, 50 or 100 ppm [0, 92 or 183 mg/m^3] ethylene oxide (> 99% pure) for 6 h per day on 5 days per week for up to 102 weeks, at which time the experiment was terminated. Mean body weights of treated males and females were similar to those of controls. At the end of the study, 28/50 control, 31/50 low-dose and 34/50 high-dose males, and 25/50 control, 24/50 low-dose and 31/50 high-dose females were still alive.

Table 7. Carcinogenicity studies of inhalation exposure to ethylene oxide in experimental mice

Strain	Sex	No./group at start	Purity	Dose and duration of exposure	Duration of study	Incidence of tumours	Result	Comments	Reference
Strain A/J	F	30	≥ 99.7%	0, 70, 200 ppm, 6 h/day, 5 days/week 0, 200 ppm, 6 h/day, 5 days/week	Up to 6 months Up to 6 months	Lung[a]: 8/30, 16/28, 25/29 Lung[a]: 8/29, 12/28	$p < 0.001$ (trend) NS	Two independent experiments; tumour multiplicities increased with dose in both experiments ($p < 0.05$)	Adkins *et al.* (1986)
$B6C3F_1$	M	50	> 99%	0, 50, 100 ppm, 6 h/day, 5 days/week	102 weeks	Lung[b]: 11/50, 19/50, 26/50 Harderian gland[c]: 1/43, 9/44, 8/42[d]	$p = 0.002$ (trend) $p < 0.03$ (trend)		National Toxicology Program (1987)
$B6C3F_1$	F	50	> 99%	0, 50, 100 ppm, 6 h/day, 5 days/week	102 weeks	Lung[b]: 2/49, 5/48, 22/49 Harderian gland[c]: 1/46, 6/46[d], 8/47 Lymphoma: 9/49, 6/48, 22/49 Uterus[e]: 0/49, 2/47, 5/49 Mammary gland[f]: 1/49, 8/48, 6/49	$p < 0.001$ (trend) $p < 0.04$ (trend) $p = 0.023$ (trend) $p < 0.03$ (trend) $p = 0.012$ (low dose only)		National Toxicology Program (1987); Picut *et al.* (2003)

F, female; M, male; NS, not significant

[a] Mice with one or more pulmonary adenomas/total mice at risk

[b] Mice with one or more tumours/total mice at risk, alveolar/bronchiolar adenomas and carcinomas combined

[c] Papillary cystadenomas

[d] A cystadenocarcinoma was also present in an animal with a cystadenoma.

[e] Adenocarcinomas, including one tumour in a low-dose mouse originally reported as an adenoma

[f] Carcinomas

Table 8. Carcinogenicity studies of inhalation exposure to ethylene oxide in experimental rats

Strain	Sex	No./group at start	Purity	Dose and duration of exposure	Duration of study	Incidence of tumours	Result	Comments	Reference
Fischer 344	M	80	99.7%	0, 50, 100 ppm, 7 h/day, 5 days/week	2 years	Brain[a]: 0/76, 2/77, 5/79 Mononuclear-cell leukaemia: 24/77, 38/79, 30/76 Peritesticular mesothelioma: 3/78, 9/79, 21/79	$p < 0.05$ (high dose) $p = 0.03$ (low dose) $p = 0.002$ (high dose)		Lynch *et al.* (1984a)
Fischer 344	M	120	> 99.9%	0, 10, 33, 100 ppm, 6 h/day, 5 days/week	2 years	Brain[b]: 1/181, 0/92, 3/85, 6/87 Mononuclear-cell leukaemia: 13/97, 9/51, 12/39, 9/30 Peritesticular mesothelioma: 2/97, 2/51, 4/39, 4/30 Subcutaneous fibroma: 3/97, 9/51, 1/39, 11/30	$p < 0.05$ (trend) $p < 0.05$ (trend) $p < 0.005$ (trend) $p < 0.01$ (high dose)	Early deaths due to viral sialo-dacryoadenitis; no increases in tumour incidence up to 18 months; sites other than brain include only necropsies after 24 months.	Snellings *et al.* (1984); Garman *et al.* (1985, 1986)
Fischer 344	F	120	> 99.9%	0, 10, 33, 100 ppm, 6 h/day, 5 days/week	2 years	Brain[b]: 0/187, 1/94, 2/90, 2/78 Mononuclear-cell leukaemia: 11/116, 11/54, 14/48, 15/26	$p < 0.05$ (trend) $p < 0.005$ (trend)		

F, female; M, male

[a] Brain tumours were gliomas. Focal proliferations of glial cells (termed 'gliosis') were also observed in two low-dose rats and four high-dose rats.

[b] Brain tumours included gliomas only. Numbers include rats killed both at 18 months and at the conclusion of the 2-year study.

The incidence of alveolar/bronchiolar carcinomas in male mice was 6/50 control, 10/50 low-dose and 16/50 high-dose ($p = 0.019$, incidental tumour test for trend). A slight increase in the incidence of alveolar/bronchiolar adenomas also occurred. The combined incidence of lung tumours was 11/50 control, 19/50 low-dose and 26/50 high-dose ($p = 0.002$, incidental tumour test for trend). In females, the incidence of alveolar/bronchiolar adenomas (2/49 control, 4/48 low-dose and 17/49 high-dose) and alveolar/bronchiolar carcinomas (0/49 control, 1/48 low-dose and 7/49 high-dose) and the combined incidence of lung tumours (2/49 control, 5/48 low-dose and 22/49 high-dose) were all significantly increased ($p < 0.001$, incidental tumour test for trend). The incidence of papillary cystadenoma of the Harderian gland increased significantly in animals of each sex (males: 1/43 control, 9/44 low-dose and 8/42 high-dose; females: 1/46 control, 6/46 low-dose and 8/47 high-dose; $p < 0.04$, incidental tumour test for trend in both sexes). In addition, one papillary cystadenocarcinoma of the Harderian gland was observed in one high-dose male and one in a low-dose female. In females, the incidence of malignant lymphomas was 9/49 control, 6/48 low-dose and 22/49 high-dose mice ($p = 0.023$, life-table test for trend). An increase in the incidence of uterine adenocarcinomas was observed in 0/49 control, 2/47 low-dose and 5/49 high-dose females ($p < 0.03$, incidental tumour test for trend). The incidence of mammary gland carcinomas in females was 1/49 control, 8/48 low-dose ($p = 0.012$, incidental pair-wise tumour test) and 6/49 high-dose mice (National Toxicology Program, 1987). Because of the rarity of primary epithelial tumours of the uterus in long-term inhalation studies in mice, data on the pathology and incidence of uterine tumours in $B6C3F_1$ mice from 2-year National Toxicology Program inhalation bioassays of bromoethane, chloroethane and ethylene oxide were reviewed. Diagnoses of uterine adenocarcinoma in the 1987 bioassay of ethylene oxide were confirmed (Picut *et al.*, 2003). [The Working Group noted that the diagnosis of uterine adenoma in one low-dose female was revised to adenocarcinoma.]

3.1.2 *Rat*

Groups of 80 male weanling Fischer 344 rats were exposed by inhalation to 0 (control; filtered air), 50 or 100 ppm [92 or 180 mg/m^3] ethylene oxide (purity, 99.7%) vapour for approximately 7 h per day on 5 days per week for 2 years. The mortality rate was increased in the two treated groups over that in controls, and the increase was significant for the high-dose group ($p < 0.01$). Mononuclear-cell leukaemia was observed in 24/77 control rats, in 38/79 rats exposed to 50 ppm ethylene oxide and in 30/76 exposed to 100 ppm. The overall increase in the incidence of mononuclear-cell leukaemia was significant ($p = 0.03$) in the low-dose group, but the increase could not be ascertained in the high-dose group because of excessive mortality. Peritoneal mesotheliomas in the region of the testis developed in 3/78 control, 9/79 low-dose and 21/79 high-dose rats; the increase was significant for the high-dose group ($p = 0.002$). Gliomas (mixed cell type) were found in 0/76 control, 2/77 low-dose and 5/79 high-dose animals ($p < 0.05$, pair-wise comparison for the high dose). Focal proliferation of glial cells (termed ‘gliosis’)

was observed in two rats exposed to 50 ppm and four rats exposed to 100 ppm ethylene oxide. [The Working Group noted that lesions such as those described as 'gliosis' are probably glial tumours, and that true gliosis is a reactive lesion and not a neoplasm.] The incidence of other neoplasms was comparable in the control and treated groups and was not associated with exposure to ethylene oxide. A high incidence of proliferative lesions described as 'multifocal cortical hyperplasia' and 'cortical nodular hyperplasia' was observed in the adrenal cortex of animals exposed to ethylene oxide (Lynch *et al*., 1984a).

Three groups of 120 male and three groups of 120 female Fischer 344 rats, 8 weeks of age, were exposed by inhalation to 10, 33 or 100 ppm [18, 59 or 180 mg/m^3] ethylene oxide (purity, > 99.9%) vapour for 6 h per day on 5 days per week for up to 2 years. Two control groups (I and II), each of 120 male and 120 female rats, were exposed in inhalation chambers to room air. All animals that died or were killed when moribund and those killed at scheduled intervals of 6, 12, 18 and 24 (females)–25 (males) months were examined. During month 15 of exposure, mortality increased in both treated and control groups due to a viral sialodacryoadenitis. Mortality was higher in the groups exposed to 33 and 100 ppm ethylene oxide than in the other groups and was also higher in females than in males. Up to 18 months of exposure, no significant increase in tumour incidence was observed. In treated rats killed after 18 months, the incidence of brain tumours classified as 'gliomas, malignant reticulosis and granular-cell tumours' was increased in animals of each sex. The incidence of brain tumours (gliomas only) among rats killed at 18 and 24–25 months was: males: 1/181 (controls), 0/92 (10 ppm), 3/85 (33 ppm) and 6/87 (100 ppm) ($p < 0.05$, Cox's test for adjusted trend and Fisher's exact test for high-dose versus control); and females: 0/187 (controls), 1/94 (10 ppm), 2/90 (33 ppm) and 2/78 (100 ppm) ($p < 0.05$, Cox's test for adjusted trend). In females killed after 24 months of exposure, mononuclear-cell leukaemia was found in 5/60 (control I), 6/56 (control II), 11/54 (10 ppm), 14/48 (33 ppm) and 15/26 (100 ppm) animals; the incidence of leukaemia was reported by the authors to be significantly increased in the 100-ppm group ($p < 0.001$) and in a mortality-adjusted trend test ($p < 0.005$). In males, mononuclear-cell leukaemia was found in 5/48 (control I), 8/49 (control II), 9/51 (10 ppm), 12/39 (33 ppm) and 9/30 (100 ppm) animals ($p < 0.05$, mortality-adjusted trend test). Peritoneal mesotheliomas originating in the testicular serosa were found in 1/48 (control I), 1/49 (control II), 2/51 (10 ppm), 4/39 (33 ppm) and 4/30 (100 ppm) males ($p < 0.005$, trend test). The incidence of subcutaneous fibromas in male rats of the high-dose group was also significantly increased: 1/48 (control I), 2/49 (control II), 9/51 (10 ppm), 1/39 (33 ppm) and 11/30 (100 ppm) ($p < 0.01$) (Snellings *et al*., 1984; Garman *et al*., 1985, 1986).

3.2 Oral administration

Rat

Groups of 50 female Sprague-Dawley rats, approximately 100 days of age, were administered 7.5 or 30.5 mg/kg bw ethylene oxide (purity, 99.7%) in a commercial vegetable oil [composition unspecified] by gastric intubation twice weekly for 107 weeks (average total dose, 1186 or 5112 mg/kg bw, respectively). Controls comprised one group of 50 untreated female rats and a second group of 50 female rats treated with vegetable oil alone. The survival rate of rats in the high-dose group was lower than that of the control groups. Treatment with ethylene oxide resulted in a dose-dependent increase in the incidence of forestomach tumours, which were mainly squamous-cell carcinomas. Such tumours were not found in the untreated or vehicle controls. In the low-dose group, 8/50 animals developed squamous-cell carcinomas, 4/50 had carcinomas *in situ* and 9/50 had papillomas, hyperplasia or hyperkeratosis of the forestomach. In the high-dose group, 31/50 animals developed malignant tumours of the stomach; 29 were squamous-cell carcinomas of the forestomach and two were fibrosarcomas, one of which was located in the glandular stomach. In addition, 4/50 had carcinomas *in situ* and 11/50 had papillomas, hyperplasia or hyperkeratosis of the forestomach. Many of the stomach tumours found in the high-dose group metastasized or grew invasively into neighbouring organs. There was no increase in the incidence of tumours at other sites in the treated animals over that in controls (Dunkelberg, 1982).

3.3 Dermal application

Mouse

Thirty female ICR/Ha Swiss mice, 8 weeks of age at the start of treatment, received topical applications of approximately 100 mg of a 10% solution of ethylene oxide (purity, 99.7%) in acetone on the clipped dorsal skin three times a week for life. The median survival time was 493 days. No skin tumour was observed (Van Duuren *et al*., 1965). [The Working Group noted the high volatility of ethylene oxide which would tend to reduce the dose that the animals received.]

3.4 Subcutaneous administration

Mouse

Groups of 100 female NMRI mice, 6–8 weeks of age, received subcutaneous injections of 0.1, 0.3 or 1.0 mg/mouse ethylene oxide (purity, 99.7%) in tricaprylin once a week for 95 weeks (mean total dose, 7.3, 22.7 or 64.4 mg/mouse). Groups of 200 untreated and 200 tricaprylin-treated mice served as controls. The survival rate of the group given the highest dose was reduced. Ethylene oxide induced a dose-dependent increase in

the incidence of sarcomas at the injection site. Sarcomas occurred in 0/200 untreated controls, 4/200 animals treated with tricaprylin alone, and 5/100, 8/100 and 11/100 animals that received 0.1, 0.3 and 1 mg ethylene oxide, respectively [$p < 0.001$, Cochran-Armitage test for trend]. No significant increase in the incidence of tumours at other sites was observed (Dunkelberg, 1981).

3.5 Induction of enzyme-altered foci in a two-stage liver system

Rat

Litters of Sprague-Dawley rats, 3–5 days of age, were exposed with their dams by inhalation to 0 ppm (five male and nine female rats), 33 ppm (60 mg/m^3, 10 females), 55 ppm (100 mg/m^3, four males and seven females) or 100 ppm (183 mg/m^3, four males and eight females) ethylene oxide [purity unspecified] for 8 h per day on 5 consecutive days per week for 3 weeks. One week later, the offspring were administered, as a promoting agent, 10 mg/kg bw Clophen A 50 (a mixture of polychlorinated biphenyls [not otherwise specified]) orally by gavage twice a week for up to 8 additional weeks, at which time the experiment was terminated. The livers were examined for adenosine triphosphatase-deficient and γ-glutamyltranspeptidase-positive foci. In females that received the two highest doses, but not in males, the number and total area of adenosine triphosphatase-deficient foci increased significantly ($p < 0.05$, Student's *t* test) in comparison with the controls that received Clophen A 50 only. There was no significant difference in the number or total area of γ-glutamyltranspeptidase-positive foci between controls and animals given the high dose of ethylene oxide (Denk *et al*., 1988).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

(a) Absorption, distribution and excretion

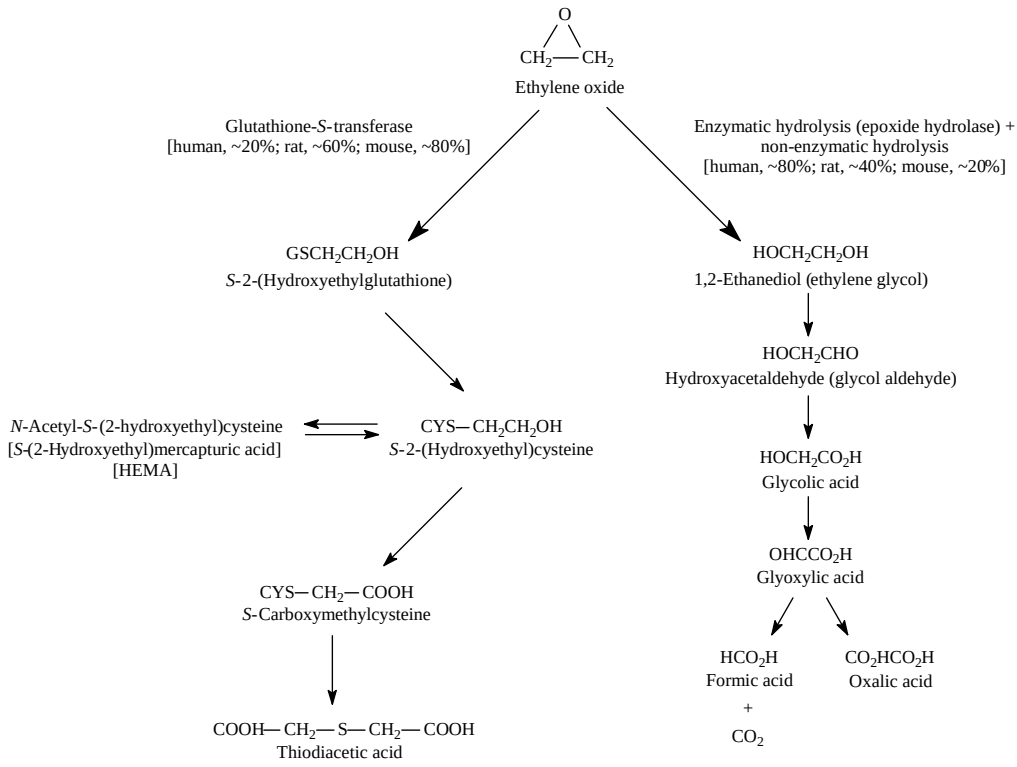
Ethylene oxide is readily taken up by the lungs and is absorbed relatively efficiently into the blood. A study of workers exposed to ethylene oxide revealed an alveolar retention of 75–80%, calculated from hourly determinations of ethylene oxide concentrations in the environmental air that ranged from 0.2 to 24.1 mg/m^3 [0.11–13.2 ppm] and in alveolar air that ranged from 0.05 to 6 mg/m^3 [0.03–3.3 ppm] (Brugnone *et al*., 1985, 1986). At steady state, therefore, 20–25% of inhaled ethylene oxide that reached the alveolar space was exhaled as the unchanged compound and 75–80% was taken up by the body and metabolized. Blood samples taken from workers 4 h after the workshift 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 samples determined for the blood:air partition coefficient *in vitro* was explained by incomplete saturation of tissues and limitation of the metabolic rate by the rate of lung uptake (Brugnone *et al*., 1986).

(b) Metabolism

The following overall schema describes the mammalian metabolism of ethylene oxide (Figure 2). Ethylene oxide is converted (*a*) by enzymatic and non-enzymatic hydrolysis to ethylene glycol, which is partly excreted as such and partly metabolized further via glycolaldehyde, glycolic acid and glyoxalic acid to oxalic acid, formic acid and carbon dioxide; and (*b*) by conjugation with glutathione (GSH) followed by further metabolism to *S*-(2-hydroxyethyl)cysteine, *S*-(2-carboxymethyl)cysteine and *N*-acetylated derivatives (*N*-acetyl-*S*-(2-hydroxyethyl)cysteine (also termed *S*-(2-hydroxyethyl)mercapturic acid or HEMA) and *N*-acetyl-*S*-(2-carboxymethyl)cysteine) (Wolfs *et al.*, 1983; Popp *et al.*, 1994), which are partly converted to thiodiacetic acid (Scheick *et al*., 1997).

Figure 2. Metabolism of ethylene oxide



Adapted from Wolfs *et al.* (1983); Scheick *et al.* (1997)

Blood concentrations of ethylene glycol were determined at the end of day 3 of a normal working week in sterilization personnel who were exposed to ethylene oxide. TWA concentrations of ethylene oxide determined over 8 h ranged from 0.3 to 52 ppm [0.55–95.2 mg/m^3] (overall mean, 4.2 ppm [7.7 mg/m^3]). The mean concentrations of ethylene glycol in the blood of exposed subjects were twice as high (90 mg/L) as those in controls (45 mg/L) (Wolfs *et al*., 1983).

The concentration of thioethers excreted in urine collected at the end of sterilization processes was found to be twice as high in nonsmoking personnel (10.2 mmol/mol creatinine) exposed to peak concentrations of 1–200 ppm [1.83–366 mg/m^3] ethylene oxide as that in unexposed workers (5.46 mmol/mol creatinine). The concentration of ethylene oxide in air was not monitored routinely (Burgaz *et al*., 1992).

The glutathione-*S*-transferase (GST) activity for ethylene oxide in human liver cytosolic fractions was low (too low to determine the Michaelis-Menten constant [K_m] value). The maximum velocity (V_{max}) varied from 7.6 to 10.6 nmol/min/mg protein. Epoxide hydrolase (EH) activity in the microsomal fraction of the human liver averaged 1.8 nmol/min/mg protein. The K_m for hydrolysis has been estimated to be approximately 0.2 mM, but non-enzymatic hydrolysis was significant and precluded accurate determination (Fennell & Brown, 2001).

Metabolism of ethylene oxide to the GSH conjugate and ethylene glycol is generally considered to be the major pathway for the elimination of DNA-reactive ethylene oxide. However, strongly suggestive evidence *in vitro* was presented by Hengstler *et al.* (1994) that glycolaldehyde is formed by further metabolism of ethylene glycol and that this derivative leads to DNA–protein cross-links and to DNA strand breaks (as measured with the alkaline elution assay) after in-vitro incubation with human peripheral mononuclear blood cells.

(c) GST polymorphisms

Ethylene oxide is a substrate of the GST isoenzyme T1 (Hayes *et al*., 2005). This isoenzyme is polymorphic and a relatively large population (in Caucasians about 20%, in Asians almost 50%) has a homozygous deletion (null genotype) (Bolt & Thier, 2006). As expected, these individuals show a significantly higher amount of hydroxyethyl valine in their haemoglobin due to the metabolism of endogenous ethylene to endogenous ethylene oxide (Thier *et al*., 2001). Nevertheless, the influence of this genetic trait on the formation of these haemoglobin adducts by workplace exposure to exogenous ethylene oxide is much less clear, as discussed below.

In the cytoplasm of erythrocytes obtained from 36 individuals, ethylene oxide was eliminated three to six times faster in samples from so-called conjugators (defined by a standardized conjugation reaction of methyl bromide and GSH; 75% of the population) than in those from the remaining 25% (who lack this GST-specific activity). In the latter samples, disappearance did not differ from that of controls. In this experiment, the disappearance of ethylene oxide was investigated in the gas phase of closed vials that contained GSH and cytoplasm of erythrocytes (Hallier *et al.*, 1993).

In contrast, several publications reported no or an unclear association between GSTT1 status and hydroxyethyl adducts after workplace exposure to ethylene oxide (HEMA formation) (Haufroid *et al.*, 2007) or exposure to ethylene oxide from cigarette smoking (hydroxyethyl valine in haemoglobin) (Müller *et al.*, 1998). Other studies reported a relatively weak but significant association (0.17 ± 0.03 *N*-(2-hydroxyethyl)valine formed in haemoglobin versus 0.08 ± ≤ 0.01; $p \leq 0.02$) after exposure to ethylene oxide of individuals who had homozygous deletion of the *GSTT1* gene compared with those who had at least one copy of the gene (Yong *et al.*, 2001).

The different impact of the *GSTT1* polymorphism is due to the fact that, in the study of Hallier *et al.* (1993), cytoplasm of erythrocytes was exposed to ethylene oxide, while in the subsequent studies, the whole human organism was exposed to ethylene oxide. Similarly, Föst *et al.* (1995) also observed that, when ethylene oxide is added to whole blood of various individuals, the impact of the *GSTT1* polymorphism is significant.

In-vitro incubation of mononuclear blood cells from various individuals with polymorphisms in GSTs (*GSTM1, GSTT1* and *GSTP1*) did not demonstrate a significant influence on ethylene oxide-induced DNA strand breaks (measured by the Comet assay) and on micronucleus formation in binucleated cells (Godderis *et al.*, 2006). This lack of an effect is presumably due to the null or negligible contribution of GSTM1 and GSTP1 enzymes to ethylene oxide metabolism and to a too small (20%) contribution of GSTT1 activity in the human whole organism (Figure 2).

(d) Haemoglobin adducts

Ethylene oxide is an electrophilic agent that alkylates nucleophilic groups in biological macromolecules. Haemoglobin adducts have been used to monitor tissue doses of ethylene oxide (Calleman *et al.*, 1978; Farmer *et al.*, 1987; Osterman-Golkar & Bergmark, 1988; Ehrenberg, 1991; Ehrenberg & Törnqvist, 1992). The results have shown measurable increases in hydroxyethyl haemoglobin adducts that are dependent on the workplace exposure concentration (reported and discussed in IARC, 1994).

Cigarette smoke contains ethylene oxide, and hydroxyethyl valine in the haemoglobin of smokers correlates with the number of cigarettes smoked (Fennell *et al.*, 2000; Bono *et al.*, 2002; Wu *et al.*, 2004). The umbilical cord blood of smoking pregnant women contained increased levels of hydroxyethyl valine, which were quantitatively related to the increased levels in maternal blood (Farmer *et al.*, 1996a). Levels of this adduct were significantly higher in the haemoglobin of newborns from smoking compared with those from nonsmoking mothers (147 ± 68 compared with 42 ± 18 pmol/g haemoglobin; $p \leq 0.01$) (Tavares *et al.*, 1994). In *GSTT1*-null individuals, levels of hydroxyethyl valine were significantly elevated when normalized to smoking status or levels of cotinine. The lack of functional *GSTT1* was estimated to increase the internal dose of ethylene oxide derived from cigarette smoke by 50–70% (Fennell *et al.*, 2000). Ethylene oxide is also formed endogenously in humans (Bolt, 1996). This may limit the sensitivity of this adduct as a biomarker for cigarette smoking; indeed, the formation of hydroxyethyl valine was

not associated with exposure to secondhand smoke or with tea or alcoholic beverage consumption, age or gender (Wu *et al.*, 2004).

(e) Toxicokinetic modelling

A recent model by Fennell and Brown (2001) determined a half-life of ethylene oxide in the human body of 47.6 min, taking into account the enzymatic disposition of ethylene oxide by human liver microsomal EH (mEH) and cytosolic GST by their K_m and V_{max} characteristics, as well as a possible depletion of GSH. This half-life value was quite close to the 42-min value obtained by Filser *et al.* (1992). In this human model, the majority of ethylene oxide is metabolized by hydrolysis, and only approximately 20% is converted to GSH conjugates. There is little change in metabolism with increasing exposure concentration. At given exposure concentrations of ethylene oxide (1, 100 and 300 ppm [1.83, 183 and 549 mg/m^3]), blood concentration was sensitive to alveolar ventilation and to K_m and V_{max} for liver mEH but relatively insensitive to the liver GSH concentration and the rate of GSH synthesis in the liver.

4.1.2 *Experimental systems*

(a) Absorption, distribution and excretion

Pulmonary uptake of ethylene oxide is expected to be rapid and dependent only upon the alveolar ventilation rate and concentration in the inspired air, since this compound is very soluble in blood (IPCS, 1985). Ethylene oxide is absorbed rapidly through the respiratory tract in rats (Filser & Bolt, 1984; Koga *et al.*, 1987; Tardif *et al.*, 1987), mice (Ehrenberg *et al.*, 1974; Tardif *et al.*, 1987) and rabbits (Tardif *et al.*, 1987). Close to 100% of inhaled ethylene oxide was absorbed by mice that were exposed for 1–2 h to 2–55 mg/m^3 (1.1–30 ppm) (Ehrenberg *et al.*, 1974).

The permeation rate of a solution of 1% ethylene oxide in water (w/v) through excised human skin at 30 ºC was determined to be 0.125 mg/(cm^2 × h) (Baumbach *et al.*, 1987).

The pharmacokinetics of inhaled ethylene oxide have been investigated in male Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al.*, 1992). The studies were carried out in closed exposure chambers of 6.4 and 9.5 L occupied by two and three rats, respectively. The initial concentrations of ethylene oxide vapour in the chamber atmospheres were up to about 1100 ppm [2000 mg/m^3]. Filser and Bolt (1984) showed that ethylene oxide is rapidly taken up by the lungs because the clearance due to uptake, which reflects the rate of transfer of ethylene oxide from the atmosphere into the organism, was 11 100 mL/h (185 mL/min) for two Sprague-Dawley rats that weighed 500 g. Johanson and Filser (1992) calculated a value of 58 mL/min for one Sprague-Dawley rat that weighed 250 g by allometric scaling, according to the method of Filser (1992). This value represents 50% of the alveolar ventilation (117 mL/min; Arms & Travis, 1988), which indicates that about 50% of the amount inhaled into the lung is exhaled again without becoming systemically available via the bloodstream. A possible

explanation for this finding is that there is a 'wash in-wash out' effect in the upper airways (Johanson & Filser, 1992), which may be more effective in rats than in humans (Filser *et al*., 1993). The maximal accumulation of ethylene oxide in the body of Sprague-Dawley rats, determined as the thermodynamic partition coefficient for whole body:air, was 30. Because of fast metabolic elimination, the concentration ratio at steady state for whole body:air, calculated for two animals that weighed 500 g, was only 1.52 over the entire dose range. A re-calculation of this parameter according to Filser (1992) for one Sprague-Dawley rat that weighed 250 g yielded a value of 1.88, which is similar to the coefficient for venous blood:environmental air found in workers exposed to ethylene oxide under steady-state conditions (see above).

An almost uniform distribution of ethylene oxide within the body was concluded from the similar tissue:air partition coefficients for organs of male Fischer 344 rats determined *in vitro*: fat, 44.1; muscle, 48.3; brain, 58.7; lung, 60.9; liver, 61.6; blood, 64.1; and testes, 83 (Krishnan *et al*., 1992).

Elimination of ethylene oxide was described by first-order kinetics over the whole concentration range examined in both Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al*., 1992). At steady state, the clearance due to metabolism in relation to the concentration in the atmosphere was 10 600 mL/h (177 mL/min) for two Sprague-Dawley rats that weighed 500 g (Filser & Bolt, 1984). Re-calculation for one Sprague-Dawley rat that weighed 250 g according to the method of Filser (1992) gave a value of almost 55 mL/min. On the basis of the finding that clearance due to metabolism in relation to the concentration in the atmosphere is nearly identical to that due to uptake, uptake of ethylene oxide by inhalation was concluded to be the rate-limiting step for metabolism of this compound. Alveolar retention in one Sprague-Dawley rat that weighed 250 g was calculated as 47% on the basis of a ventilation rate of 117 mL/min and clearance of metabolism in relation to the concentration in the atmosphere of 55 mL/min. The half-life was reported to be 6 min in two animals that weighed 500 g (Bolt & Filser, 1987).

Clearance rates of ethylene oxide from the blood, brain, muscle and testes were nearly identical. Following a 4-h inhalation exposure of B6C3F$_1$ mice and Fischer 344 rats to 100 ppm [183 mg/m^3], the average blood elimination half-lives were 2.4–3.2 min in mice and 11–14 min in rats (Brown *et al*., 1996, 1998).

In male Fischer 344/N rats exposed by nose-only inhalation for 60 min to 5 ppm [9.2 mg/m^3] ethylene oxide, a steady-state blood level of about 60 ng/g was reached after 15 min (Maples & Dahl, 1993).

After 4-h inhalation exposures of B6C3F$_1$ mice to 0, 50, 100, 200, 300 or 400 ppm [0, 91.5, 183, 366, 549 or 732 mg/m^3] ethylene oxide, concentrations of ethylene oxide in the blood increased linearly with inhaled concentrations of less than 200 ppm, but the blood concentration increased more rapidly than linearly above that level. GSH levels in the liver, lung, kidney and testes decreased as exposures increased above 200 ppm, which indicated that, at low concentrations, GSH conjugation is responsible for the disappearance of ethylene oxide but, at higher concentrations when tissue GSH begins to be

depleted, the elimination occurs via a slower hydrolysis process, which leads to a greater than linear increase in blood concentrations of ethylene oxide (Brown *et al.*, 1998).

(b) Metabolism

For an overview of mammalian metabolism of ethylene oxide, see Figure 2.

For early identification and quantification of mammalian metabolites of ethylene oxide, see IARC (1994).

After intravenous injection of 1 and 10 mg/kg bw ethylene oxide to male Sprague-Dawley rats, HEMA was excreted as a constant percentage of the dose: about 30% from 0 to 12 h and 5% from 12 to 24 h. Following administration of 100 mg/kg bw ethylene oxide, the corresponding percentages were 16% and 5%, respectively. These results indicate that the capacity for GSH conjugation at the high dose could have been exceeded within the first 12 h (Gérin & Tardif, 1986).

Ethylene glycol, 2-hydroxyethylmercapturic acid, 2-methylthioethanol and 2-mercaptoethanol were identified as metabolites in the urine of male Wistar rats exposed for 6 h to 500 ppm [915 mg/m^3] ethylene oxide (Koga *et al.*, 1987). The amounts of ethylene glycol in the urine of male Wistar rats collected during 6-h exposures to 50, 100, 200, 300 and 500 ppm [91.5, 183, 366, 549 and 915 mg/m^3] ethylene oxide and up to 20 h thereafter were 0.2, 0.35, 1.0, 2.5 and 4.2 mg, and thus increased disproportionately to the exposure concentrations (Koga *et al.*, 1985). These findings may indicate a relative decrease in GSH conjugation.

The pattern of excretion of ethylene oxide metabolites in mice, rats and rabbits was investigated in urine collected 24 h after treatment with ethylene oxide, either intravenously (20 and 60 mg/kg bw) or by inhalation for 6 h (about 200 ppm [366 mg/m^3]). Marked species differences were seen (Table 9), since metabolites that resulted from conjugation of ethylene oxide with GSH were found in the urine of male Swiss CD-1 mice and male Sprague-Dawley rats but not in that of rabbits [strain not given]. HEMA was excreted in the urine of mice and rats, but *S*-(2-hydroxyethyl)cysteine and *S*-(carboxymethyl)cysteine were present only in the urine of mice. Ethylene glycol, the reaction product of the enzymatic and non-enzymatic hydrolysis of ethylene oxide, was found in the urine of all three species (Tardif *et al.*, 1987).

(c) GSH depletion

Treatment of animals with ethylene oxide lowered the concentration of GSH in various tissues. Immediately after a 4-h exposure of male Swiss-Webster mice and Fischer 344 rats to atmospheric concentrations of 100, 400 and 900 ppm [183, 732 and 1647 mg/m^3] (mice) and 100, 600 and 1200 ppm [183, 1098 and 2196 mg/m^3] (rats) ethylene oxide, concentration-related decreases in GSH levels were observed in the kidney, heart, lung, brain, stomach, spleen, testis and liver of both species, in the blood of mice but not of rats and in bone marrow, which was examined in rats only. In both species, the GSH levels were reduced to a greater extent in the liver, lung and stomach

Table 9. Urinary excretion of ethylene oxide metabolites within 24 h after treatment of mice, rats and rabbits with ethylene oxide intravenously or by inhalation

Treatment	Urinary metabolites (µmol/100 g bw) (mean values)			
	N-Acetyl-*S*-(2-hydroxy-ethyl)cysteine	*S*-(2-Hydroxy-ethyl)cysteine	*S*-(Carboxy-methyl)cysteine	Ethylene glycol
20 mg/kg intravenously				
Mouse	3.75	2.62	0.85	1.48
Rat	14.00	ND	ND	2.68
Rabbit	ND	ND	ND	0.95
60 mg/kg intravenously				
Mouse	9.53	6.80	4.30	3.55
Rat	32.28	ND	ND	8.59
Rabbit	ND	ND	ND	3.76
200 ppm [366 mg/m^3], 6 h inhalation				
Mouse	4.63	2.62	2.83	0.77
Rat	19.61	ND	ND	1.84
Rabbit	ND	ND	ND	2.56

Adapted from Tardif *et al*. (1987)
ND, not detected

than in other organs. After exposure to the highest concentrations, GSH levels in the tissues were depressed to 20–30% of the control values (McKelvey & Zemaitis, 1986).

Concentrations of GSH in the hepatic cytosol of male Wistar rats decreased to 37% of that of controls after a single exposure (4 h) to 500 ppm [915 mg/m^3] ethylene oxide, to 13% after exposure to 1500 ppm [2745 mg/m^3] (Katoh *et al*., 1990) and to 5% after exposure to 2500 ppm [4575 mg/m^3] (Nakashima *et al*., 1987). Immediately after the last of a series of repeated exposures of male Wistar rats to 500 ppm [915 mg/m^3] ethylene oxide for 6 h per day on 3 days per week for 6 weeks, the hepatic GSH concentration was decreased by 50%. Control values were reached again 12 h thereafter (Katoh *et al*., 1989).

The consequences of GSH depletion on the occurrence of non-linear increases in the concentration of ethylene oxide in the blood with increasing external doses of ethylene oxide is discussed in Section 4.1.2(*a*). Haemoglobin adduct formation in mice and rats exposed to 0, 3, 10, 33, 100 and 300 (rats only) ppm [0, 5.49, 18.3, 60.39, 183 and 549 mg/m^3] ethylene oxide for 6 h per day on 5 days per week for 4 weeks was linear in both species up to 33 ppm, after which the slope increased more than proportionately (Walker *et al*., 1992a). The dose-related decrease in hepatic GSH concentration (Brown

et al., 1998) provides a plausible explanation for the more than proportional increase in hydroxyethylated haemoglobin (see below). In both rats and mice, depletion of GSH was already considerable following a single exposure to high levels (i.e. 550 mg/m^3) of ethylene oxide (McKelvey & Zemaitis, 1986; Brown *et al.*, 1998).

(d) Haemoglobin adducts

Binding of ethylene oxide to haemoglobin has been reviewed in some detail (IARC, 1994). In-vitro treatment of mouse, rat and human cells with ethylene oxide demonstrated the formation of adducts with *S*-cysteine, histidine and *N*-terminal valine in haemoglobin. The second-order rate constants were approximately the same for histidine and valine across the three species; however, large species differences were seen with respect to *S*-cysteine (Segerbäck, 1990). In studies of the fate of ethylene oxide in mice, single exposures to this epoxide produced in a dose-dependent manner haemoglobin adducts that disappeared at a rate predicted by the normal life-span of the red blood cells. These data suggested that stable haemoglobin adducts would accumulate over the lifetime of the erythrocyte during chronic exposures to ethylene oxide and provided the basis for the concept of using haemoglobin adducts to monitor integral doses of alkylating agents. Single intraperitoneal injections of and multiple inhalation exposures to ethylene oxide generally yielded linear dose–response relationships between dose and histidine and valine haemoglobin adduct levels. However, chronic inhalation exposures of male Fischer 344 rats to 10, 33 or 100 ppm [18.3, 60.4 or 183 mg/m^3] ethylene oxide under cancer bioassay conditions (6 h per day on 5 days per week for 2 years) resulted in a non-linear dose–response curve for N^{τ}-(2-hydroxyethyl)histidine (Osterman-Golkar *et al.*, 1983). Comparisons of the data from single-dose or 4-week inhalation exposure studies of rats versus a study of rats exposed for 2 years suggested that the dose–response relationship between the concentration of ethylene oxide and the formation of haemoglobin adducts can change over time during repeated exposure to this epoxide (Walker *et al.*, 1992a).

More recent studies on haemoglobin adducts in ethylene oxide-exposed mice and rats have focused on (*a*) potential species differences in the relationships between exposure, accumulation of haemoglobin adducts over time during repeated exposures and the dynamics in loss of adducts after discontinuation of exposures (Fennell *et al.*, 1992; Walker *et al.*, 1992a); and (*b*) comparisons of the formation and persistence of adducts in haemoglobin and DNA (Walker *et al.*, 1993), and the relationships between exposure, levels of haemoglobin adducts as a marker of dose and induction of somatic mutations (Tates *et al.*, 1999). Results of species comparisons of the formation and persistence of *N*-(2-hydroxyethyl)valine have been summarized previously (IARC, 1994). In brief, dose-related levels of *N*-(2-hydroxyethyl)valine were reported to be similar in mice and rats exposed to ethylene oxide (3–100 ppm [5.49–183 mg/m^3] for 4 weeks), while the adducts were lost in a species-specific pattern related to a differing life-span of red blood cells in each species. However, the relationships between *N*-(2-hydroxyethyl)valine in haemoglobin and *N*7-(2-hydroxyethyl)guanine in DNA varied with length of exposure, interval since exposure, species and tissue, which led the authors to conclude that the

haemoglobin adducts were unlikely to provide accurate predictions of DNA adducts in tissues under conditions in which the actual scenario of exposure to ethylene oxide is unknown (Walker *et al.*, 1993). Tates *et al.* (1999) measured *N*-(2-hydroxyethyl)valine to determine blood levels of ethylene oxide in rats and then used the estimated blood doses to compare the mutagenic effects of ethylene oxide via three routes of exposure, including single intraperitoneal injections, ingestion via the drinking-water or inhalation; comparisons of the mutagenic responses from alternative treatments with ethylene oxide are considered below. Other studies that measured the formation of *N*-(2-hydroxyethyl)valine in mice and rats after inhalation of ethylene oxide have typically used this haemoglobin adduct as a marker of exposure for comparison with DNA adducts in the same animals (Walker *et al.*, 2000; Rusyn *et al.*, 2005). In general, the same relationships were found as those reported in the earlier rodent studies of ethylene oxide that are summarized above.

(e) Toxicokinetic modelling

A physiologically based pharmacokinetic model has been developed for dosimetry of inhaled and intravenously injected ethylene oxide in rats (Krishnan *et al.*, 1992). The model enables the determination of tissue distribution, metabolic pathways, i.e. hydrolysis by EH and conjugation with GSH by GST, depletion of hepatic and extrahepatic GSH and binding of ethylene oxide to haemoglobin and DNA. The biochemical parameters used in the model were obtained by fitting data obtained after inhalation of ethylene oxide in closed chambers to data on tissue GSH concentrations (McKelvey & Zemaitis, 1986) and on levels of hydroxyethyl adducts in haemoglobin and tissue DNA (Potter *et al.*, 1989). The model was validated by comparing simulated and published data on urinary excretion of HEMA after inhalation and intravenous administration of ethylene oxide (Gérin & Tardif, 1986; Tardif *et al.*, 1987) and on levels of hydroxyethyl adducts in haemoglobin and tissue DNA after exposure for 6 h to 300 ppm [549 mg/m^3] ethylene oxide (Walker *et al.*, 1990, 1992a). The second-order rate constants obtained for the binding of ethylene oxide to amino acid residues in haemoglobin are similar to those published by Segerbäck (1990). According to the model, total adduct formation in haemoglobin and DNA accounted for 0.25% and 0.001% of the inhaled dose, respectively. After exposure to atmospheric concentrations of up to 500 ppm [915 mg/m^3] ethylene oxide, the model predicted first-order kinetics for whole-body elimination, but non-linearity in individual metabolic pathways and exhalation. Comparison of the predictions for low and 500-ppm exposures indicated that the share of GSH conjugation decreased from 38 to 27%, whereas the share of hydrolysis increased from 31 to 36% and that of exhalation from 23 to 28% (Krishnan *et al.*, 1992).

More recent physiologically based pharmacokinetic models of uptake and metabolism of ethylene oxide in mice, rats and humans were published by Csanády *et al.* (2000) and Fennell and Brown (2001). These models predicted adequately blood and tissue concentrations of ethylene oxide in rats and mice (with the exception of the testes). Simulations by the model of Fennell and Brown (2001) indicate that, in mice, rats and humans, about

80%, 60% and 20% of ethylene oxide, respectively, would be metabolized via GSH conjugation. Nevertheless, modelling 6-h inhalation exposures gave simulated ethylene oxide areas under the curve and blood peak concentrations that were similar for mice, rats and humans (Fennell & Brown, 2001). Thus, exposure to a given concentration of ethylene oxide in air results in similar predicted blood ethylene oxide areas under the curve for mice, rats and humans.

4.1.3 *Comparison of rodent and human data*

A striking difference between rodents and humans in the metabolism of ethylene oxide is the predominance of the GSH pathway in mice and rats, while in humans (and in other larger animals tested to date), the pathway initiated by enzymatic and non-enzymatic hydrolysis is of greater importance (Jones & Wells, 1981; Martis *et al.*, 1982; Gérin & Tardif, 1986; Tardif *et al.*, 1987; Brown *et al.*, 1996) which is consistent with the observed levels of the ethylene oxide that conjugate GSTT1 in the order mice > rats > humans. This leads to an onset of significant GSH depletion in rodents at exposure concentrations that were used in toxicological investigations, namely between 33 and 100 ppm [60.4 and 183 mg/m^3] ethylene oxide in ambient air (see above). However, it is important to note that carcinogenicity in rats was already observed at the lower exposure concentration of 10 ppm [18.3 mg/m^3] ethylene oxide in one study (see Section 3).

Although the physiologically based pharmacokinetic models constructed by Fennell and Brown (2001) indicated profound differences in the relative contribution of the different metabolic pathways, when differences in uptake and metabolism are taken into account, simulated blood peak concentrations and areas under the curve were similar for mice, rats and humans (human levels within about 15% of rat and mouse levels). Thus, exposure to a given concentration of ethylene oxide in air gives similar predicted blood levels of ethylene oxide and areas under the curve for mice, rats and humans (in the range of exposures used in the rodent cancer bioassays, i.e. 100 ppm [183 mg/m^3] and below; above these concentrations, the differences in GSH depletion may be expected to lead to significant differences in the levels of ethylene oxide in blood with comparable concentrations in the ambient air).

Human erythrocytes are rich (but polymorphic) in the ethylene oxide-metabolizing enzyme GSTT1, but, in the experimental animals investigated, this protective enzyme is not present in the erythrocytes (Hallier *et al.*, 1993). This adds a further complexity to species differences in the metabolism of ethylene oxide.

4.2 Genetic and related effects

The direct reaction of ethylene oxide with DNA is thought to initiate the cascade of genetic and related events that lead to cancer (Swenberg *et al.*, 1990). Ethylene oxide is a direct alkylating agent and reacts with nucleophiles without the need for metabolic transformation. It uses the general S_N2 mechanism and has a high Swain-Scott substrate

constant (Golberg, 1986), both of which favour efficient *N*7-alkylation of guanine (and other ring nitrogens to a lesser extent) due to the electron density distribution of purines and the steric availability of the *N*7-position of guanine (Kolman *et al*., 2002). The reactivity of this agent is due to the strain of the oxirane ring and the partial positive charge on the carbon atoms, which give the compound its electrophilic character and predispose the ring to open and form a hydroxyethylcarbonium ion (Golberg, 1986). Several published reviews contain details of earlier studies of the reactivity and genetic toxicity of ethylene oxide in humans and experimental systems (Kolman *et al*., 1986; Dellarco *et al*., 1990; Walker *et al*., 1990; IARC, 1994; Natarajan *et al*., 1995; Health Canada, 1999; Preston, 1999; Thier & Bolt, 2000; Health Canada, 2001; Kolman *et al*., 2002; WHO, 2003). This update is focused primarily upon recently published in-vivo studies of ethylene oxide-exposed humans and rodent models that shed light on the mode of action and the extent to which species comparisons may be pertinent to an evaluation of the carcinogenicity of this chemical in humans.

4.2.1 *Humans*

(a) DNA adducts

For nearly 20 years, it has been known that the endogenous formation of ethylene and its conversion to ethylene oxide leads to 2-hydroxyethylation of DNA to yield background levels of *N*7-(2-hydroxyethyl)guanine (7-HEG) in unexposed humans (reviewed in Bolt, 1996; Farmer & Shuker, 1999; Walker *et al*., 2000; Marsden *et al*., 2007). Ethylene is generated *in vivo* during normal physiological processes such as lipid peroxidation, oxidation of methionine, oxidation of haeme in haemoglobin and/or through the metabolizing activity of intestinal bacteria (reviewed in Thier & Bolt, 2000). Walker *et al.* (2000) described a series of studies of background levels of these adducts in different tissues of unexposed humans, and showed that lower endogenous levels of 7-HEG have typically been found with more sensitive detection methods than those employed in reports on the impact of endogenously versus exogenously derived ethylene oxide (Bolt, 1996). Farmer and Shuker (1999) suggested that, in order to estimate the increase in cancer risk attributable to a given external exposure, it is clearly important to establish and consider background levels of corresponding DNA damage so that the scale of the incremental increase can be calculated. It is mainly for this reason that more sensitive and specific analytical methods have been developed for the measurement of background and treatment-induced levels of 7-HEG than for any other single DNA adduct (reviewed in Zhao *et al.*, 1999; Liao *et al.*, 2001; Kao & Giese, 2005).

Since endogenous DNA damage through lipid peroxidation is thought to contribute significantly to cancer in humans (Marnett, 2002), Zhao and Hemminki (2002) investigated the association between age and endogenous formation of 7-HEG in 34 younger and older healthy human subjects (mean ages, 39.8 and 82.8 years, respectively). Steady-state levels of 7-HEG in the DNA of peripheral blood lymphocytes were similar in the younger and older subjects (means, 3.8 ± 3.4 and 3.0 ± 2.7 adducts/10^7 nucleotides,

respectively), which suggests that endogenous DNA damage by ethylene oxide that arises from ethylene generated by lipid peroxidation and the repair of such damage is independent of age as a contributing factor to cancer risk.

A single study has been performed to examine the formation of 7-HEG in humans exposed to ethylene oxide in the workplace (Yong *et al*., 2007). 7-HEG was quantified, using the method of Kao and Giese (2005), in peripheral blood granulocytes from 58 hospital workers exposed to ethylene oxide during the unloading of sterilizers or when working adjacent to sterilizers. Cumulative exposure to ethylene oxide (ppm–h) was estimated during the 4-month period before the collection of blood samples. There was considerable interindividual variation in the levels of 7-HEG in both unexposed control and ethylene oxide-exposed workers, ranging from 1.6 to 241 adducts/10^7 nucleotides. The mean levels in the unexposed, low-ethylene oxide exposure (< 32 ppm–h) [58.6 mg/m^3–h] and high-ethylene oxide exposure (> 32 ppm–h) groups were 3.8, 16.3 and 20.3 adducts/10^7 nucleotides, respectively, but the differences were not statistically significant after adjustment for cigarette smoking and other potential confounders. Since the life-span of granulocytes is less than 1 day compared with a life-span of up to several years for lymphocytes, the large inter-individual variation in 7-HEG levels as well as elevated values in individual ethylene oxide-exposed workers may have reflected very recent transient peak exposures that were not captured in the overall 4-month cumulative exposure estimates.

(b) Mutations and other genetic related effects (see also Table 10)

Studies of human exposures to ethylene oxide have focused upon individuals who were employed in the operation of hospital or factory sterilization units and workers who were involved in ethylene oxide manufacturing or processing. Selected studies showed that exposure to ethylene oxide results in chromosomal alterations that are related to both the level and duration of exposure, while a single study suggested that exposure to ethylene oxide causes gene mutations. Several other biomarkers of DNA damage (unscheduled DNA synthesis, DNA single-strand breaks, premature centromere division and DNA–protein cross-links) have been studied by only a few investigators; results are summarized at the end of this section.

Preston (1999) provided a critical assessment of the cytogenetic effects of ethylene oxide *in vitro* and *in vivo*, discussed the basic guidelines for cytogenetic assays in population monitoring and hazard identification, and exhaustively reviewed reports of human cytogenetic studies of ethylene oxide through to 1996. Occupational exposures to ethylene oxide have resulted in increased levels of chromosomal events, which included sister chromatid exchange, chromosomal aberrations and micronucleus formation in blood cells. Cytogenetic end-points can serve as markers of exposure to ethylene oxide and DNA damage. Moreover, several large prospective studies have demonstrated that, on a population basis, increased levels of chromosomal aberration or micronucleus formation are indicative of an increased risk for cancer development (Hagmar *et al*., 1998;

Table 10. Cytogenetic observations in humans exposed to ethylene oxide

No. of exposed	No. of controls	Exposure time (years)		Ethylene oxide in air (ppm)[a]		Cytogenetic effects[b]			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
12	8			0−36[c]				+	Garry *et al*. (1979)
12	11	1−8	4	0.5−1		−			Pero *et al.* (1981)
5	11	0.8−3	1.6	5−10		+			
18 (factory I)	11	0.5−8	3.2		< 1	+	+[d]	−	Högstedt *et al*. (1983,
10 (factory II)	9	0.5−8	1.7		< 1	+	−	−	1990)
9 (low-dose task)	13				13[e]			−	Yager *et al*. (1983)
5 (high-dose task)	13				501[e]			+	
14	14			< 0.07−4.3[f]				−	Hansen *et al*. (1984)
10 (nonsmokers)	15	0.5−10	5.7	[15–123]				+	Laurent *et al*. (1984)
15 (smokers)	7	0.5−10	4.5					+	
22 (low exposure)	22	1−4	3	0.2−0.5[f]	0.35	(+)		+	Sarto *et al*. (1984a,b;
10 (moderate exposure)	10			0−9.3[f]	1.84			+	1987)
19 (high exposure)	19			3.7−20[f]	10.7	+		+	
13 (work site I)	12		3.2	0.5[f]		−		−	Stolley *et al*. (1984);
22 (21)[g] (work site II)	19 (20)[g]		3.1	5−10[f]		−		(+)	Galloway *et al*. (1986)
26 (25)[g] (work site III)	22 (21)[g]		4	5−20[f]		(+)		+	
33 (production workers)	32	1−14		≤ 0.05−8	≤ 0.01[h]	+			Clare *et al.* (1985)
50	141	1−10		1−40[f]		+		+	Richmond *et al*. (1985)
36	35	1−14		0.05−8	0.12	−			van Sittert *et al*. (1985)

Table 10 (contd)

No. of exposed	No. of controls	Exposure time (years)		Ethylene oxide in air (ppm)[a]		Cytogenetic effects[b]			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
22 (sterilization unit)	10	1–8		0−2.6		+			Karelová *et al*. (1987)
21 (factory workers)	20	2–17		0−4.5		+			
25 (laboratory workers)	20	1–15		0−4.8		+			
9	27	0.5−12	5	0.025−0.38[f]			−		Sarto *et al*. (1990)
3	27			> 0.38[i]			+		
34	23		8	0.008−2.4[f]	< 0.3	−	−	+	Mayer *et al*. (1991)
5	10	0.1−4	8.6		0.025		−	−	Sarto *et al*. (1991)
5	10	4−12		< 1−4.4	0.38		−	+	
9 (hospital workers)	8	2−6	4	22−72	0.025[j]	+	−	+	Tates *et al*. (1991)
15 (factory workers)	15	3-27	12	14−400	5[j]	+	+	+	
10	10		3	60−69		+		+	Lerda & Rizzi (1992)
32	8		5.1	0−0.3[f]	0.04		−	+	Schulte *et al*. (1992)
11	8		9.5	0.13−0.3[f]	0.16		−	+	
47	47				< 1	–		–	Tomkins *et al*. (1993)
14 (hospital workers, nonsmokers)	14			0.5−208[f]				−	Popp *et al*. (1994)
11 (hospital workers, smokers)	11			0.5−417[f]				−	
75	22	3−14	7	2−5		+	+		Ribeiro *et al*. (1994)

Table 10 (contd)

No. of exposed	No. of controls	Exposure time (years)		Ethylene oxide in air (ppm)[a]		Cytogenetic effects[b]			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
28 (hospital workers)	8			0−0.30	0.08		−	+	Schulte *et al.* (1995)
10 (hospital workers)				0.13−0.30	0.17		−	+	
7 (production workers)	7	Accidental		28−429			−	−	Tates *et al.* (1995)
7 (production workers)		< 5		< 0.005−0.02			−	−	
7 (production workers)		> 15		< 0.005−0.01			−	−	
9 (low exposure, hospital workers)	48		4	2.7−10.9	2.7	+		−	Major *et al.* (1996)
27 (high exposure, hospital workers)	10		15	2.7−82	5.5	+		+	

CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange; TWA, time-weighted average
Blanks not studied

[a] 1 ppm = 1.83 mg/m^3 ethylene oxide

[b] +, positive; –, negative

[c] Maximum concentration measured during purge cycle

[d] Positive for erythroblasts and polychromatic aberrations; negative for peripheral blood lymphocytes

[e] Average 6-month cumulative dose in mg ethylene oxide

[f] TWA

[g] Numbers in parentheses are for chromosomal aberrations evaluated by Galloway *et al.* (1986)

[h] Calculated by linear extrapolation

[i] Exposed acutely from sterilizer leakage

[j] Estimated 40-h TWA based on haemoglobin adducts

Liou *et al.*, 1999; Smerhovsky *et al.*, 2001; Hagmar *et al.*, 2004; Boffetta *et al.*, 2007; Bonassi *et al.*, 2007).

The induction of increased frequencies of sister chromatid exchange has been found to be a sensitive indicator of genotoxic exposure to ethylene oxide in humans (Tates *et al.*, 1991). Evaluation of the impact of exposure to ethylene oxide on changes in the frequency of sister chromatid exchange should take into consideration the observation that the available studies assessed a wide range of exposure concentrations and conditions, including moderately high-dose acute exposures and chronic exposures at varying concentrations of ethylene oxide. Some interesting trends are apparent across studies. Those that failed to find significant increases in frequencies of sister chromatid exchange following exposure to ethylene oxide were primarily studies of workers who were exposed to approximately 5 ppm [9.15 mg/m^3] or less (TWA) (Högstedt *et al.*, 1983; Yager *et al.*, 1983; Hansen *et al.*, 1984; Stolley *et al.*, 1984; Sarto *et al.*, 1991; Tates *et al.*, 1995), although a number of studies report increased frequencies of sister chromatid exchange in workers exposed to less than 5 ppm (TWA) (Sarto *et al.*, 1984a, 1987; Mayer *et al.*, 1991; Tates *et al.*, 1991; Schulte *et al.*, 1992, 1995). Significant increases in frequencies of sister chromatid exchange were commonly found in studies that evaluated individuals who were exposed to concentrations of ethylene oxide > 5 ppm (TWA) (Garry *et al.*, 1979; Yager *et al.*, 1983; Laurent *et al.*, 1984; Sarto *et al.*, 1984a; Stolley *et al.*, 1984; Tates *et al.*, 1991; Lerda & Rizzi, 1992; Schulte *et al.*, 1992). It should be noted that comparisons among studies are complicated by differences in the methodology used to record and report exposure concentrations of ethylene oxide; this problem is further complicated by the fact that, although workers were generally exposed to low average levels in some studies, acute high exposures were noted to have occurred (Tates *et al.*, 1991). In spite of these complications, several studies found significant differences in sister chromatid exchange frequencies in individuals and/or groups exposed to levels of ethylene oxide higher than the designated low-exposure group from the same or similar environment (Yager *et al.*, 1983; Sarto *et al.*, 1984a; Stolley *et al.*, 1984; Tates *et al.*, 1991; Schulte *et al.*, 1992). These findings support the observation that sister chromatid exchange frequencies varied with level and frequency of exposure to ethylene oxide. Two studies investigated changes in frequencies of sister chromatid exchange over time and found that they remained elevated for at least 6 months even when exposures were decreased or ceased after the first assessment (Sarto *et al.*, 1984b; Stolley *et al.*, 1984). A relationship between changes in frequencies of sister chromatid exchange and cigarette smoking has also been found by several investigators (Sarto *et al.*, 1991); because data on exposure to cigarette smoke as a potential confounding factor was not consistently included in all studies, it is not always possible to determine the primary cause for the observed changes in sister chromatid exchange frequency (Sarto *et al.*, 1991).

Because increased frequencies of some classes of chromosomal aberration are associated with an increased risk for the development of cancer (Sorsa *et al.*, 1990), several studies have investigated the induction of increased frequencies of chromosomal aberrations in individuals exposed to ethylene oxide. Workers exposed to a range of

concentrations from 0.01 ppm to 200 ppm [0.02–366 mg/m^3] have been evaluated; the majority of the studies found significant increases in chromosomal aberrations (Pero *et al.*, 1981; Högstedt *et al.*, 1983; Sarto *et al.*, 1984b; Richmond *et al.*, 1985; Galloway *et al.*, 1986; Karelová *et al.*, 1987; Högstedt *et al.*, 1990; Tates *et al.*, 1991; Lerda & Rizzi, 1992; Ribeiro *et al.*, 1994; Major *et al.*, 1996). One study found significant increases in chromosomal aberrations in individuals exposed to concentrations of ethylene oxide of approximately 1 ppm [1.83 mg/m^3] and below (Högstedt *et al.*, 1983), although the majority of positive studies evaluated exposure conditions in which higher concentrations of ethylene oxide were present. Other studies of workers exposed to concentrations of ethylene oxide of approximately 1 ppm [1.83 mg/m^3] TWA and below did not find evidence of increased chromosomal aberrations (van Sittert *et al.*, 1985; Mayer *et al.*, 1991). Chromosomal aberration frequencies have been found to correlate with exposure concentrations of ethylene oxide and/or duration of exposure (Clare *et al.*, 1985; Galloway *et al.*, 1986; Tates *et al.*, 1991; Lerda & Rizzi, 1992). As noted for studies of sister chromatid exchange, chromosomal aberrations can be influenced by concurrent exposure to cigarette smoke and by ageing; these factors, combined with differences in the methods used to determine the magnitude of the exposures to ethylene oxide, limit the conclusions that can be drawn from comparisons between studies (Preston, 1999). As reported for sister chromatid exchange, the validity of these comparisons is supported by the observation that some investigators found significant increases in chromosomal aberrations in high-dose groups but not in low-dose groups exposed in the same or similar environments (Sarto *et al.*, 1984b; Galloway *et al.*, 1986).

Fewer investigators have evaluated the impact of exposure to ethylene oxide on the frequencies of micronucleated cells, and comparisons are therefore limited for this cytogenetic marker. The available studies showed a combination of positive and negative effects. Högstedt *et al.* (1990) and Ribeiro *et al.* (1994) found increased frequencies of micronucleated lymphocytes in workers while Tates *et al.* (1991) found significant increases in micronucleus frequencies in workers exposed to high but not to low doses. Exposure concentrations in all of these studies varied, but ranged from < 1 ppm to 400 ppm [1.83–732 mg/m^3] ethylene oxide. Studies that evaluated individuals exposed to levels of ethylene oxide ≤ 1 ppm gave negative results (Högstedt *et al.*, 1983; Sarto *et al.*, 1990, 1991; Tates *et al.*, 1995).

Two studies determined micronucleus frequencies in tissues other than lymphocytes. Ribeiro *et al.* (1994) evaluated both peripheral blood lymphocytes and exfoliated buccal cells in individuals exposed to 2–5 ppm [3.66–9.15 mg/m^3] ethylene oxide (TWA); micronucleus frequencies in buccal cells had a negative association while lymphocytes had a significantly positive association with the effects of ethylene oxide. Sarto *et al.* (1990) found significant increases in micronucleus frequencies in nasal mucosal cells but not in exfoliated buccal cells of workers exposed to ethylene oxide at concentrations below 0.38 ppm [0.7 mg/m^3] (TWA) (some workers experienced acute exposure to ethylene). Exposure to cigarette smoke and age were not discussed as potential confounders in these studies. These mixed results led to the conclusion by Tates *et al.* (1991)

that the relative sensitivity of end-points for the detection of exposure to ethylene oxide in humans was in the following order: haemoglobin adducts > sister chromatid exchange > chromosomal aberrations > micronucleus formation > hypoxanthine–guanine phosphoribosyl transferase gene (*HPRT*) mutants. However, *HPRT* mutation is a marker of heritable DNA change and thus should not be expected to be sensitive as an indicator of exposure.

Several studies reported individual differences in the magnitude of response for one or more biomarkers that were not accounted for by adjusting for known confounders such as smoking or age (Laurent *et al.*, 1984; Sarto *et al.*, 1984a). Yong *et al.* (2001) evaluated the relationship between levels of haemoglobin adducts, sister chromatid exchange frequencies and *GSTT1* genotype and found that individuals who had the null *GSTT1* genotype had significantly higher levels of haemoglobin adducts and lower frequencies of sister chromatid exchange than individuals who had at least one positive allele. Because these findings were made after most of the above reports, the impact of individual genotype upon the markers listed above is not known. The results of Yong *et al.* (2001) suggest that studies that found significant variations between individuals may have detected the impact of *GSTT1* genotype upon the study end-points, and support the need to consider genotype in future studies of ethylene oxide-induced effects.

Only three reports have addressed the issue of whether occupational exposure to ethylene oxide is associated with the induction of gene mutations. In the first study, the T-cell cloning assay was used to measure *HPRT* mutant frequencies in peripheral blood lymphocytes from nine ethylene oxide-exposed hospital workers and 15 ethylene oxide-exposed factory workers (Tates *et al.*, 1991). Hospital workers included nurses and technicians who were involved in the sterilization of medical equipment and were exposed to ethylene oxide once or twice a week for about 10 min. The concentrations of ethylene oxide ranged from 20 to 25 ppm [36.6–45.8 mg/m^3] in the sterilization room and from 22 to 72 ppm [40.3.–131.8 mg/m^3] in front of the sterilizer immediately after opening (as measured by GC). The hospital workers were matched for age, sex and smoking habits with a control group of eight unexposed administrative workers. The factory workers were employed at a plant that was involved in the production of ethylene oxide-sterilized disposable medical equipment, and were similarly matched with a group of 15 unexposed controls in the same factory. During a 4-month monitoring period (equivalent to the lifespan of erythrocytes in humans), five workers were engaged in 'daily' sterilization activities, two workers were involved in 'daily' sterilization except for leave periods of 7 or 11 days and eight remaining workers were 'occasionally' exposed to ethylene oxide during exposure control, packing and quality control of sterilized products. Before the collection of samples in early 1990, the mean duration of exposure of factory workers was 12 years (range, 3–27 years), with average ambient exposure levels from 1989 onwards that were estimated to be about 17 ppm [~31 mg/m^3] ethylene oxide. Based on measurements of *N*-(2-hydroxyethy)valine haemoglobin adducts that integrate exposure over time, average exposures to ethylene oxide in the 4 months before blood sampling were

estimated to be a 40-h TWA of 0.025 ppm [0.046 mg/m^3] for hospital workers and 5 ppm [9.15 mg/m^3] for factory workers (Tates *et al.* 1991).

The average *HPRT* mutant frequencies in hospital workers ($12.4 \pm 9.9 \times 10^{-6}$) and factory workers ($13.8 \pm 4.4 \times 10^{-6}$) were remarkably similar and showed increases of 55% and 60%, respectively, above background in their respective control groups ($8.0 \pm 3.6 \times 10^{-6}$ and $8.6 \pm 4.4 \times 10^{-6}$); however, the mutagenic response was significantly elevated only in the factory workers. Tates *et al.* (1991) suggested that the statistically significant increase in the mutant frequency in the factory workers, and not the hospital workers, may have been due to some extent to the larger study populations of factory workers and their controls, but the investigators concluded that the difference was more probably due to the higher exposure concentrations and tissue doses of ethylene oxide in factory workers. The mean cloning efficiency was uniformly lower in the exposed factory workers than in their control group (as well as the hospital workers exposed and their control groups), which might exaggerate the effect of exposure to ethylene oxide on *HPRT* mutant frequency in factory workers; however, adjustments (using multiplicative and additive models) for the observed effect of cloning efficiency, age and smoking status in factory workers appeared to justify the conclusion that the increased *HPRT* mutant frequency in this group was due to exposure to ethylene oxide. [While the Working Group recognized the importance of this study, it also noted inconsistencies in the results with regard to the response in relation to the exposure concentrations and duration of exposure, as well as the apparent similarity of the mutational spectrum of the exposed group and that of control populations.]

In a follow-up study of workers in an ethylene oxide production plant, Tates *et al.* (1995) again used the T-cell cloning assay to measure *HPRT* mutant frequencies in three exposed and one unexposed groups (seven subjects per group). Group I workers were incidentally exposed to acute high concentrations of ethylene oxide, while group II and III workers were chronically exposed to low concentrations of ethylene oxide for < 5 years and > 15 years, respectively. No significant differences in mutant frequencies were observed between any combination of worker or control groups, which implies that incidental exposure to high levels of ethylene oxide (28−429 ppm [52–785 mg/m^3]) or chronic exposure to low concentrations of ethylene oxide (< 0.005−0.02 ppm [< 0.01–0.04 mg/m^3]) did not cause any measurable permanent gene mutations in lymphocytes.

A few investigations extended the number of biomarkers evaluated to characterize ethylene oxide-induced effects to include unscheduled DNA synthesis and induction of DNA single-strand breaks, premature centromere division and DNA–protein cross-links. Induction of unscheduled DNA synthesis was decreased compared with controls in workers exposed to a range of concentrations of ethylene oxide that included levels below 1 ppm [mg/m^3] (TWA) (Pero *et al.*, 1981; Sarto *et al.*, 1987; Mayer *et al.*, 1991); the significance of this finding was not clear, but one investigator considered the decrease to reflect diminished DNA repair capacity (Pero *et al.*, 1981). DNA single-strand breaks were not found to be increased in ethylene oxide-exposed workers in one study (Mayer *et al.*, 1991), but were shown to be increased by alkaline elution in peripheral blood

mononuclear cells from hospital and factory workers occupied in sterilizing medical devices (Fuchs *et al.*, 1994). In nonsmokers, significantly higher elution was observed in ethylene oxide-exposed workers than controls, with remarkable individual differences in susceptibility according to *GSTT1* polymorphism. In smokers, a similar trend was observed in ethylene oxide-exposed subjects but the response was smaller and non-significant. Only one study evaluated premature centromere division as a potential indicator of genomic instability (Major *et al.*, 1999); an increase in this phenomenon was observed in ethylene oxide-exposed subjects that correlated with increased chromosomal aberrations. Another study measured DNA–protein cross-links by alkaline filter elution in hospital workers and found a significant correlation between reduced elution rates and exposure to ethylene oxide (Popp *et al.*, 1994).

4.2.2 *Experimental systems*

(a) DNA adducts

The relative amounts of adducts found in two independent studies of the reactivity of ethylene oxide with double-stranded DNA *in vitro* (Segerbäck, 1990; Li *et al.*, 1992) are compared in tables in Segerbäck (1994) and Kolman *et al.* (2002). *In vitro,* the reaction of ethylene oxide with nucleic acids occurs mainly at ring nitrogens, and leads to the formation of 7-HEG, O^6-(2-hydroxyethyl)guanine (O^6-HEG) and reaction products with *N*1, *N*3, *N*7 and N^6 of adenine and *N*3 of cytosine, uracil and thymine. Depending upon the in-vitro study, 7-HEG accounted for up to 90% of alkylation products and smaller amounts of other DNA adducts were formed. Segerbäck (1990) reported that treatment of calf thymus DNA with ^{14}C-labelled ethylene oxide resulted in the formation of 7-HEG, *N*3-(2-hydroxyethyl)adenine (3-HEA) and O^6-HEG at a ratio of 100:4.4:0.5. In contrast, Walker *et al.* (1992b) found that the ratio of the steady-state concentrations of 7-HEG, 3-HEA and O^6-HEG was 100:0.3:0.4 following repeated exposures of rats to ethylene oxide, which indicates that 3-HEA and O^6-HEG do not accumulate *in vivo* at levels predicted by the in-vitro ratios of these adducts and 7-HEG.

Many studies have evaluated DNA adducts as a molecular dosimeter following single or repeated exposures of mice and rats to ethylene oxide by intraperitoneal injection or inhalation. 7-HEG has consistently been found to be the predominant adduct; relatively small amounts of O^6-HEG and 3-HEA were identified in a single investigation of in-vivo adduct formation in tissues of ethylene oxide-exposed rats (Walker *et al.*, 1992b). Time-course studies have demonstrated that 7-HEG accumulated at steady-state levels after 4 weeks of inhalation exposure of mice and rats to high concentrations of ethylene oxide (100 ppm [183 mg/m^3] in mice and 100 or 300 ppm [183 or 549 mg/m^3] in rats) for 6 h per day on 5 days per week (Walker *et al.*, 1990, 1992b; Rusyn *et al.*, 2005); in contrast, adducts reached a plateau after 1 week of exposure during in-vivo conversion of exogenous ethylene to low levels of ethylene oxide (~6 ppm [~11 mg/m^3]) in most tissues of mice and rats exposed to 3000 ppm ethylene by inhalation 6 h per day on 5 days per week for 4 weeks (Walker *et al.*, 2000; Rusyn *et al.*, 2005). These data support the

hypothesis that, after repeated exposures of rodents to high concentrations of ethylene oxide, the discrepancies between the time to steady state (i.e. ≥ 28 days) and the DNA adduct half-life values [cited in the previous review of ethylene oxide (IARC, 1994); i.e. half-life of 1.0−2.3 days in most mouse tissues and 2.9−4.8 days in most rat tissues (Walker *et al.*, 1992b)] were most probably related to a greater dependence on spontaneous depurination for loss of 7-HEG than at lower levels of exposures to ethylene oxide at which DNA repair is not saturated (Walker *et al.*, 2000). In contrast, the patterns of elimination of 7-HEG adducts after a single 6-h exposure of rats to 40 or 3000 ppm ethylene exhibited slow linear loss of adducts from the brain, liver and spleen in animals exposed to endogeneously formed low-level ethylene oxide equivalents of ~1−6 ppm [1.83–11 mg/m^3] (Rusyn *et al.*, 2005).

Dose–response studies in mice and rats exposed to 3, 10, 33 or 100 ppm [5.49, 18.3, 60.4 or 183 mg/m^3] ethylene oxide for 6 h per day on 5 days per week for 4 weeks showed that the formation of 7-HEG at any given exposure level was similar for all tissues (brain, liver, lung, spleen) except the testis (within a factor of three) (Walker *et al.*, 1992b; Wu *et al.*, 1999a; Rusyn *et al.*, 2005), which suggests that, since not all of these tissues are targets for cancer, other critical factors may be involved in the species and tissue specificity for tumour induction by this chemical (Walker *et al.*, 1992b). Nevertheless, in rats exposed by inhalation for 6 h per day on 5 days per week for 4 weeks to 0, 50, 100 or 200 ppm [0, 91.5, 183 or 366 mg/m^3] ethylene oxide, statistically significant linear relationships were found between mean levels of 7-HEG and increases in *Hprt* mutant frequencies and between 7-HEG and elevations in sister chromatid exchange or so-called high-frequency cells (van Sittert *et al.*, 2000). Similar relationships between levels of 7-HEG and *Hprt* mutant frequencies were observed in mice exposed for 4 weeks to the same concentrations of ethylene oxide, except that the dose–response curves were sublinear and showed a greater effect per unit dose for both biomarkers with increasing exposure (see Fig. 4 in Walker *et al.*, 1997a). Thus, while levels of ethylene oxide-induced 7-HEG do not correlate directly with species/tissue susceptibility, they appear to predict the occurrence of increased frequencies of gene mutations and sister chromatid exchange in the lymphocytes of ethylene oxide-exposed rodents.

Background levels of 7-HEG in mice and rats have been investigated in numerous reports on the validation of sensitive methods to measure this adduct and/or establish the relative contribution of DNA damage that arises from endogenously and exogenously derived ethylene oxide (Föst *et al.*, 1989; Walker *et al.*, 1992b; Eide *et al.*, 1999; Wu *et al.*, 1999a,b; Zhao *et al.*, 1999; van Sittert *et al.*, 2000; Walker *et al.*, 2000; Rusyn *et al.*, 2005; Marsden *et al.*, 2007). Most recently, Marsden *et al.* (2007) developed a highly sensitive LC–MS/MS assay with selected reaction monitoring and established the lowest background levels of 7-HEG (1.1−3.5 adducts/10^8 nucleotides) reported to date for multiple tissues (colon, heart, kidney, liver, lung, spleen and stomach) in male Fischer 344 rats, which corroborated and extended an earlier report of ~2.6 7-HEG adducts/10^8 nucleotides in liver DNA from unexposed male Lewis rats (van Sittert *et al.*, 2000). Marsden *et al.* (2007) also measured exogenously derived 7-HEG following intra-

peritoneal administration of a single dose or three daily doses of 0.01–1.0 mg/kg ethylene oxide. These relatively low doses resulted in modest but generally dose-related increases in the observed concentrations of 7-HEG (i.e. background levels from endogenous ethylene oxide plus levels from exogenous ethylene oxide) in various tissues, except at the lowest concentration for which the measured amounts of 7-HEG in various tissues did not differ from those detected in control animals. The latter finding indicates that any increase in 7-HEG was negligible compared with the endogenous DNA damage already present.

(b) Mutations and other genetic effects (see Table 11 for details and references)

As a direct-acting alkylating agent, ethylene oxide has displayed genotoxic activity in nearly all studies in experimental systems, with a few notable dose-related exceptions in ethylene oxide-exposed rodents. *In vitro*, ethylene oxide caused DNA damage and gene mutations in bacteriophage, bacteria, fungi, insects and mammalian cells, and gene conversion in yeast (IARC, 1994). Given the general consistency of the in-vitro data in Table 11, only studies conducted in in-vivo systems are detailed here.

In-vivo exposure to ethylene oxide induced DNA damage and heritable mutations in germ cells of rodents, which caused alkali-labile sites and single-strand breaks in mouse sperm and spermatids, and chromosomal aberrations in mouse spermatocytes. It also induced dominant lethal effects in mice and rats, and heritable translocations in mice. Reviews of these studies have been published (Health Canada, 1999, 2001; WHO, 2003). It should be noted that these (mostly dose-related) effects were observed at relatively high concentrations of ethylene oxide (165–300 ppm [302–549 mg/m^3]; lower doses not tested) that exceeded the high dose used in the rodent carcinogenicity studies of this chemical.

Results of in-vivo gene mutation studies in rodents have given consistently positive results following ingestion, injection or inhalation of ethylene oxide. The in-vivo mutagenicity of ethylene oxide at the *Hprt* locus of somatic cells was demonstrated following intraperitoneal injection and after repeated inhalation exposures of mice. The impact of age-dependent trafficking on the 'manifestation' of mutant T-cells in rodents was taken into consideration in designing the *Hprt* mutation studies (Walker *et al.*, 1999). In young adult male *lacI* transgenic mice of B6C3F$_1$ origin, inhalation exposures for 4 weeks to ethylene oxide resulted in significant non-linear increases in the frequencies of *Hprt* mutations in splenic T cells. The average induced mutant frequencies (i.e. the average observed mutant frequency after treatment minus the average background mutant frequency) following exposures to 50, 100 or 200 ppm [91.5, 183 or 366 mg/m^3] ethylene oxide were 1.6, 4.6 and 11.9×10^{-6}; greater relative mutagenic efficiencies (mutations per unit dose) were observed at higher than at lower concentrations. The small but significant mutagenic response induced at 50 ppm ethylene oxide (the low-dose concentration in the carcinogenicity bioassay in mice) was only 73% above the average control animal value of $2.2 \pm 0.3 \times 10^{-6}$ ($p = 0.009$). In a follow-up study of rodents exposed for 4 weeks to

Table 11. Genetic and related effects of ethylene oxide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, Chinese hamster V79 cells *in vitro*	+	NT	0.5 mM 1 h[c]	Herrero *et al.* (1997)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus *in vitro*	+	+	0.5 mM 5 h[c]	Tan *et al.* (1981)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus *in vitro*	+	NT	≤ 2000 ppm 1h	Zamora *et al.* (1983)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	NT	12 344 ppm 30 min	Zhong *et al.* (1992)
Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	NT	3500 ppm 30 min	Zhong *et al.* (1992)
Cell transformation, mouse C3H/10T1/2 cells	+	NT	2.5 mM 1 h[c]	Kolman *et al.* (1989)
DNA single-strand breaks, human diploid fibroblasts *in vitro*	+	NT	2.5 mM 1 h[c]	Nygren *et al.* (1994)
DNA double-strand breaks, human diploid fibroblasts *in vitro*	+	NT	10 mM 1 h[c]	Nygren *et al.* (1994)
DNA single-strand breaks, human lymphocytes *in vitro*	+	NT	0.5 mM 1 h[c]	Hengstler *et al.* (1997)
Unscheduled DNA synthesis, human lymphocytes *in vitro*	+	NT	0.1mM 24 h[c]	Pero *et al.* (1981)
Gene mutation, human fibroblasts *in vitro*	+	NT	2.5 mM 1 h[c]	Kolman *et al.* (1992)
Gene mutation, human fibroblasts *in vitro*	+	NT	5 mM 1 h[c]	Bastlová *et al.* (1993)
Sister chromatid exchange, human fibroblasts *in vitro*	+	NT	36 ppm 24 h[c]	Star (1980)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.22 mM 20 min	Garry *et al.* (1982)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	~1 mM 3 s[c]	Tucker *et al.* (1986)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	~2.5 mM 1 h[c]	Agurell *et al.* (1991)
Sister chromatid exchange, human lymphocytes *in vitro*	+[d]	NT	1000 ppm 90 min	Hallier *et al.* (1993)
Chromosomal aberrations, human amniotic cell line *in vitro*	+	NT	≥ 5 mM 1 h	Poirier & Papadopoulo (1982)
DNA single-strand breaks, mouse spermatids *in vivo*	+		1800 ppm inh 1 h	Sega *et al.* (1988)

Table 11 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, mouse spermatids *in vivo*	+		100 mg/kg × 1 ip	Sega & Generoso (1988)
Gene mutation, mouse spleen T lymphocytes, *Hprt* locus *in vivo*	+		600 mg/kg ip	Walker & Skopek (1993)
Gene mutation, mouse spleen T lymphocytes, *Hprt* locus *in vivo*	+		50 ppm inh[e] × 4 wk	Walker *et al*. (1997a)
Gene mutation, mouse and rat spleen T lymphocytes, *Hprt* locus *in vivo*	+		200 ppm inh[e] × 4 wk	Walker *et al*. (1997b, 2000)
Gene mutation, mouse lung, *LacI* transgene *in vivo*	+		200 ppm inh[e] × 4 wk	Sisk *et al*. (1997)
Gene mutation, mouse bone marrow, spleen, *LacI* transgene *in vivo*	–		200 ppm inh[e] × 4 wk	Sisk *et al*. (1997)
Gene mutation, male Lewis rat spleen T lymphocytes, *Hprt* locus *in vivo*	+		20 mg/kg × 1 ip	Tates *et al*. (1999)
Gene mutation, male Lewis rat spleen T lymphocytes, *Hprt* locus *in vivo*	+		2 mM dw, 30 d	Tates *et al*. (1999)
Gene mutation, male Lewis rat spleen T lymphocytes, *Hprt* locus *in vivo*	+		50 ppm inh[e] × 4 wk	Tates *et al*. (1999)
Gene mutation, male Lewis rat spleen T lymphocytes, *Hprt* locus *in vivo*	+		200 ppm inh[e] × 4 wk	van Sittert *et al*. (2000)
Gene mutation, mouse bone marrow, *LacI* transgene *in vivo*	+		100 ppm inh[e] × 48 wk	Recio *et al*. (2004)
Gene mutation, mouse testis, *LacI* transgene *in vivo*	+		25 ppm inh[e] × 48 wk	Recio *et al*. (2004)
Mouse specific locus, spermatogonial stem cells *in vivo*	–		255 ppm inh 6 h/d[f]	Russell *et al*. (1984)

Table 11 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mouse specific locus, spermatogonial stem cells *in vivo*	(+)		200 ppm inh 6 h/d[g]	Lewis *et al*. (1986)
Sister chromatid exchange, rabbit lymphocytes *in vivo*	+		50 ppm inh[e] × 12 wk	Yager & Benz (1982)
Sister chromatid exchange, male Fischer 344 rat lymphocytes *in vivo*	+		451 ppm inh 6 h	Kligerman *et al*. (1983)
Sister chromatid exchange, monkey lymphocytes *in vivo*	+		50 ppm inh 7 h/d[h], 5 d/wk, 2 years	Lynch *et al*. (1984b); Kelsey *et al*. (1988)
Sister chromatid exchange, rabbit lymphocytes *in vivo*	+		200 ppm inh 6 h/d, 40 days	Yager (1987)
Sister chromatid exchange, Swiss mouse bone-marrow cells *in vivo*	+		30 mg/kg ip × 1	Farooqi *et al*. (1993)
Sister chromatid exchange, male Fischer 344 rat bone-marrow cells and spleen *in vivo*	+		100 ppm inh[e], 3 mo	Ong *et al*. (1993)
Sister chromatid exchange, male Fischer 344 rat lymphocytes *in vivo*	+		150 ppm inh[e] × 1–4 wk	Preston & Abernethy (1993)
Sister chromatid exchange, male Lewis rat splenic lymphocytes *in vivo*	(+)		50–200 ppm inh[e] × 4 wk	van Sittert *et al*. (2000); Lorenti Garcia *et al*. (2001)
Micronucleus formation, mouse and rat bone-marrow cells *in vivo*	+		100 mg/kg × 1 iv	Appelgren *et al*. (1978)
Micronucleus formation, mouse bone-marrow cells *in vivo*	+		125 mg/kg × 1 ip	Jenssen & Ramel (1980)
Micronucleus formation, rat bone-marrow cells *in vivo*	+		100 ppm inh[e], 3 mo	Hochberg *et al*. (1990)
Micronucleus formation, Swiss mouse bone-marrow cells *in vivo*	+		30 mg/kg ip × 1	Farooqi *et al*. (1993)

Table 11 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male Fischer 344 rat and B6C3F$_1$ mouse bone marrow *in vivo*	+		200 ppm inh[e] × 4 wk	Vergnes & Pritts (1994)
Micronucleus formation, male Lewis rat splenic lymphocytes *in vivo*	–		50–200 ppm inh[e] × 4 wk	van Sittert *et al*. (2000); Lorenti Garcia *et al*. (2001)
Chromosomal aberrations, male Swiss mouse bone-marrow cells *in vivo*	+		200 ppm inh 6 h/d	Ribeiro *et al*. (1987a)
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	+		30 mg/kg × 1 ip	Farooqi *et al*. (1993)
Chromosomal aberrations, male Fischer 344 rat lymphocytes *in vivo*	–		450 ppm inh 6 h/d, 3 d	Kligerman *et al*. (1983)
Chromosomal aberrations, monkey lymphocytes *in vivo*	+		100 ppm inh 7 h/d, 5 d/wk, 2 years	Lynch *et al*. (1984b)
Chromosomal aberrations, male Fischer 344 rat lymphocytes *in vivo*	–		150 ppm inh[e] × 1–4 wk	Preston & Abernethy (1993)
Chromosomal aberrations, male Lewis rat lymphocytes *in vivo*	–		50–200 ppm inh[e] × 4 wk	van Sittert *et al*. (2000); Lorenti Garcia *et al*. (2001)
Chromosomal aberrations, mouse spermatocytes treated *in vivo*	+		400 ppm inh 6 h/d	Ribeiro *et al*. (1987a)
Dominant lethal mutation, mouse *in vivo*	–		25 mg/kg × 1 iv	Appelgren *et al*. (1977)
Dominant lethal mutation, mouse *in vivo*	+		150 mg/kg × 1 ip	Generoso *et al*. (1980)
Dominant lethal mutation, mouse *in vivo*	+		250 ppm inh[e] × 2 wk	Generoso *et al*. (1983)

Table 11 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal mutation, mouse *in vivo*	+		300 ppm inh 6 h/d × 4 d	Generoso *et al*. (1986)
Dominant lethal mutation, mouse *in vivo*	+		204 ppm inh 6 h/d [j]	Generoso *et al*. (1990)
Dominant lethal mutation, rat *in vivo*	+		1000 ppm inh 4 h	Embree *et al*. (1977)
Mouse heritable translocation	+		30 mg/kg ip × 5 d/wk, 5 wk	Generoso *et al*. (1980)
Mouse heritable translocation	+		165 ppm 6 h/d [j]	Generoso *et al*. (1990)
Binding (covalent) to calf thymus DNA *in vitro*	+	NT	2M 10 h	Li *et al.* (1992)
Binding (covalent) to human DNA *in vitro*	+	NT	1 mM 3 h	Pauwels & Veulemans (1998)
Binding (covalent) to mouse DNA *in vivo*	+		1.15 ppm inh 75 min	Ehrenberg *et al.* (1974)
Binding (covalent) to rat DNA *in vivo*	+		20.4 µmol/kg × 1 ip	Osterman-Golkar *et al.* (1983)
Binding (covalent) to rat DNA *in vivo*	+		100 ppm inh 4 h	Föst *et al.* (1989)
Binding (covalent) to rat DNA *in vivo*	+		1 ppm inh 6 h	Potter *et al.* (1989)
Binding (covalent) to rat DNA *in vivo*	+		300 ppm inh[e] × 4 wk	Walker *et al.* (1990)
Binding (covalent) to mouse DNA *in vivo*	+		300 ppm inh 1 h	Sega *et al.* (1991)
Binding (covalent) to mouse DNA *in vivo*	+		33 ppm inh[e] × 4 wk	Walker *et al.* (1992b)
Binding (covalent) to rat DNA *in vivo*	+		10 ppm inh[e] × 4 wk	Walker *et al.* (1992b)
Binding (covalent) to rat DNA *in vivo*	+		~ 4.6 ppm inh 6 h × 1	Bolt & Leutbecher (1993)
Binding (covalent) to rat DNA *in vivo*	+		300 ppm inh 12 h/d × 3	Eide *et al.* (1995)
Binding (covalent) to mouse DNA *in vivo*	+		10 ppm inh[e] × 4 wk	Wu *et al.* (1999a)
Binding (covalent) to rat DNA in vivo	+		3 ppm inh[e] × 4 wk	Wu *et al.* (1999a)
Binding (covalent) to rat DNA *in vivo*	+		50 ppm inh[e] × 4 wk	van Sittert *et al*. (2000)

Table 11 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to mouse and rat DNA *in vivo*	+		~ 1 ppm inh[e] × 4 wk	Walker *et al.* (2000); Rusyn *et al.* (2005)
Binding (covalent) to rat DNA *in vivo*	+		0.1 mg/kg × 1 ip	Marsden *et al.* (2007)
Binding (covalent) to rat haemoglobin *in vivo*	+		1 ppm inh 6 h	Potter *et al*. (1989)
Binding (covalent) to mouse haemoglobin *in vivo*	+		300 ppm inh 1 h	Sega *et al.* (1991)
Binding (covalent) to mouse haemoglobin *in vivo*	+		≥ 33 ppm inh[e] × 4 wk	Walker *et al*. (1993)
Binding (covalent) to rat haemoglobin in vivo	+		≥ 10 ppm inh[e] × 4 wk	Walker et al. (1993)
Sperm morphology, mouse *in vivo*	+		200 ppm inh 6 h/d × 5	Ribeiro *et al*. (1987b)

[a] +, positive; –, negative; (+), weak positive; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; d, day; dw, drinking-water; inh, inhalation; inj, injection; ip, intraperitoneal; iv, intravenous; mo, month; wk, week
[c] Concentration in the culture medium
[d] Single concentration, positive only for non-conjugators of glutathione
[e] 6 h/day, 5 days/week
[f] Sixty days total over a 5-month period
[g] Five days/week; 6–7 months; mating started 7th week of exposure and continued throughout exposure period
[h] Five days/week; 2 years (study group from Lynch *et al*., 1984b)
[i] Five days/week; 16 days
[j] Five days/week; 6 weeks then daily 2.5 weeks

0 or 200 ppm [366 mg/m^3] ethylene oxide, *Hprt* mutant frequency measurements in splenic T cells of young adult male Fischer 344 rats and B6C3F$_1$ mice were significantly increased by five- to sixfold over background (1.7 and 1.3×10^{-6} in control mice and rats, respectively).

Ethylene oxide-induced mutagenesis at the *Hprt* locus in splenic T cells of rats was confirmed in two additional investigations in young adult male Lewis rats exposed to ethylene oxide by single intraperitoneal injection, in the drinking-water or by inhalation. Significant mutagenic effects were observed by all three routes of exposure; plots of the mutagenicity data against blood dose (estimated from haemoglobin adducts) were a common denominator, which showed that, at equal blood doses, injection of ethylene oxide led to higher mutant frequencies than treatment in the drinking-water, which was more mutagenic than exposure via inhalation. In a follow-up study of inhalation exposure of male Lewis rats to the same concentrations of ethylene oxide, modest dose-related increases in *Hprt* mutant frequencies were found but the only significant elevation was in the group exposed to the highest dose of ethylene oxide. The results of these studies of *Hprt* gene mutation led to the conclusion that ethylene oxide is only weakly mutagenic in adult rats.

Ethylene oxide-induced gene mutations have been demonstrated in multiple tissues of *LacI* transgenic mice. Following 4 weeks of inhalation exposure of male Big Blue™ mice to ethylene oxide, the frequencies of *LacI* gene mutations in exposed animals were significantly increased over control values in lung but not bone marrow or spleen. When Big Blue mice were exposed to ethylene oxide for 12, 24 and 48 weeks, clear dose-related mutagenic responses in bone marrow were observed only after 48 weeks of exposure, with *LacI* mutant frequencies significantly increased only in the 100- and 200-ppm [183- and 366-mg/m^3] exposure groups. In testes of the same mice, dose-related increases in mutant frequencies were found in the groups exposed to 25, 50 or 100 ppm [45.8, 91.5 or 183 mg/m^3] but not the group exposed to 200 ppm [366 mg/m^3] ethylene oxide.

Recent assessments of the carcinogenicity of ethylene oxide have included discussions and summary tables of the results from many studies on chromosomal effects in ethylene oxide-exposed humans (Health Canada, 1999, 2001; WHO, 2003), but no critical review of cytogenetic studies in ethylene oxide-exposed rodents has been made since 1990 (Dellarco *et al.*, 1990). Several studies have shown that repeated exposures of rats to levels equal to or greater than those used in rodent carcinogenicity studies of ethylene oxide (> 50 ppm [> 91.5 mg/m^3]) induced dose-related increases in sister chromatid exchange. Treatment of rats and mice with high acute doses of ethylene oxide by intraperitoneal or intravenous injection or orally (i.e. routes of exposure that are less relevant to humans) also caused increases in the frequencies of micronucleus formation and chromosomal aberrations.

In contrast, following inhalation exposure (i.e. a route of exposure relevant to humans), only concentrations of ethylene oxide that exceeded those used in rodent cancer bioassays induced micronucleus formation or chromosomal aberrations in mice and rats. Modest but significant increases in bone marrow micronuclei were observed in male

Fischer 344 rats and B6C3F$_1$ mice following 4 weeks of exposure for 6 h per day on 5 days per week to 200 ppm [366 mg/m^3] ethylene oxide, but not to 40, 1000 or 3000 ppm exogenous ethylene which is converted *in vivo* to about 1–6 ppm [1.83–11 mg/m^3] ethylene oxide (Vergnes & Pritts, 1994). In contrast, no increases in the frequencies of chromosomal aberrations or micronucleus formation were found in peripheral blood/splenic lymphocytes from rats exposed to ethylene oxide. Furthermore, another study showed that 4 weeks of exposure of rats to ethylene oxide failed to cause an increase in translocations (e.g. the percentage of translocations in controls and rats treated with 200 ppm [366 mg/m^3] was 0.1% and 0.09%, respectively). [The Working Group noted, however, that strong conclusions cannot be drawn about the clastogenic potential of the ethylene oxide treatment regimen used in the 4-week inhalation study (van Sittert *et al.*, 2000; Lorenti Garcia *et al.*, 2001) because cytogenetic studies were initiated 5 days after the final day of exposure, a suboptimal time, and the power of the fluorescent in-situ hybridization studies were limited by analysis of only a single chromosome and the small numbers of rats per group examined.] In studies of the potential clastogenicity of styrene, a group of rats exposed to 150 ppm [274.5 mg/m^3] ethylene oxide by inhalation for 6 h per day on 5 days per week for 1, 2, 3 or 4 weeks was included based upon the expectation that, with appropriate sampling times shortly after exposure, a positive response for chromosomal aberrations would be produced in lymphocytes of ethylene oxide-exposed animals. However, the frequency of chromosomal aberrations in ethylene oxide-exposed rats was not increased over that in air controls at any sampling time.

(c) Mutational spectra

Walker and Skopek (1993) reported limited data on mutational spectra for *Hprt* mutant T-cell clones from B6C3F$_1$ mice given repeated intraperioneal injections of ethylene oxide. Molecular analyses using polymerase chain reaction-based denaturing gradient gel electrophoresis and sequencing for mutations in the exon 3 region of *Hprt* suggested the involvement of both modified guanine and adenine bases in ethylene oxide-induced mutagenesis. Additional data on *Hprt* gene mutational spectra were presented in a preliminary report following 4-week inhalation exposures of mice to 0, 50, 100 or 200 ppm [0, 91.5, 183 or 366 mg/m^3] ethylene oxide or rats to 0 or 200 ppm [366 mg/m^3] ethylene oxide for 6 h per day on 5 days per week (Walker *et al.*, 1997b). The types of mutation in ethylene oxide-exposed mice or rats were independent of the route of exposure; these mutations included a combination of base substitutions, frameshifts and small deletions; the most common lesions were a +1G in a run of six guanines (base pairs 207–212) and several different small deletions in the region of base pairs 275–280. These data suggest that ethylene oxide mutagenesis may involve similar mechanisms in mice and rats.

In ethylene oxide-exposed Big Blue™ mice, molecular analyses of mutations in the *lacI* transgene of bone marrow showed a decrease in mutations at G:C base pairs and an increase at A:T base pairs, which were exclusively A:T→T:A transversions and accounted for 25.4% (14/55) of the mutations isolated (Recio *et al.*, 2004). In contrast,

A:T→T:A transversions occurred at a frequency of 1.4% (1/70) in concurrent control mice. The mutational spectrum in the testes of ethylene oxide-exposed mice indicated that small increases across most mutational types were sufficient to produce an overall increase in the *LacI* mutation frequency, although this was not significant for a specific type compared with controls.

4.2.3 *Mechanism of mutation induction*

Although abundant data *in vitro* and *in vivo* indicate that genotoxicity plays a major role in the carcinogenicity of ethylene oxide, and its mutagenicity is deemed to be a consequence of high reactivity with DNA, the lesions responsible for ethylene oxide-induced mutations *in vivo* have not yet been identified. Possible precursor lesions for the induction of mutations by ethylene oxide are thought to include the formation of (*a*) 7-HEG and other *N*-alkylated bases that may 'predispose' to mutational events, (*b*) O^6-HEG as a promutagenic adduct, (*c*) hydroxyethyl adducts of the DNA backbone and (*d*) secondary reactive oxygen species. Systematic studies of oxidative damage from exposure to ethylene oxide *in vivo* have not been performed and this potential source of mutation is not considered in depth here. Since the publication of the first data on mutational spectra following exposure of human cells *in vitro* (Bastlová *et al.*, 1993) and exposure of mice to ethylene oxide *in vivo* (Walker & Skopek, 1993), several lines of research have been pursued in a limited fashion (*a*) to assess the hypothesis that adducts that lead to abasic (apurinic/apyrimidinic) sites may contribute to the mutagenesis of ethylene oxide; (*b*) to identify and characterize promutagenic adducts that may account for certain types of mutations induced by ethylene oxide; and (*c*) to evaluate the means by which adducts could cause large deletions and chromosomal alterations produced by ethylene oxide.

As the major DNA adduct of ethylene oxide, 7-HEG is unlikely to be directly promutagenic because *N*7-alkylguanine adducts formed from small epoxides such as ethylene oxide and propylene oxide do not cause distortion of the double helix and do not interfere with hydrogen bonding (Albertini & Sweeney, 2007); rather, *N*7-alkylguanine adducts are hypothesized to result in mutation by loss of the adduct via depurination or the action of DNA glycosylases. The resulting abasic sites are non-coding and may result in base substitutions and strand breaks. The action of apurinic endonuclease creates a single-strand break which, if unresolved, can lead to DNA double-strand breaks and, possibly, chromosomal alterations (Vogel & Natarajan, 1982). Current data suggest that depurination of *N*7-alkylguanine adducts results largely in G→T transversions and, to a lesser extent, G→A transitions and G→C transversions (Loeb & Preston, 1986; Takeshita *et al.*, 1987). Approximately half of the base substitutions at the *Hprt* locus of splenic T cells of mice and rats exposed to ethylene oxide were at A:T base pairs (Walker & Skopek, 1993; Walker *et al.*, 1997b), and it is feasible that these mutations could originate from spontaneous or glycosylase-mediated depurination of 3-HEA and other *N*-hydroxyethyl adducts of adenine/thymine, with subsequent by-pass replication of abasic sites. While chemically induced abasic sites have been found to be important in the mutagenic

mechanisms for only a few exogenous chemicals (Loeb & Preston, 1986), the fact that > 98% of the DNA adducts produced by ethylene oxide lead to abasic sites denoted the need to investigate their role in the mutagenesis of this agent (Walker & Skopek, 1993). In order for these mutagenic events to occur at a rate sufficient to result in ethylene oxide-induced changes in mutational spectra (including increases in base substitutions and deletions), accumulation of abasic sites that arise from high levels of 7-HEG would be expected to occur over time (Rusyn *et al.*, 2005).

A study was recently completed to test the hypothesis that exposure to ethylene oxide results in the accumulation of abasic sites and induces changes in the expression of genes for base-excision DNA repair, which predisposes to point mutations and chromosomal aberrations in Fischer 344 rats exposed by inhalation for 6 h per day on 5 days per week for 4 weeks to 0 or 100 ppm [183 mg/m^3] ethylene oxide or 0 to 3000 ppm ethylene (~ 6 ppm ethylene oxide) (Rusyn *et al.*, 2005). The resulting data demonstrated that, while 7-HEG accumulates with repeated exposures, *N*-hydroxyethylation of DNA by ethylene oxide is repaired without accumulation of abasic sites, and that the mechanisms proposed above probably play a minor role in the mutagenicity of this agent. The same conclusions would apply to the minor accumulation of 3-HEA and other *N*-hydroxyethyl adducts of adenine/thymine, and the induction of strand-breaks or point mutations at A:T base pairs by ethylene oxide.

Several investigators have proposed that the mutagenicity of ethylene oxide may involve the action of minor promutagenic adducts, such as O^6-HEG, *N*3-(2-hydroxyethyl)-2′-deoxyuridine (3-HEdU) and possibly ring-opened 7-HEG (Solomon, 1999; van Sittert *et al.*, 2000; Rusyn *et al.*, 2005; Marsden *et al.*, 2007). O^6-HEG is considered to be a miscoding lesion due to mispairing with thymine during DNA replication (Ellison *et al.*, 1989); however, this adduct is formed at extremely low levels *in vivo* in ethylene oxide-exposed rats (and presumably other species) and is efficiently removed from DNA by the repair protein O^6-alkylguanine–DNA alkyltransferase and excision repair (Ludeke & Kleihues, 1988). In addition, only a few G→A mutations were found in *p53* exons 6 and 7 of ethylene oxide-induced mammary gland carcinomas (Houle *et al.*, 2006) and these transitions were not significantly increased in reporter genes of ethylene oxide-exposed mice and rats. While O^6-HEG may contribute minimally to the mutagenicity of ethylene oxide *in vivo*, O^6-alkylguanine adducts are probably important promutagenic lesions for epoxides with an S_N1 character, such as styrene oxide (Latif *et al.*, 1988; Solomon, 1999). *N*3-(2-Hydroxyethyl)-2′-cytidine in DNA rapidly deaminates to stable, potentially mutagenic 3-HEdU lesions (Solomon, 1999). This lesion has been shown to block DNA replication by bacterial polymerases *in vitro* and to cause G:C→A:T and G:C→T:A base substitutions (Bhanot *et al.*, 1994; Zhang *et al.*, 1995). In contrast, the formation of cytosine or uracil adducts has not been shown in rodents exposed to ethylene oxide, and, if formed, 3-HEdU would be expected to be at very low levels and to contribute to a minor extent to the mutagenicity of ethylene oxide *in vivo*. 7-HEG can undergo imidazole ring-opening to chemically stable, potentially mutagenic lesions (Solomon, 1999). Formation of ethylene oxide-induced ring-opened 7-HEG has not been demonstrated *in*

vivo but is worthy of investigation in the future. Exploration of other potential pro-mutagenic adducts of ethylene oxide should not only include site-directed mutagenesis studies to characterize the mutagenic specificity and efficiency of these adducts, but also experiments *in vivo* to demonstrate whether or not these adducts occur at biologically relevant concentrations in ethylene oxide-exposed rodents.

Because exposure to ethylene oxide is associated with cytogenetic effects in humans and induces deletions in human cells *in vitro* and in rodents *in vivo*, it is also essential to determine which DNA adducts and mechanism(s) are responsible for large-scale mutational events. The suspected metabolite of ethylene oxide, glycolaldehyde, has been shown to form DNA–protein crosslinks and single-strand breaks *in vitro* (Hengstler *et al.*, 1994), but no information is available on the formation of this intermediate *in vivo* or its potential effects following exposures to ethylene oxide. The lack of accumulation of abasic sites during repeated high-dose exposures of rats to ethylene oxide (Rusyn *et al.*, 2005) does not favour a mechanism that involves the induction of strand breaks via abasic sites that result from labile DNA adducts (Lindahl, 1979). Another postulated mechanism for ethylene oxide-induced strand scissions is via the formation of a β-hydroxyethyl phosphotriester adduct that requires the interaction of the β-hydroxyethyl group with the phosphate backbone (Agurell *et al.*, 1991). 2-Hydroxyethylation of phosphate groups introduces extreme instability into the sugar–phosphate backbone, since the resultant phosphotriester breaks down through a dioxaphospholane ring intermediate (Eisenbrand *et al.*, 1986). This alternative mechanism for ethylene oxide-induced strand breaks and chromosomal damage deserves further investigation both *in vitro* and *in vivo*.

4.2.4 *Alterations in oncogenes and suppressor genes in tumours*

The ability of ethylene oxide to induce alterations in *ras* proto-oncogenes and the *p53* tumour-suppressor gene has been investigated in immunohistochemical and molecular studies of mutations in control and chemically induced neoplasms of B6C3F_1 mice. In the first investigation of spontaneous and ethylene oxide-induced mammary gland carcinomas, the *p53* and H-*ras* genes were selected for study because they are among the most commonly altered genes in human cancers, mutations in both often occur in the same cancer cell and several lines of evidence support an interaction between these genes during the multistep process of oncogenesis (Houle *et al.*, 2006). Immunohistochemistry results showed that p53 protein expression was detected in 42% (8/19) of spontaneous and 67% (8/12) of ethylene oxide-induced carcinomas. Further semiquantitative evaluations revealed that the protein levels were sixfold higher in cancers from exposed mice than in those from control mice (i.e. the average scores by the ‘quickscore method’ were 3.83 and 0.63, respectively). Mutations in *p53* exons 5−8 were detected in eight of 12 (67%) ethylene oxide-induced carcinomas; five of the eight mutations had two or more base changes. Fourteen base substitutions were identified, including eight silent mutations, five missense mutations and one nonsense mutation; no, four, seven and three mutations occurred in exons 5, 6, 7 and 8, respectively. Nine of 14 alterations (64%)

involved guanine bases, including mutations in codons 241 and 264 that occurred in more than one neoplasm from mice treated with 50 and 100 ppm [91.5 and 183 mg^3] ethylene oxide. Ethylene oxide-induced mammary carcinomas exhibited a clear dose-related response in relation to the level of p53 protein expression and the number of *p53* gene mutations. Base substitutions were found in the *p53* gene in 58% (7/12) of spontaneous mammary carcinomas, including five silent mutations, seven missense mutations and one nonsense mutation. However, in contrast to the results seen in the tumours of ethylene oxide-exposed animals, nine of the 13 mutations occurred in exon 5, none was found in exon 6 and seven were C→T transitions. H-*ras* Mutations in codon 61 (bases CAA) were detected in 33% (4/12) and 26% (5/19) of ethylene oxide-induced and spontaneous mammary carcinomas, respectively. All ethylene oxide-induced alterations were missense mutations localized to the second base (or mutations at adenine), whereas 80% (4/5) of spontaneous mutations occurred in the first base of codon 61 (or mutations at cytosine) with the remaining mutation found in the third base of codon 61 (Houle *et al*., 2006).

Analyses for cancer gene mutations were extended to other target tissues by evaluating the occurrence K-*ras* gene mutations in lung, Harderian gland and uterine neoplasms from ethylene oxide-exposed and control $B6C3F_1$ mice (Hong *et al.*, 2007). Specifically, the base triplets for codons 12 (GGT), 13 (GGC) and 61 (CAA) were examined for mutations. K-*ras* Gene mutations were identified in 100% (23/23) of the ethylene oxide-induced lung neoplasms and 25% (27/108) of the spontaneous lung neoplasms. Codon 12 mutations were most common in the lung neoplasms from both exposed and control mice; however, 97% (21/23) of the ethylene oxide-induced tumours had a G→T transversion at the second base compared with only one in 108 spontaneous tumours that exhibited this mutation, while 11 spontaneous tumours had a G→A transition in the second base compared with only two ethylene oxide-induced tumours. In Harderian gland neoplasms, 86% (18/21) of ethylene oxide-induced tumours compared with 7% (2/27) of spontaneous tumours contained K-*ras* gene mutations. Codon 13 G→C and codon 12 G→T transversions were the predominant mutations in ethylene oxide-induced Harderian gland neoplasms, but these did not occur in the spontaneous counterparts. Spontaneous uterine carcinomas were not examined, but 83% (5/6) of such neoplasms from ethylene oxide-exposed mice had K-*ras* gene mutations, all of which were codon 13 C→T transitions. Dose-related trends for K-*ras* gene mutations were found for all three cancer types in mice treated with 50 and 100 ppm [91.5 and 183 mg/m^3] in the cancer bioassay of ethylene oxide.

These data on *p53* and *ras* gene mutational spectra show distinct differences in the locations and types of base substitutions in spontaneous versus ethylene oxide-induced neoplasms in mice. Futhermore, the profile of K-*ras* gene mutations in ethylene oxide-induced lung and Harderian gland neoplasms was different from that described for other chemically induced tumours of the same tissues, which suggests that ethylene oxide induces a chemical-specific signature for base substitutions (Hong *et al.*, 2007). Based upon the spectra of *p53* and H-*ras* gene mutations in mammary gland neoplasms, Houle *et al.* (2006) suggested that purine bases (guanine and adenine) serve as the primary

targets for mutation by ethylene oxide while mutation that involves mostly cytosine appears to be a more common spontaneous event. The larger data set for cancer gene mutations in spontaneous and ethylene oxide-induced neoplasms of the lung, Harderian gland, uterus and mammary gland indicates that ethylene oxide probably increases the mutant fraction for most types of base substitution across proto-oncogenes and tumour-suppressor genes, as was found in the *Hprt* gene of T cells and the *LacI* gene of testes from ethylene oxide-exposed mice. The overall data suggest that a common mechanism for ethylene oxide is as a point mutagen in oncogenes and tumour-suppressor genes of multiple tissues in mice.

4.3 Mechanisms of carcinogenesis

Ethylene oxide is a direct-acting alkylating agent that has been shown to have genotoxic and mutagenic activity in numerous assays in both somatic and germ cells, and in both prokaryotic and eukaryotic organisms (IARC, 1994). It is active in a wide range of in-vitro and in-vivo systems. Increases in both gene mutations and chromosomal alterations, two general classes of cancer-related genetic changes, have been observed. In-vitro and in-vivo studies have shown that ethylene oxide can bind to cellular macromolecules, which results in a variety of DNA, RNA and protein adducts. The major DNA adduct recovered *in vivo* is 7-HEG and additional adducts such as 3-HEA and O^6-HEG are detected at much lower levels (Walker *et al*., 1992b). In-vitro studies indicate that other minor adducts can also be formed from the reaction of ethylene oxide with the $N1$ and N^6 of adenine and the $N3$ of cytosine, uracil and thymine (IARC, 1994; Tates *et al*., 1999; Kolman *et al*., 2002). While the exact mechanism by which these adducts lead to mutation is unknown, a number of mechanisms could be involved, including the mispairing of altered bases or the formation of apurinic/apyrimidinic sites via DNA repair or chemical depurination/depyrimidination combined with the insertion of another base, which would typically be an adenine opposite an apurinic site (Tates *et al*., 1999; Houle *et al*., 2006). These lesions can also lead to the formation of single-strand breaks and, subsequently, to chromosomal breakage. In addition, the putative ethylene oxide metabolite, glycolaldehyde, has been shown to form DNA–protein cross-links and DNA single-strand breaks (Hengstler *et al*., 1994).

In-vivo studies using reporter genes such as *Hprt* or the *LacI* transgene have shown that ethylene oxide can significantly increase the frequency of mutations following exposure in both mice and rats (Walker *et al*., 1993; Sisk *et al*., 1997; Walker *et al*., 1997a,b; Tates *et al*., 1999; Recio *et al*., 2004). The type of mutation that is recovered appears to be influenced by the assay system involved. In mouse splenic and/or thymic T lymphocytes, mutations in *Hprt* could be detected after shorter exposures (4-week inhalation exposure or multiple intraperitoneal injections over 1 week) and appeared to consist of larger deletion mutations as well as base-pair substitutions and frameshift mutations (Walker *et al*., 1993, 1997a,b). The latter point mutations appeared to originate primarily from either altered G or altered A bases (Walker *et al*., 1993; IARC, 1994). In the inhalation study,

significant increases in *LacI* mutations were not seen in the spleen, bone marrow or germ cells of mice after 4 weeks of exposure to ethylene oxide (Sisk *et al*., 1997). A modest but significant increase in *LacI* mutants was seen in the lungs of mice exposed to 200 ppm [366 mg/m^3] ethylene oxide. In a follow-up study with prolonged exposure (up to 48 weeks), significant increases in *LacI* mutants were seen in the bone marrow and testes of ethylene oxide-exposed transgenic mice (Recio *et al*., 2004). DNA sequence analysis of mutants obtained from the bone marrow showed that only AT→TA transversions were recovered at a significantly increased frequency in the exposed mice. A unique mutational spectrum was not seen in the testes.

Only limited information is available on the mutagenicity of ethylene oxide in humans. A significant increase in *HPRT* mutation frequency was reported in one group of workers with prolonged higher exposures to ethylene oxide (~5 ppm [~ 9.15 mg/m^3] TWA) but not in another group with lower exposure levels (0.025 ppm [0.046 mg/m^3] TWA) (Tates *et al*., 1991; Kolman *et al*., 2002).

In two recent studies, an elevated frequency of mutations or a change in mutational spectra has been seen in the tumours of ethylene oxide-treated mice (Houle *et al*., 2006; Hong *et al*., 2007). In the Hong *et al*. (2007) study, K-*ras* mutations were detected in 100% (23/23) of ethylene oxide-induced lung tumours compared with 25% (27/108) of spontaneous tumours. Codon 12 G→T transversions occurred frequently in the ethylene oxide-induced lung neoplasms (21/23) but infrequently in spontaneous lung neoplasms (1/108). Similarly, K-*ras* mutations were present in 86% (18/21) of Harderian gland tumours from ethylene oxide-treated animals but were only seen in 7% (2/27) of the spontaneous tumours in this organ. Codon 13 G→C and codon 12 G→T transversions were common in the ethylene oxide-induced but absent in the spontaneous Harderian gland tumours (0/27). K-*ras* Mutations were also seen in 83% (5/6) of ethylene oxide-induced uterine tumours, all of which exhibited a G→C transition in codon 13. The incidence in spontaneous uterine tumours was not reported. A similar study by Houle *et al*. (2006) provided evidence of the involvement of H-*ras* and *p53* mutations in mammary gland tumours induced by ethylene oxide in mice. The mutation frequency was only slightly elevated for H-*ras* (33% in treated versus 26% in spontaneous) or *p53* (67% in the ethylene oxide-treated versus 58% in the control animals), but the mutational spectra of tumours obtained from control and treated animals differed significantly. The mutational spectra were generally consistent with a targeting of G and A bases by ethylene oxide (Houle *et al*., 2006; Hong *et al*., 2007). The high frequencies of mutation present in these genes, particularly mutations in the critical codons of K-*ras* and inactivation of *p53*, indicate that mutations are induced in the tumours of ethylene oxide-treated mice and that the changes probably play an important role in ethylene oxide-induced tumorigenesis in these tissues.

Little is known about the mechanisms that might lead to ethylene oxide-induced tumours in humans. However, activating mutations in the *ras* family of oncogenes and inactivation of *p53* have been shown to play critical roles in the development of both spontaneous and chemically induced cancers (Pedersen-Bjergaard *et al*., 2006; Zarbl,

2006). For example, activating mutations in *ras* genes have been shown to occur in up to 30% of cases of acute myelogenous leukaemia (Byrne & Marshall, 1998). In most cases, N-ras is activated, although activation of K-ras is occasionally seen (Byrne & Marshall, 1998; Bowen *et al*., 2005; Christiansen *et al*., 2005). The mutations typically occur in codons 12, 13 and 61, sites that are critical for the normal regulation of ras activity. The activating mutations lead to the generation of constitutively activated ras proteins that cannot be switched off and inappropriately generate proliferative signals within the cell (Byrne & Marshall, 1998). Some patients who lack *ras* mutations still exhibit an overexpression of *ras* genes and this has been considered as further evidence for the involvement of dysregulated ras signalling in leukaemogenesis (Byrne & Marshall, 1998).

Acute myelogenous leukaemia in patients who have previously been treated with alkylating agents frequently exhibits distinctive characteristics that allow it to be distinguished from acute myelogenous leukaemia induced by other agents (such as topoisomerase II inhibitors) or that occurs spontaneously (Pedersen-Bjergaard & Rowley, 1994; Pedersen-Bjergaard *et al*., 2006). One of the hallmarks of leukaemias induced by alkylating agents is that they frequently exhibit loss of chromosomes 5 or 7 (-5, -7) or loss of part of the long arms of these chromosomes (5q-, 7q-). In addition, mutations in *p53* are frequently seen in leukaemias with the -5/5q- karyotype, and mutations in *p53* and *ras* are seen in a subset of those that exhibit the -7/7q- karyotype (Christiansen *et al*., 2001; Pedersen-Bjergaard *et al*., 2006). Although ethylene oxide has not been investigated specifically for its ability to induce losses in chromosomes 7 and 5 or deletions of the long arms of chromosomes 7 and 5 (7q- or 5q-), it has been reported to induce similar types of chromosomal alterations and deletions in a variety of experimental models and/or in the lymphocytes of exposed workers (IARC, 1994; Major *et al*., 1996, 1999). The detection of elevated levels of chromosomal aberrations and micronuclei in the peripheral blood lymphocytes of ethylene oxide-exposed workers is of particular note, as multiple prospective studies have reported that individuals with increased levels of chromosomal aberrations or micronuclei in these cells are at increased risk for developing cancer (Hagmar *et al*., 1998; Liou *et al*., 1999; Smerhovsky *et al*., 2001; Hagmar *et al*., 2004; Boffetta *et al*., 2007; Bonassi *et al*., 2007).

A comparison of the evidence for ethylene oxide-induced genetic and related changes in experimental animals and humans is summarized in Table 12.

Table 12. Comparison of the evidence for key ethylene oxide-induced genetic and related changes in humans, human cells and experimental animals

End-point	In-vivo exposure		In-vitro exposure
	Animals	Humans	Human cells
Haemoglobin adduct formation	Strong	Strong	Strong
DNA adduct formation	Strong	Weak*	Strong
Mutations in reporter genes in somatic cells	Strong	Weak*	Strong
Mutations in cancer-related genes in tumours	Strong	NI	NA
Increased levels of cancer-related proteins in tumours	Strong	NI	NA
Chromosomal alterations in somatic cells			
Sister chromatid exchange	Strong	Strong	Strong
Structural chromosomal aberrations	Strong[+]	Strong	Moderate
Micronucleus formation	Strong[+]	Strong	NI

NA, not applicable; NI, no information
* Possibly due to a lack of adequate studies
[+] Positive responses were seen only at exposure concentrations above those used in rodent cancer bioassays.

5. Summary of Data Reported

5.1 Exposure data

Ethylene oxide is a flammable gas. It was first produced in the 1910s by the chlorohydrin process, which has gradually been replaced by the direct oxidation of ethylene since the 1930s. Ethylene oxide is used predominantly for the production of other chemicals, such as ethylene glycols, ethoxylates, ethanol amines and glycol ethers, and its use is increasing. It is also used as a sterilant to treat hospital equipment, disposable medical items, spices and other products. The highest human exposures occur in occupational settings. Historically, 8-h time-weighted average exposures above 20 mg/m^3 were common when the chlorohydrin process was used, while average exposures of 2–20 mg/m^3 were reported during direct oxidation of ethylene and in plants that produce sterilized medical items. The largest group of exposed workers is hospital personnel who perform sterilization operations. The average levels of exposure in hospitals and production facilities have decreased significantly (on average to below 1 ppm [2 mg/m^3]) in western Europe and North America following the introduction of new occupational exposure limits in the mid-1980s. High peak exposures may still occur in some short-term work tasks. Ethylene oxide may occur in indoor air at levels generally < 0.2 mg/m^3,

probably due to small amounts in tobacco smoke. Ethylene oxide residues have also been detected in some spices and other food products.

5.2 Cancer in humans

Epidemiological evidence of the risk for human cancer from ethylene oxide derives 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 a 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 two cohorts that were published after that time. Many of the cohort members employed at chemical factories were also exposed to other chemicals.

By far the most informative epidemiological investigation was a study by the National Institute of Occupational Safety and Health of more than 18 000 employees at 14 industrial facilities in the USA where ethylene oxide was used to sterilize medical supplies or food spices, or to test the sterilizing equipment. This investigation benefited not only from greater statistical power than other studies (as a consequence of its large size), but also from a lower potential for confounding by concomitant exposure to other chemicals and from incorporation of detailed quantitative assessments of individual exposures to ethylene oxide. For these reasons, the Working Group gave greatest weight to the National Institute for Occupational Safety and Health study when assessing the balance of epidemiological evidence on ethylene oxide, although findings from other studies were also taken into account.

In examining the epidemiological evidence, the Working Group focused in particular on lymphatic and haematopoietic cancers and on cancers of the breast, stomach, pancreas and brain. These sites were selected because excess risks had been suggested by one or more epidemiological study or because tumours at the same site had been reported in rodent bioassays.

Evaluation of the 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 exact specification of tumours on death certificates.

The Working Group found some epidemiological evidence for associations between ethylene oxide and lymphatic and haematopoietic cancers, and specifically lymphoid tumours (i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia). In the most recent follow-up of the National Institute for Occupational Safety and Health cohort, no overall excess of deaths from non-Hodgkin lymphoma or multiple myeloma was observed in comparison with national death rates. However, in an internal analysis, mortality from lymphoid tumours (as defined above) was associated with measures of cumulative exposure to ethylene oxide among men. No corresponding association was found among women. Other studies did not point consistently to an increase

in the risk for non-Hodgkin 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 have not been confirmed by later studies. In the latest analysis of the National Institute for Occupational Safety and Health cohort, mortality from 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 for leukaemia.

The numbers of cases of Hodgkin lymphoma in published studies were too few to draw meaningful conclusions.

Four of the cohort studies provided useful information on the association between exposure to ethylene oxide and breast cancer. The National Institute for Occupational Safety and Health study and a cohort study of hospital sterilization workers in the United Kingdom examined mortality from breast cancer and found no overall excess risk. Three studies examined the incidence of breast cancer: the National Institute for Occupational Safety and Health study and a cohort study from Sweden found no overall excess risk for breast cancer, while another cohort study from New York State, USA, found a borderline significant excess risk of about 60%. Cancer incidence was recognized to be underestimated in the National Institute for Occupational Safety and Health study, which was thus negatively biased for the investigation of overall cancer incidence.

A study conducted within the National Institute for Occupational Safety and Health 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, these findings were not supported by more recent, larger studies (including the National Institute for Occupational Safety and Health investigation); nor did the balance of epidemiological evidence point to an increased risk for brain cancer in humans exposed to ethylene oxide.

5.3 Cancer in experimental animals

Ethylene oxide was tested for carcinogenicity in one experiment by intragastric intubation in female rats, by inhalation in female mice in one experiment and in both sexes of mice in another experiment, by inhalation in both sexes of rats in one experiment and in male rats of the same strain in another experiment. It was also tested in single studies in female mice by skin application and by subcutaneous injection.

In the experiment of intragastric intubation in female rats, ethylene oxide produced tumours of the forestomach, which were mainly squamous-cell carcinomas. In one study in both sexes of mice, inhalation of ethylene oxide resulted in increases in the incidence of alveolar/bronchiolar lung tumours and tumours of the Harderian gland in animals of each sex and of uterine adenocarcinomas, mammary carcinomas and malignant lymphomas in females. In a pulmonary tumour bioassay in female strain A/J mice, inhalation of ethylene oxide increased the number of pulmonary adenomas per mouse. In both experiments in which male and female rats of one strain were exposed by inhalation, ethylene oxide increased the incidence of mononuclear-cell leukaemia and brain tumours in animals of each sex and of peritoneal mesotheliomas in the region of the testis and subcutaneous fibromas in males. Ethylene oxide produced local sarcomas in female mice following subcutaneous injection. In a limited study in female mice treated by skin application, no skin tumours were observed.

5.4 Mechanistic and other relevant data

Inhaled ethylene oxide is readily taken up by the lungs, is absorbed efficiently into the blood and is systemically distributed in rodents and in humans. Ethylene oxide is converted by both enzymatic and non-enzymatic hydrolysis to ethylene glycol, which is excreted or further metabolized, and by conjugation with glutathione mediated by the polymorphic glutathione-*S*-transferase T1. Glycolaldehyde, which is potentially formed by further metabolism of ethylene glycol, can cause DNA–protein crosslinks and DNA strand breaks. A striking difference in the metabolism of ethylene oxide between rodents and humans is the predominance of the glutathione conjugation pathway in mice and rats, while the pathway initiated by enzymatic and non-enzymatic hydrolysis is of greater importance in humans. Simulations indicated that, in mice, rats and humans, about 80%, 60% and 20%, respectively, would be metabolized via glutathione conjugation. Despite these differences, physiologically based pharmacokinetic models of uptake and metabolism of ethylene oxide in the range of exposures used in rodent bioassays (100 ppm [180 mg/m^3] and below) yielded simulated blood peak concentrations and areas under the curve that were similar for mice, rats and humans.

Ethylene oxide is a direct-acting alkylating agent that forms adducts with proteins and DNA. Haemoglobin adducts have been used for biomonitoring purposes, in view of the significant correlation between cumulative exposure over 4 months (the lifespan of human erythrocytes) and levels of adducts in the haemoglobin of ethylene oxide-exposed workers. Endogenous hydroxyethyl adducts of haemoglobin and DNA are also found in both humans and experimental animals in the absence of known exogenous exposure to ethylene oxide. *N*7-Hydroxyethylguanine is quantitatively the major DNA adduct formed, but does not appear to be directly promutagenic. Minor promutagenic adducts proposed for ethylene oxide are either induced at very low levels or have not been shown to occur in humans or experimental animals.

Studies of workers exposed to ethylene oxide in hospital and factory sterilization units and in ethylene oxide manufacturing and processing plants have been fairly consistent in showing chromosomal damage in peripheral blood lymphocytes, which included sister chromatid exchange in 21 of 33 study groups, chromosomal aberrations in 18 of 24 study groups and micronucleus formation in four of 18 study groups. In ethylene oxide-exposed workers, the frequency of micronucleus formation was also elevated in the bone marrow in one study and in nasal mucosal cells in another study, but not in exfoliated buccal cells in two studies. In general, the degree of chromosomal damage was correlated with level and duration of exposure, and the majority of positive results were found in studies that evaluated individuals who were exposed to time-weighted average concentrations greater than 5 ppm [9 mg/m^3] ethylene oxide. Elevated levels of chromosomal aberrations and micronucleus formation in peripheral blood lymphocytes have been associated with increased risks for cancer in humans. One study suggested an elevation in gene mutations in one of two groups of ethylene oxide-exposed workers. Several other biomarkers of DNA damage, which include unscheduled DNA synthesis, DNA single-strand breaks, premature centromere divisions and DNA–protein crosslinks, have been studied by only a few investigators.

Collectively, the genotoxicity data in experimental systems consistently demonstrate that ethylene oxide is a mutagen and clastogen across all phylogenetic levels tested. Ethylene oxide induced unscheduled DNA synthesis, DNA strand breaks, gene mutation, sister chromatid exchange and chromosomal aberrations in cultured human cells, as well as mutations, chromosomal aberrations, micronucleus formation and cell transformation in rodent cells *in vitro*. It also induced gene mutation, specific locus mutation, sister chromatid exchange, chromosomal aberrations, micronucleus formation, dominant lethal mutation and heritable translocation in somatic and/or germ cells in rodents treated *in vivo*. Analogous genetic and related effects of ethylene oxide were observed in non-mammalian systems. Unequivocal data show that ethylene oxide caused dose-related increases in point mutations in both reporter genes and cancer genes of multiple tissues from mice and rats exposed to 25–200 ppm [45–360 mg/m^3] ethylene oxide, doses that encompassed all but the lowest concentration used in rodent cancer bioassays. In these in-vivo studies, ethylene oxide was consistently a relatively weak point mutagen. Ethylene oxide induced sister chromatid exchange but not chromosomal aberrations or micronucleus formation in rodents exposed by inhalation to concentrations that were used in carcinogenicity studies. In contrast, assays for the potential clastogenic effects of inhalation of this concentration range of ethylene oxide by rodents have not been performed uniformly in an optimal fashion. Rodent studies that were positive for clastogenicity following exposure to higher doses of ethylene oxide or via a less relevant route of exposure may also be informative for a risk for chromosomal events under some exposure scenarios in ethylene oxide-exposed humans. With regard to the mode of action of ethylene oxide as a genotoxic carcinogen in rodents, there is clear evidence that point mutations in *ras* proto-oncogenes and the *p53* tumour-suppressor gene (which are involved in a range of human cancers) are key events in the development of cancers, but

the role of chromosomal alterations as primary mutational events in the carcinogenicity of ethylene oxide in mice and rats is still uncertain.

6. Evaluation and Rationale

6.1 Carcinogenicity in humans

There is *limited evidence* in humans for the carcinogenicity of ethylene oxide.

6.2 Carcinogenicity in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethylene oxide.

6.3 Overall evaluation

Ethylene oxide is *carcinogenic to humans (Group 1).*

6.4 Rationale

In making the overall evaluation, the Working Group also took into consideration the following supporting evidence:

(*a*) Ethylene oxide is a direct-acting alkylating agent that reacts with DNA.

(*b*) Ethylene oxide induces a dose-related increase in the frequency of ethylene oxide-derived haemoglobin adducts in exposed humans and rodents.

(*c*) Ethylene oxide induces a dose-related increase in the frequency of ethylene oxide-derived DNA adducts in exposed rodents.

(*d*) Ethylene oxide consistently acts as a mutagen and clastogen at all phylogenetic levels.

(*e*) Ethylene oxide induces heritable translocations in the germ cells of exposed rodents.

(*f*) Ethylene oxide induces a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.

(*g*) Prospective studies have shown that elevated levels of chromosomal aberrations and micronucleus formation in peripheral blood lymphocytes are associated with increased risks for cancer in humans.

7. References

ACGIH® Worldwide (2005) *Documentation of the TLVs® and BEIs® with Other Worldwide Occupational Exposure Values — 2005 CD ROM*, Cincinnati, OH, American Conference of Governmental Industrial Hygienists

Adkins, B., Jr, Van Stee, E.W., Simmons, J.E. & Eustis, S.L. (1986) Oncogenic response of strain A/J mice to inhaled chemicals. *J. Toxicol. environ. Health*, **17**, 311–322

Agurell, E., Cederberg, H., Ehrenberg, L., Lindahl-Kiessling, K., Rannug, U. & Törnqvist, M. (1991) Genotoxic effects of ethylene oxide and propylene oxide: A comparative study. *Mutat. Res.*, **250**, 229–237

Albertini, R.A. & Sweeney, L.M. (2007) Propylene oxide: Genotoxicity profile of a rodent nasal carcinogen. *Crit. Rev. Toxicol.*, **37**, 489–520

Allied Signal Chemicals (1993) *Ethylene Oxide*, Morristown, NJ

Ambroise, D., Moulin, J.-J., Squinazi, F., Protois, J.-C., Fontana, J.-M. & Wild, P. (2005) Cancer mortality among municipal pest-control workers. *Int. Arch. occup. environ. Health*, **78**, 387–393

Anon. (2004) Product focus — EO-EG, *Chem. Week*, **April 28**, 38

Anon. (2005) Chemical profile: Ethylene oxide. *Eur. chem. News*, **25 July** (electronic archive)

Appelgren, L.-E., Eneroth, G. & Grant, C. (1977) Studies on ethylene oxide: Whole-body autoradiography and dominant lethal test in mice. *Proc. Eur. Soc. Toxicol.*, **18**, 315–317

Appelgren, L.-E., Eneroth, G., Grant, C., Landström, L.-E. & Tenghagen, K. (1978) Testing of ethylene oxide for mutagenicity using the micronucleus test in mice and rats. *Acta pharmacol. toxicol.*, **43**, 69–71

Arms, A.D. & Travis, C.C. (1988) *Reference Physiological Parameters in Pharmacokinetic Modeling* (Report No EPA 600 6-88/004), Washington DC, US Environmental Protection Agency.

Bailey, E., Farmer, P.B. & Shuker, D.E.G. (1987) Estimation of exposure to alkylating carcinogens by the GC–MS determination of adducts to hemoglobin and nucleic acid bases in urine. *Arch. Toxicol.*, **60**, 187–191

Bastlová, T., Andersson, B., Lambert, B. & Kolman, A. (1993) Molecular analysis of ethylene oxide-induced mutations at the HPRT locus in human diploid fibroblasts. *Mutat. Res.*, **287**, 283–292

Baumbach, N., Herzog, V. & Schiller, F. (1987) [In vitro studies on the permeation of ethylene oxide through human skin]. *Dermatol. Monatsschr.*, **173**, 328–332 (in German)

Benson, L.O. & Teta, M.J. (1993) Mortality due to pancreatic and lymphopoietic cancers in chlorohydrin production workers. *Br. J. ind. Med.*, **50**, 710–716

Bhanot, O.S., Singh, U.S. & Solomon, J.J. (1994) The role of 3-hydroxethyluridine in mutagenesis by ethylene oxide. *J. biol. Chem.*, **269**, 30056–30064

Bisanti, L., Maggini, M., Raschetti, R., Alegiani, S.S., Ippolito, F.M., Caffari, B., Segnan, N. & Ponti, A. (1993) Cancer mortality in ethylene oxide workers. *Br. J. ind. Med.*, **50**, 317–324

Boeniger, M. (1988a) *Health Hazard Evaluation Report, University of Cincinnati Hospital, Cincinnati, OH* (Report No. HETA 86-508), Cincinnati, OH, National Institute for Occupational Safety and Health

Boeniger, M. (1988b) *Health Hazard Evaluation Report, Humana Audubon Hospital, Louisville*, KY (Report No. HETA 87-378), Cincinnati, OH, National Institute for Occupational Safety and Health

Boffetta, P., van der Hel, O., Norppa, H., Fabianova, E., Fucic, A., Gundy, S., Lazutka, J., Cebulska-Wasilewska, A., Puskailerova, D., Znaor, A., Kelecsenyi, Z., Kurtinaitis, J., Rachtan, J., Forni, A., Vermeulen, R. & Bonassi, S. (2007) Chromosomal aberrations and cancer risk: Results of a cohort study from Central Europe. *Am. J. Epidemiol.*, **165**, 36–43

Bolt, H.M. (1996) Quantification of endogenous carcinogens: The ethylene oxide paradox. *Biochem. Pharmacol.*, **52**,1–5

Bolt, H.M. & Filser, J.G. (1987) Kinetics and disposition in toxicology. Example: Carcinogenic risk estimate for ethylene. *Arch. Toxicol.*, **60**, 73–76

Bolt, H.M. & Leutbecher, M. (1993) Dose–DNA adduct relationship for ethylene oxide. *Arch. Toxicol.*, **67**, 712–713

Bolt, H.M. & Thier, R. (2006) Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. *Curr. Drug Metab.*, **7**, 613–628

Bolt, H.M., Peter, H. & Föst, U. (1988) Analysis of macromolecular ethylene oxide adducts. *Int. Arch. occup. environ. Health*, **60**, 141–144

Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M.P., Bolognesi, C., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Hagmar, L., Joksic, G., Martelli, A., Migliore, L., Mirkova, E., Scarfi, M.R., Zijno, A., Norppa, H. & Fenech, M. (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28**, 625–631

Bono, R., Vincenti, M., Saglia, U., Pignata, C., Russo, R., & Gilli, G. (2002) Tobacco smoke and formation of N-(2-hydroxyethyl)valine in human hemoglobin. *Arch. environ. Health*, **57**, 416–421

Bowen, D.T., Frew, M.E., Hills, R., Gale, R.E., Wheatley, K., Groves, M.J., Langabeer, S.E., Kottaridis, P.D., Moorman, A.V., Burnett, A.K. & Linch, D.C. (2005) RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood*, **106**, 2113–2119

Brown, C.D., Wong, B.A. & Fennell, T.R. (1996) In vivo and in vitro kinetics of ethylene oxide metabolism in rats and mice. *Toxicol. appl. Pharmacol.*, **136**, 8–19

Brown, C.D., Asgharian, B., Turner, M.J. & Fennell, T.R. (1998) Ethylene oxide dosimetry in the mouse. *Toxicol. appl. Pharmacol.*, **148**, 215–221

Brugnone, F., Perbellini, L., Faccini, G. & Pasini, F. (1985) Concentration of ethylene oxide in the alveolar air of occupationally exposed workers. *Am. J. ind. Med.*, **8**, 67–72

Brugnone, F., Perbellini, L., Faccini, G.B., Pasini, F., Bartolucci, G.B. & DeRosa, E. (1986) Ethylene oxide exposure. Biological monitoring by analysis of alveolar air and blood. *Int. Arch. occup. environ. Health*, **58**, 105–112

Burgaz, S., Rezanko, R., Kara, S. & Karakaya, A.E. (1992) Thioethers in urine of sterilization personnel exposed to ethylene oxide. *J. clin. pharm. Ther.*, **17**, 169–172

Byrne, J.L. & Marshall, C.J. (1998) The molecular pathophysiology of myeloid leukaemias: Ras revisited. *Br. J. Haematol.*, **100**, 256–264

Calleman, C.J., Ehrenberg, L., Jansson, B., Osterman-Golkar, S., Segerbäck, D., Svensson, K. & Wachtmeister, C.A. (1978) Monitoring and risk assessment by means of alkyl groups in hemoglobin in persons occupationally exposed to ethylene oxide. *J. environ. Pathol. Toxicol*., **2**, 427–442

Chessor, E., Verhoeven, M., Hon, C.-Y. & Teschke, K. (2005) Evaluation of a modified scavenging system to reduce occupational exposure to nitrous oxide in labor and delivery rooms. *J. occup. environ. Hyg*., **2**, 314–322

Chien, Y.C., Liu, H.H., Lin Y.C., Su, P.C., Li, L.H., Chang, C.P. Dang, D.T. & Chen C.Y. (2007) Ethylene oxide sterilization in the medical-supply manufacturing industry: Assessment and control of worker exposure. *J. biomed. Mat. Res. B, appl. Biomat.*, **83**, 527–537

Christiansen, D.H., Andersen, M.K. & Pedersen-Bjergaard, J. (2001) Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. *J. clin. Oncol.*, **19**, 1405–1413

Christiansen, D.H., Andersen, M.K., Desta, F. & Pedersen-Bjergaard, J. (2005) Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia*, **19**, 2232–2240

Clare, M.G., Dean, B.J., de Jong, G. & van Sittert, N.J. (1985) Chromosome analysis of lymphocytes from workers at an ethylene oxide plant. *Mutat. Res*., **156**, 109–116

Coggon, D., Harris, E.C., Poole, J. & Palmer, K.T. (2004) Mortality of workers exposed to ethylene oxide: Extended follow up of a British cohort. *Occup. environ. Med.*, **61**, 358–362

Csanády, G.A., Denk, B., Pütz, C., Kreuzer, P.E., Kessler, W., Baur, C., Gargas, M.L. & Filser, J.G. (2000) A physiological toxicokinetic model for exogenous and endogenous ethylene and ethylene oxide in rat, mouse, and human: Formation of 2-hydroxyethyl adducts with hemoglobin and DNA. *Toxicol. appl. Pharmacol*., **165**, 1–26

Cummins, K.J., Schultz, G.R., Lee, J.S., Nelson, J.H. & Reading, J.C. (1987) The development and evaluation of a hydrobromic acid-coated sampling tube for measuring occupational exposures to ethylene oxide. *Am. ind. Hyg. Assoc. J*., **48**, 563–573

Currier, M.F., Carlo, G.L., Poston, P.L. & Ledford, W.E. (1984) A cross sectional study of employees with potential occupational exposure to ethylene oxide. *Br. J. ind. Med*., **41**, 492–498

Dahlgran, J.R. & Shingleton, C.R. (1987) Determination of ethylene oxide in ethoxylated surfactants and demulsifiers by headspace gas chromatography. *J. Assoc. off. anal. Chem*., **70**, 796–798

Daniel, G., Hoffman, W. & McDonald, B. (2004) OSHA compliance issues: Ethylene oxide in a commercial sterilization operation. *J. occup. environ. Hyg.*, **1**, D121–D125

Danielson, J.W., Snell, R.P. & Oxborrow, G.S. (1990) Detection and quantitation of ethylene oxide, 2-chloroethanol, and ethylene glycol with capillary gas chromatography. *J. chromatogr. Sci*., **28**, 97–101

Dellarco, V.L., Generoso, W.M., Sega, G.A., Fowle, J.R., III & Jacobson-Kram, D. (1990) Review of the mutagenicity of ethylene oxide. *Environ. mol. Mutag.*, **16**, 85–103

Denk, B., Filser, J.G., Oesterle, D., Deml, E. & Greim, H. (1988) Inhaled ethylene oxide induces preneoplastic foci in rat liver. *J. Cancer Res. clin. Oncol*., **114**, 35–38

Deschamps, D., Laurent, A.-M., Festy, B. & Conso, F. (1989) [Study of six ethylene oxide sterilization units in the Poor Law Administration of Paris.] *Arch. Mal. prof*., **50**, 641–649 (in French)

Devanney, M.T. (2007) *CEH Marketing Research Report – Ethylene Oxide* (Abstract), Zürich, SRI Consulting [available at http://www.sriconsulting.com/CEH/Public/Reports/654.5000/ accessed 26.02.2008]

Dever, J.P., George, K.F., Hoffman, W.C. & Soo, H. (2004) Ethylene oxide. In: Kroschwitz, J.I. & Howe-Grant, M., eds, *Kirk Othmer Encyclopedia of Chemical Technology*, Vol. 10, New York, John Wiley & Sons, pp. 632–673 (on line)

Dunkelberg, H. (1981) [Carcinogenic activity of ethylene oxide and its reaction products 2-chloroethanol, 2-bromoethanol, ethylene glycol and diethylene glycol. I. Carcinogenicity of ethylene oxide in comparison with 1,2-propylene oxide after subcutaneous administration in mice.] *Zbl. Bakt. Hyg. I. Abt. Orig. B*, **174**, 383–404 (in German)

Dunkelberg, H. (1982) Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. *Br. J. Cancer*, **46**, 924–933

Ehrenberg, L. (1991) Detection and measurement of protein adducts: Aspects of risk assessment. *Prog. clin. biol. Res.*, **372**, 79–87

Ehrenberg, L. & Törnqvist, M. (1992) Use of biomarkers in epidemiology: Quantitative aspects. *Toxicol. Lett.*, **64–65**, 485–492

Ehrenberg, L., Hiesche, K.D., Osterman-Golkar, S. & Wennberg, I. (1974) Evaluation of genetic risks of alkylating agents: Tissue doses in the mouse from air contaminated with ethylene oxide. *Mutat. Res.*, **24**, 83–103

Eide, I., Hagemann, R., Zahlsen, K., Tabreke, E., Törnqvist, M., Kumar, R., Vodicka, P. & Hemminki, K. (1995) Uptake, distribution, and formation of hemoglobin and DNA adducts after inhalation of C2−C8 1-alkenes (olefins) in the rat. *Carcinogenesis*, **16**, 1603–1609

Eide, I, Zhao, C., Kumar, R., Hemminki, K., Wu, K.-Y. & Swenberg, J.A. (1999) Comparison of ^{32}P-postlabeling and high-resolution GC/MS in quantifying N7-(2-hydroxethyl)guanine adducts. *Chem. Res. Toxicol.*, **12**, 979–984

Eisenbrand, G., Muller, N., Denkel, E. & Sterzel, W. (1986) DNA adducts and DNA damage by antineoplastic and carcinogenic N-nitrosocompounds. *J. Cancer Res. clin. Oncol.*, **112**, 196–204

Elliott, L.J., Ringenburg, V.L., Morelli-Schroth, P., Halperin, W.E. & Herrick, R.F. (1988) Ethylene oxide exposures in hospitals. *Appl. ind. Hyg.*, **3**, 141–145

Ellison, K.S., Dogliotti, E., Connors, T.D., Basu, A.K. & Essigmann, J.M. (1989) Site-specific mutagenesis by O^6-alkylguanines located in the chromosomes of mammalian cells: Influence of the mammalian O^6-alkylguanine–DNA alkyltransferase. *Proc. natl Acad. Sci. USA*, **86**, 8620–8624

Embree, J.W., Lyon, J.P. & Hine, C.H. (1977) The mutagenic potential of ethylene oxide using the dominant-lethal assay in rats. *Toxicol. appl. Pharmacol.*, **40**, 261–267

Environmental Protection Agency (1992a) Ethylene oxide; tolerances for residues. *US Code fed. Regul.*, **Title 40**, Part 180.151, p. 311

Environmental Protection Agency (1992b) Ethylene oxide. *US Code fed. Regul.*, **Title 40**, Part 185.2850, p. 456

Environmental Protection Agency (2000) *Control of Emissions of Hazardous Air Pollutants from Motor Vehicles and Motor Vehicle Fuels. EPA Technical Support Document* (EPA 420-R-00-023), Washington DC

Farmer, P.B. & Shuker, D.E.G. (1999) What is the significance of increases in background levels of carcinogen-derived protein and DNA adducts? Some considerations for incremental risk assessment. *Mutat. Res.*, **424**, 275–286

Farmer, P.B., Bailey, E., Gorf, S.M., Törnqvist, M., Osterman-Golkar, S., Kautiainen, A. & Lewis-Enright, D.P. (1986) Monitoring human exposure to ethylene oxide by the determination of haemoglobin adducts using gas chromatography–mass spectrometry. *Carcinogenesis*, **7**, 637–640

Farmer, P.B., Neumann, H.G., & Henschler, D. (1987) Estimation of exposure of man to substances reacting covalently with macromolecules. *Arch Toxicol*., **60**, 251–260

Farmer, P.B., Cordero, R., & Autrup, H. (1996a) Monitoring human exposure to 2-hydroxyethylating carcinogens. *Environ Health Perspect*., **104**, 449–452

Farmer, P.B., Sepai, O., Lawrence, R., Autrup, H., Sabro Nielsen, P., Vestergard, A.B., Waters, R., Leuratti, C., Jones, N.J., Stone, J., Baan, R.A., van Delft, J.H., Steenwinkel, M.J., Kyrtopoulos, S.A., Souliotis, V.L., Theodorakopoulos, N., Bacalis, N.C., Natarajan, A.T., Tates, A.D., Haugen, A., Andreassen, A., Ovrebo, S., Shuker, D.E., Amaning, K.S., Schouft, A., Ellul, A., Garner, R.C., Dingley, K.H., Abbondandolo, A.A., Merlo, F., Cole, J., Aldrich, K., Beare, D., Capulas, E., Rowley, G., Waugh, A.P.W., Povey, A.C., Haque, K., Kirsch-Volders, M., van Hummelen, P. & Castelain, P. (1996b) Biomonitoring human exposure to environmental carcinogenic chemicals. *Mutagenesis*, **11**, 363–381

Farooqi, Z., Tornqvist, M., Ehrenberg, L. & Natarajan, A.T. (1993) Genotoxic effects of ethylene oxide and propylene oxide in mouse bone marrow cells. *Mutat. Res.*, **288**, 223–228

Fennell, T.R. & Brown, C.D. (2001) A physiologically based pharmacokinetic model for ethylene oxide in mouse, rat, and human. *Toxicol. appl. Pharmacol*., **173**, 161–175

Fennell, T.R., Sumner, S.C. & Walker, V.E. (1992) A model for the formation and removal of hemoglobin adducts. *Cancer Epidemiol. Biomarkers Prev*., **1**, 213–219

Fennell, T.R., MacNeela, J.P., Morris, R.W., Watson, M., Thompson, C.L. & Bell, D.A. (2000) Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: Effects of glutathione S-transferase T1-null and M1-null genotypes. *Cancer Epidemiol. Biomarkers Prev*., **9**, 705–712

Filser, J.G. (1992) The closed chamber technique-uptake, endogenous production, excretion, steady-state kinetics and rates of metabolism of gases and vapors. *Arch. Toxicol*., **66**, 1–10

Filser, J.G. & Bolt, H.M. (1984) Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. *Arch. Toxicol*., **55**, 219–223

Filser, J.G., Denk, B., Törnqvist, M., Kessler, W. & Ehrenberg, L. (1992) Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene. *Arch. Toxicol*., **66**, 157–163

Filser, J.G., Schwegler, U., Csanády, G.A., Greim, H., Kreuzer, P.E. & Kessler, W. (1993) Species-specific pharmacokinetics of styrene in rat and mouse. *Arch. Toxicol*., **67**, 517–530

Filser, J.G., Kreuzer, P.E., Greim, H. & Bolt H.M. (1994) New scientific arguments for regulation of ethylene oxide residues in skin-care products. *Arch. Toxicol*., **68**, 401–405

Flores, G.H. (1983) Controlling exposure to alkylene oxides. *Chem. Eng. News*, **79**, 39–43

Föst, U., Marczynsk, B., Kasemann, R. & Peter, H. (1989) Determination of 7-(2-hydroxyethyl)guanine with gas chromatography/mass spectrometry as a parameter for genotoxicity of ethylene oxide. *Arch. Toxicol*., **Suppl. 13**, 250–253

Föst, U., Hallier, E., Ottenwälder, H., Bolt, H.M. & Peter, H. (1991) Distribution of ethylene oxide in human blood and its implications for biomonitoring. *Hum. exp. Toxicol.*, **10**, 25–31

Föst, U., Törnqvist, M., Leutbecher, M., Granath, F., Hallier, E., & Ehrenberg, L. (1995) Effects of variation in detoxification rate on dose monitoring through adducts. *Hum. exp. Toxicol.*, **14**, 201–203

Fowles, J., Mitchell, J. & McGrath, H. (2001) Assessment of cancer risk from ethylene oxide residues in spices imported into New Zealand. *Food. chem. Toxicol.*, **39**, 1055–1062

Fuchs, J., Wullenweber, U., Hengstler, J.G., Bienfait, H.G., Hiltl, G. & Oesch. F. (1994) Genotoxic risk for humans due to work place exposure to ethylene oxide: Remarkable individual differences in susceptibility. *Arch. Toxicol.*, **68**, 343–348

Galloway, S.M., Berry, P.K., Nichols, W.W., Wolman, S.R., Soper, K.A., Stolley, P.D. & Archer, P. (1986) Chromosome aberrations in individuals occupationally exposed to ethylene oxide and in a large control population. *Mutat. Res.*, **170**, 55–74

Gardner, M.J., Coggon, D., Pannett, B. & Harris, E.C. (1989) Workers exposed to ethylene oxide: A follow up study. *Br. J. ind. Med.*, **46**, 860–865

Garman, R.H., Snellings, W.M. & Maronpot, R.R. (1985) Brain tumors in F344 rats associated with chronic inhalation exposure to ethylene oxide. *Neurotoxicology*, **6**, 117–138

Garman, R.H., Snellings, W.M. & Maronpot, R.R. (1986) Frequency, size and location of brain tumours in F-344 rats chronically exposed to ethylene oxide. *Food chem. Toxicol.*, **24**, 145–153

Garry, V.F., Hozier, J., Jacobs, D., Wade, R.L. & Gray, D.G. (1979) Ethylene oxide: Evidence of human chromosomal effects. *Environ. Mutag.*, **1**, 375–382

Garry, V.F., Opp, C.W., Wiencke, J.K. & Lakatua, D. (1982) Ethylene oxide induced sister chromatid exchange in human lymphocytes using a membrane dosimetry system. *Pharmacology*, **25**, 214–221

Generoso, W.M., Cain, K.T., Krishna, M., Sheu, C.W. & Gryder, R.M. (1980) Heritable translocation and dominant-lethal mutation induction with ethylene oxide in mice. *Mutat. Res.*, **73**, 133–142

Generoso, W.M., Cumming, R.B., Bandy, J.A. & Cain, K.T. (1983) Increased dominant-lethal effects due to prolonged exposure of mice to inhaled ethylene oxide. *Mutat. Res.*, **119**, 377–379

Generoso, W.M., Cain, K.T., Hughes, L.A., Sega, G.A., Braden, P.W., Gosslee, D.G. & Shelby, M.D. (1986) Ethylene oxide dose and dose-rate effects in the mouse dominant-lethal test. *Environ. Mutag.*, **8**, 1–7

Generoso, W.M., Cain, K.T., Cornett, C.V., Cacheiro, N.L.A. & Hughes, L.A. (1990) Concentration–response curves for ethylene-oxide-induced heritable translocations and dominant lethal mutations. *Environ. mol. Mutag.*, **16**, 126–131

Gérin, M. & Tardif, R. (1986) Urinary N-acetyl-S-2-hydroxyethyl-L-cysteine in rats as biological indicator of ethylene oxide exposure. *Fundam. appl. Toxicol.*, **7**, 419–423

Glaser, Z.R. (1979) Ethylene oxide: Toxicology review and field study results of hospital use. *J. environ. Pathol. Toxicol.*, **12**, 173–208

Godderis, L., Aka, P., Mateuca, R., Kirsch-Volders, M., Lison, D. & Veulemans, H. (2006) Dose-dependent influence of genetic polymorphisms on DNA damage induced by styrene oxide, ethylene oxide and gamma-radiation. *Toxicology*, **219**, 220–229

Golberg, L. (1986) *Hazard Assessment of Ethylene Oxide*, Boca Raton, FL, CRC Press, pp. 3–7

Greenberg, H.L., Ott, M.G. & Shore, R.E. (1990) Men assigned to ethylene oxide production or other ethylene oxide related chemical manufacturing: A mortality study. *Br. J. ind. Med.*, **47**, 221–230

Greife, A.L., Hornung, R.W., Stayner, L.G. & Steenland, K.N. (1988) Development of a model for use in estimating exposure to ethylene oxide in a retrospective cohort mortality study. *Scand. J. Work Environ. Health*, **14** (Suppl. 1), 29–30

Hagmar, L., Welinder, H., Lindén, K., Attewell, R., Osterman-Golkar, S. & Törnqvist, M. (1991) An epidemiological study of cancer risk among workers exposed to ethylene oxide using hemoglobin adducts to validate environmental exposure assessments. *Int. Arch. occup. environ. Health*, **63**, 271–277

Hagmar, L., Mikoczy, Z. & Welinder, H. (1995) Cancer incidence in Swedish sterilant workers exposed to ethylene oxide. *Occup. environ. Med.*, **52**, 154–156

Hagmar, L., Bonassi, S., Strömberg, U., Brogger, A., Knudsen, L.E., Norppa, H. & Reuterwall, C. (1998) Chromosomal aberrations in lymphocytes predict human cancer: A report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res.*, **58**, 4117–4121

Hagmar, L., Strömberg, U., Bonassi, S., Hansteen, I.L., Knudsen, L.E., Lindholm, C. & Norppa, H. (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: Results from Nordic and Italian cohorts. *Cancer Res.*, **64**, 2258–2263

Hallier, E., Langhof, T., Dannappel, D., Leutbecher, M., Schröder, K., Goergens, H.W., Müller, A. & Bolt, H.M. (1993) Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: Influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. *Arch. Toxicol.*, **67**, 173–178

Hansen, J.P., Allen, J., Brock, K., Falconer, J., Helms, M.J., Shaver, G.C. & Strohm, B. (1984) Normal sister chromatid exchange levels in hospital sterilization employees exposed to ethylene oxide. *J. occup. Med.*, **26**, 29–32

Haufroid, V., Merz, B., Hofmann, A., Tschopp, A., Lison, D., & Hotz, P. (2007) Exposure to ethylene oxide in hospitals: Biological monitoring and influence of glutathione S-transferase and epoxide hydrolase polymorphisms. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 796–802

Havlicek, C.S., Hilpert, L.R., Dal, G. & Perotti, D. (1992) *Assessment of Ethylene Oxide Concentrations and Emissions from Sterilization and Fumigation Processes. Final Report*, Sacramento, CA, Prepared for Research Division, California Air Resources Board

Hayes, J.D., Flanagan, J.U. & Jowsey, I.R. (2005) Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.*, **45**, 51–88

Health Canada (1999) *Priority Substances List Assessment Report, Ethylene Oxide*, (Canadian Environmental Protection Act, 1999), Ottawa

Health Canada (2001) *Priority Substances List; Assessment Report Ethylene Oxide*, Ottawa [available at: http://www.ec.gc.ca/substances/ese/eng/psap/final/ethyleneoxide.cfm]

Heiden Associates (1988a) *An Estimate of Industry Costs for Compliance with Two Ethylene Oxide Workplace STEL Scenarios: Ethylene Oxide Production and Ethoxylation Plants*, Washington DC

Heiden Associates (1988b) *A Medical Products Industry Profile for Evaluating Compliance with Two Ethylene Oxide Workplace STEL Scenarios: 10 ppm STEL and 5 ppm STEL*, Washington DC

Hemminki, K., Mutanen, P., Saloniemi, I., Niemi, M.-L. & Vainio, H. (1982) Spontaneous abortions in hospital staff engaged in sterilising instruments with chemical agents. *Br. med. J.*, **285**, 1461–1463

Hengstler, J.G., Fuchs, J., Gebhard, S. & Oesch, F. (1994) Glycolaldehyde causes DNA–protein crosslinks: A new aspect of ethylene oxide genotoxicity. *Mutat. Res.*, **304**, 229–234

Hengstler, J.G., Fuchs, J., Tanner, B., Oesch-Bartomowicz, B., Hölz, C. & Oesch, F. (1997) Analysis of DNA single-strand breaks in human venous blood: A technique which does not require isolation of white blood cells. *Environ. mol. Mutag.*, **29**, 58–62

Herrero, M.E., Arand, M., Hengstler, J.G. & Oesch, F. (1997) Recombinant expression of human microsomal epoxide hydrolase protects V79 chinese hamster cells from styrene oxide- but not from ethylene oxide-induced DNA strand breaks. *Environ. mol. Mutag.*, **30**, 429–439

Hochberg, V., Shi, X.-C., Moorman, W. & Ong, T. (1990) Induction of micronuclei in rat bone marrow and spleen cells by varied dose-rate of ethylene oxide (Abstract No. 91). *Environ. mol. Mutag.*, **15**, 26

Hoechst Celanese Corp. (1992) *Material Safety Data Sheet: Ethylene Oxide*, Dallas, TX

Högstedt, L.C. (1988) Epidemiological studies on ethylene oxide and cancer: An updating. In: Bartsch, H., Hemminki, K. & O'Neill, I.K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, IARC, pp. 265–270

Högstedt, C., Rohlén, O., Berndtsson, B.S., Axelson, O. & Ehrenberg, L. (1979a) A cohort study of mortality and cancer incidence in ethylene oxide production workers. *Br. J. ind. Med.*, **36**, 276–280

Högstedt, C., Malmqvist, N. & Wadman, B. (1979b) Leukemia in workers exposed to ethylene oxide. *J. Am. med. Assoc.*, **241**, 1132–1133

Högstedt, B., Gullberg, B., Hedner, K., Kolnig, A.-M., Mitelman, F., Skerfving, S. & Widegren, B. (1983) Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide. *Hereditas*, **98**, 105–113

Högstedt, C., Aringer, L. & Gustavsson, A. (1986) Epidemiologic support for ethylene oxide as a cancer-causing agent. *J. Am. med. Assoc.*, **255**, 1575–1578

Högstedt, B., Bergmark, E., Törnqvist, M. & Osterman-Golkar, S. (1990) Chromosomal aberrations and micronuclei in lymphocytes in relation to alkylation of hemoglobin in workers exposed to ethylene oxide and propylene oxide. *Hereditas*, **113**, 133–138

Hong, H.-H.L., Houle, C.D., Ton, T.-V.T. & Sills, R.C. (2007) K-ras Mutations in lung tumors and tumors of other organs are consistent with a common mechanism of ethylene oxide tumorigenesis in the B6C3F1 mouse. *Toxicol. Pathol.*, **35**, 81–85

Hori, H., Yahata, K., Fujishiro, K., Yoshizumi, K., Li, D., Goto, Y. & Higashi, T. (2002) Personal exposure level and environmental ethylene oxide gas concentration in sterilization facilities of hospitals in Japan. *Appl. occup. environ. Hyg.*, **17**, 634–639

Hornung, R.W., Greife, A.L., Stayner, L.T., Steenland, N.K., Herrick, R.F., Elliott, L.J., Ringenburg, V.L. & Morawetz, J. (1994) Statistical model for prediction of retrospective exposure to ethylene oxide in an occupational mortality study. *Am. J. ind. Med.*, **25**, 825–836

Houle, C.D., Ton, T.-V.T., Clayton, N., Huff, J., Hong, H.-H.L. & Sills, R.C. (2006) Frequent *p53* and H-*ras* mutations in benzene- and ethylene oxide-induced mammary gland carcinomas from B6C3F1 mice. *Toxicol. Pathol.*, **34**, 752–762

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 157–167

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 36, *Allyl Compounds, Aldehydes, Epoxides and Peroxides*, Lyon, pp. 189–226

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon

IARC (1991) *IARC Monographs on the Evalution of Carcinogenic Risks to Humans*, Vol. 53, *Occupational Exposures in Insecticide Application, and Some Pesticides*, Lyon, pp. 179–249

IARC (1994) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 60, *Some Industrial Chemicals*, Lyon, pp. 73–159

IARC (1995) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 63, *Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals*, Lyon

IARC (1997) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 69, *Polychlorinated Dibenzo*-para-*Dioxins and Polychlorinated Dibenzofurans*, Lyon

IARC (1999a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 71, *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*, Lyon

IARC (1999b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 73, *Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances*, Lyon, pp. 131–182

IARC (2000) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 75, *Ionizing Radiation, Part 1: X- and γ-Radiation, and Neutrons*, Lyon, pp. 121–359

IARC (2004) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 83, *Tobacco Smoking*, Lyon, p. 51–1187

IARC (2006) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 88, *Formaldehyde, 2-Butoxyethanol and 1*-tert-*Butoxypropan-2-ol*, Lyon

IARC (2009) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 94, *Ingested Nitrates and Nitrites, and Cyanobacterial Peptide Toxins*, Lyon (in press)

IPCS (1985) *Ethylene Oxide* (Environmental Health Criteria 55), Geneva, World Health Organization, International Programme on Chemical Safety

IPCS-CEC (2001) *International Chemical Safety Card 0155*, Geneva, World Health Organization

IRSST (2005) [Guide to Sampling Contaminants in Workplace Air], 8th rev. Ed. [www.irsst.qc.ca/fr/_outil_100038.html] (in French)

Jensen, K.G. (1988) Determination of ethylene oxide residues in processed food products by gas–liquid chromatography after derivatization. *Z. Lebensmitt. Untersuch. Forsch.*, **187**, 535–540

Jenssen, D. & Ramel, C. (1980) The micronucleus test is part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. *Mutat. Res.*, **75**, 191–202

Johanson, G. & Filser, J.G. (1992) Experimental data from closed chamber gas uptake studies in rodents suggest lower uptake rate of chemical than calculated from literature values on alveolar ventilation. *Arch. Toxicol.*, **66**, 291–295

Jones, A.R. & Wells, G. (1981) The comparative metabolism of 2-bromoethanol and ethylene oxide in the rat. *Xenobiotica*, **11**, 763–770

Joyner, R.E. (1964) Chronic toxicity of ethylene oxide. *Arch. environ. Health*, **8**, 700–710

Kao, C.-Y. & Giese, R.W. (2005) Measurement of N7-(2'-hydroxyethyl)guanine in human DNA by gas chromatography electron capture mass spectrometry. *Chem. Res. Toxicol.*, **18**, 70–75

Kardos, L., Széles, G., Gombkötö, G., Szeremi, M., Tompa, A. & Ádány, R. (2003) Cancer deaths among hospital staff potentially exposed to ethylene oxide: An epidemiological analysis. *Environ. mol. Mutag.*, **42**, 59–60

Karelová, J., Jablonická, A. & Vargová, M. (1987) Results of cytogenetic testing of workers exposed to ethylene oxide. *J. Hyg. Epidemiol. Microbiol. Immunol.*, **31**, 119–126

Katoh, T., Higashi, K., Inoue, N. & Tanaka, I. (1989) Lipid peroxidation and the metabolism of glutathione in rat liver and brain following ethylene oxide inhalation. *Toxicology*, **58**, 1–9

Katoh, T., Higashi, K., Inoue, N. & Tanaka, I. (1990) Different responses of cytosolic and mitochondrial glutathione in rat livers after ethylene oxide exposure. *Toxicol. Lett.*, **54**, 235–239

Kauppinen, T., Toikkanen, J., Pedersen, D., Young, R., Ahrens, W., Boffetta, P., Hansen, J., Kromhout, H., Maqueda Blasco, J., Mirabelli, D., de la Orden-Rivera, V., Pannett, B., Plato, N., Savela, A., Vincent, R. & Kogevinas, M. (2000) Occupational exposure to carcinogens in the European Union. *Occup. environ. Med.*, **57**, 10–18 [data partially available on the CAREX web site: http://www.ttl.fi/NR/rdonlyres/407B368B-26EF-475D-8F2B-DA0024B853E0/0/5_exposures_by_agent_and_industry.pdf]

Kautiainen, A. & Törnqvist, M. (1991) Monitoring exposure to simple epoxides and alkenes through gas chromatographic determination of hemoglobin adducts. *Int. Arch. occup. environ. Health*, **63**, 27–31

Kelsey, K.T., Wiencke, J.K., Eisen, E.A., Lynch, D.W., Lewis, T.R. & Little, J.B (1988) Persistently elevated sister chromatid exchanges in ethylene oxide-exposed primates: The role of a sub-population of high frequency cells. *Cancer Res.*, **48**, 5045–5050

Kercher, S.L. & Mortimer, V.D. (1987) Before and after: An evaluation of engineering controls for ethylene oxide sterilization in hospitals. *Appl. ind. Hyg.*, **2**, 7–12

Kiesselbach, N., Ulm, K., Lange, H.J. & Korallus, U. (1990) A multicentre mortality study of workers exposed to ethylene oxide. *Br. J. ind. Med.*, **47**, 182–188

Kligerman, A.D., Erexson, G.L., Phelps, M.E. & Wilmer, J.L. (1983) Sister-chromatid exchange induction in peripheral blood lymphocytes of rats exposed to ethylene oxide by inhalation. *Mutat. Res.*, **120**, 37–44

Koda, S., Kumagai, S. & Ohara, H. (1999) Environmental monitoring and assessment of short-term exposures to hazardous chemicals of a sterilization process in hospital working environments. *Acta med. Okayama*, **53**, 217–223

Koga, M., Hori, H., Tanaka, I., Akiyama, T. & Inoue, N. (1985) [Quantitative analysis of urinary ethylene glycol in rats exposed to ethylene oxide]. *J. UOEH.*, **7**, 45–49 (in Japanese)

Koga, M., Hori, H., Tanaka, I., Akiyama, T. & Inoue, N. (1987) [Analysis of urinary metabolites of rats exposed to ethylene oxide]. *J. UOEH.*, **9**, 167–170 (in Japanese)

Kolman, A., Näslund, M. & Calleman, C.J. (1986) Genotoxic effects of ethylene oxide and their relevance to human cancer. *Carcinogenesis*, **7**, 1245–1250

Kolman, A., Näslund, M., Osterman-Golkar, S., Scalia-Tomba, G.-P. & Meyer, A. (1989) Comparative studies of in vitro transformation by ethylene oxide and gamma-radiation of C3H/10T1/2 cells. *Mutagenesis*, **4**, 58–61

Kolman, A., Bohušová, T., Lambert, B. & Simons, J.W.I.M. (1992) Induction of 6-thioguanine-resistant mutants in human diploid fibroblasts *in vitro* with ethylene oxide. *Environ. mol. Mutag.*, **19**, 93–97

Kolman, A., Chovanec, M., & Osterman-Golkar, S. (2002) Genotoxic effects of ethylene oxide, propylene oxide and epichlorohydrin in humans: Update review (1990–2001). *Mutat. Res.*, **512**, 173–194

Kreuzer, P.E. (1992) [Permeation Kinetics of Ethylene Oxide in Gaseous Form and Dissolved Other Matrices Through the Skin of Rats, Hamsters and Humans] (GSF-Bericht 19/92), Neuherberg, GSF-Forschungszentrum für Umwelt und Gesundheit (in German)

Kring, E.V., Damrell, D.J., Basilio, A.N., Jr, McGibney, P.D., Douglas, J.J., Henry, T.J. & Ansul, G.R. (1984) Laboratory validation and field verification of a new passive air monitoring badge for sampling ethylene oxide in air. *Am. ind. Hyg. Assoc. J.*, **45**, 697–707

Krishnan, K., Gargas, M.L., Fennell, T.R. & Andersen, M.E. (1992) A physiologically based description of ethylene oxide dosimetry in the rat. *Toxicol. ind. Health*, **8**, 121–140

Lacson, J. (2003) *CEH Marketing Research Report — Ethylene Oxide*, Zurich, SRI Consulting

La Montagne, A.D., Oakes, J.M. & Lopez Turley, R.N. (2004) Long-term ethylene oxide exposure trends in US hospitals: Relationship with OSHA regulatory and enforcement actions. *Am. J. public Health*, **94**, 1614–1619

Latif, F., Moschel, R.C., Hemminki, K. & Dipple, A. (1988) Styrene oxide as a stereochemical probe for the mechanism of aralkylation at different sites on guanosine. *Chem. Res. Toxicol.*, **1**, 364–369

Laurent, C., Frederic, J. & Leonard, A.Y. (1984) Sister chromatid exchange frequency in workers exposed to high levels of ethylene oxide in a hospital sterilization service. *Int. Arch. occup. environ. Health*, **54**, 33–43

Lerda, D. & Rizzi, R. (1992) Cytogenetic study of persons occupationally exposed to ethylene oxide. *Mutat. Res.*, **281**, 31–37

Lewis, S.E., Barnett, L.B., Felton, C., Johnson, F.M., Skow, L.C., Cacheiro, N. & Shelby, M.D. (1986) Dominant visible and electrophoretically expressed mutations induced in male mice exposed to ethylene oxide by inhalation. *Environ. Mutag.*, **8**, 867–872

Li, F., Segal, A. & Solomon, J.J. (1992) In vitro reaction of ethylene oxide with DNA and characterization of DNA adducts. *Chem.-biol. Interact.*, **83**, 35–54

Liao, P.-C., Li, C.-M., Hung, C.-W. & Chen S.-H. (2001) Quantitative detection of N^7-(2-hydroxyethyl)guanine adducts in DNA using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *J. mass Spectrom.*, **36**, 336–343

Lide, D.R., ed. (2005) *CRC Handbook of Chemistry and Physics*, 86th Ed., Boca Raton, FL, CRC Press, pp. 3–408

Lindahl, T. (1979) DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision-repair. *Prog. Nucleic Acid Res. mol. Biol.*, **22**, 135–192

Liou, S.H., Lung, J.C., Chen, Y.H., Yang, T., Hsieh, L.L., Chen, C.J. & Wu, T.N. (1999) Increased chromosome-type chromosome aberration frequencies as biomarkers of cancer risk in a blackfoot endemic area. *Cancer Res.*, **59**, 1481–1484

Loeb, L.A. & Preston, B.D. (1986) Mutagenesis by apurinic/apyrimidinic sites. *Ann. Rev. Genet.*, **20**, 201–230

Lorenti Garcia, C., Darroudi, F., Tates, A.D. & Natarajan, A.T. (2001) Induction and persistence of micronuclei, sister-chromatid exchanges and chromosomal aberrations in splenocytes and bone-marrow cells of rats exposed to ethylene oxide. *Mutat. Res*., **492**, 59–67

Lovegren, B.C. & Koketsu, M. (1977a) *BASF-Wyandotte Corporation, Geismar, Louisiana, Task II, Ethylene Oxide Survey Report of the Plant Contact, June 27–28, 1977* (PB81-229775), Springfield, VA, National Technical Information Service

Lovegren, B.C. & Koketsu, M. (1977b) *Union Carbide Corporation, Institute, West Virginia, Task II, Ethylene Oxide Survey Report of the Plant Contact, July 15–16, 1977* (PB82-106709), Springfield, VA, National Technical Information Service

Lovegren, B.C. & Koketsu, M. (1977c) *Union Carbide Corporation, Texas City, Texas, Task II, Ethylene Oxide Survey Report of the Plant Contact, June 8–9, 1977* (PB82-108218), Springfield, VA, National Technical Information Service

Lucas, L.J. & Teta, M.J. (1996) Breast cancer and ethylene oxide exposure. *Int. J. Epidemiol*., **25**, 685–686

Ludeke, B.I. & Kleihues, P. (1988) Formation and persistence of O^6-(2-hydroxyethyl)-2′-deoxyguanosine in DNA for various rat tissues following a single dose of *N*-nitroso-*N*-(2-hydroxyethyl)urea. An immuno-slot-blot study. *Carcinogenesis*, **9**, 147–151

Lynch, D.W., Lewis, T.R., Moorman, W.J., Burg, J.R., Groth, D.H., Khan, A., Ackerman, L.J. & Cockrell, B.Y. (1984a) Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. *Toxicol. appl. Pharmacol*., **76**, 69–84

Lynch, D.W., Lewis, T.R., Moorman, W.J., Burg, J.R., Gulati, D.K., Kaur, P. & Sabharwal, P.S. (1984b) Sister-chromatid exchanges and chromosome aberrations in lymphocytes from monkeys exposed to ethylene oxide and propylene oxide by inhalation. *Toxicol. appl. Pharmacol*., **76**, 85–95

Major, J., Jakab, M.G. & Tompa, A. (1996) Genotoxicological investigation of hospital nurses occupationally exposed to ethylene-oxide: I. Chromosome aberrations, sister-chromatid exchanges, cell cycle kinetics, and UV-induced DNA synthesis in peripheral blood lymphocytes. *Environ. mol. Mutag.*, **27**, 84–92

Major, J., Jakab, M.G. & Tompa, A. (1999) The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. *Mutat. Res.*, **445**, 241–249

Maples, K.R. & Dahl, A.R. (1993) Levels of epoxides in blood during inhalation of alkenes and alkenes oxides. *Inhal. Toxicol*., **5**, 43–54

Margeson, J.H., Steger, J.L. & Homolya, J.B. (1990) Chromatographic methods for analysis of ethylene oxide in emissions from stationary sources. *J. chromatogr. Sci*., **28**, 204–209

Marlowe, D.E., Lao, N.T., Eaton, A.R., Page, B.F.J. & Lao, C.S. (1987) Interlaboratory comparison of analytical methods for residual ethylene oxide in medical device materials. *J. pharm. Sci*., **76**, 333–337

Marnett, L.J. (2002) Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, **181–182**, 219–222

Marsden, D.A., Jones, D.J.L., Lamb, J.H., Tompkins, E.M., Farmer, P.B. & Brown, K. (2007) Determination of endogenous and exogenously derived N7-(2-hydroxyethyl)guanine adducts in ethylene oxide-treated rats. *Chem. Res. Toxicol*., **20**, 290–299

Martis, L., Kroes, R., Darby, T.D., & Woods, E.F. (1982) Disposition kinetics of ethylene oxide, ethylene glycol, and 2-chlorethanol in the dog. *J. Toxicol. environ. Health*, **10**, 847–856

Mayer, J., Warburton, D., Jeffrey, A.M., Pero, R., Walles, S., Andrews, L., Toor, M., Latriano, L., Wazneh, L., Tang, D., Tsai, W.-Y., Kuroda, M. & Perera, F. (1991) Biologic markers in ethylene oxide-exposed workers and controls. *Mutat. Res*., **248**, 163–176

McCammon, J., Orgel, D. & Hill, B. (1990) *Health Hazard Evaluation Report, A.E. Staley Manufacturing Co., Decatur, IL* (Report No. HETA-88-348-2081), Cincinnati, OH, National Institute for Occupational Safety and Health

McKelvey, J.A. & Zemaitis, M.A. (1986) The effects of ethylene oxide (EO) exposure on tissue glutathione levels in rats and mice. *Drug chem. Toxicol*., **9**, 51–66

Morgan, R.W., Claxton, K.W., Divine, B.J., Kaplan, S.D. & Harris, V.B. (1981) Mortality among ethylene oxide workers. *J. occup. Med*., **23**, 767–770

Mortimer, V.D., Jr & Kercher, S.L. (1989) *Control Technology for Ethylene Oxide Sterilization in Hospitals* (NIOSH Publ. No. 89–120), Cincinnati, OH, National Institute for Occupational Safety and Health

Mouilleseaux, A., Laurent, A.-M., Fabre, M., Jouan, M. & Festy, B. (1983) [Atmospheric levels of ethylene oxide in the occupational environment of sterilization and disinfection facilities.] *Arch. Mal. prof*., **44**, 1–14 (in French)

Müller, M., Krämer, A., Angerer, J. & Hallier, E. (1998) Ethylene oxide–protein adduct formation in humans: Influence of glutathione-S-transferase polymorphisms. *Int. Arch. occup. environ. Health*, **71**, 499–502

Nakashima, K., Furutani, A., Higashi, K., Okuno, F. & Inoue, N. (1987) Glutathione contents in rat livers after acute and chronic exposure to ethylene oxide. *J. UOEH*, **9**, 355–359

Natarajan, A.T., Preston, R.J., Dellarco, V., Ehrenberg, L., Generoso, W., Lewis, S. & Tates, A.D. (1995) Ethylene oxide: Evaluation of genotoxicity data and an exploratory assessment of genetic risk. *Mutat. Res*., **330**, 55–70

National Institute for Occupational Safety and Health (1987) *NIOSH Manual of Analytical Methods*, 3rd Ed., Suppl. 2 (DHHS (NIOSH) Publ. No. 84–100), Washington DC, US Government Printing Office, pp. 1614-1–1614-6

National Institute for Occupational Safety and Health (1998) *NIOSH Manual of Analytical Methods*, 4th Ed., Suppl. 2 (DHHS (NIOSH) Publ. No. 98–119), Washington DC, US Government Printing Office

National Library of Medicine (2005) *Hazardous Substances Data Bank Database*, Bethesda, MD [available at http://toxnet.nlm.nih.gov/cgi-bin/sis/search]

National Library of Medicine (2006) *Toxic Chemical Release Inventory* (*TRI*) *Data Banks* [TRI87, TRI88, TRI89, TRI90, TRI91, TRI05], Bethesda, MD

National Toxicology Program (1987) *Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F$_1$ Mice* (*Inhalation Studies*) (NTP Technical Report No. 326; NIH Publication No. 88-2582), Research Triangle Park, NC

Newman, M.A. & Freund, E. (1989) *Health Hazard Evaluation Report, Washington Hospital, Washington, PA* (Report No. HETA 89-006-2002), Cincinnati, OH, National Institute for Occupational Safety and Health

NOES (1993) *National Occupational Exposure Survey (1981–1983)*, Cincinnati, OH, National Institute for Occupational Safety and Health

Norman, S.A., Berlin, J.A., Soper, K.A., Middendorf, B.F. & Stolley, P.D. (1995) Cancer incidence in a group of workers potentially exposed to ethylene oxide. *Int. J. Epidemiol.*, **24**, 276–284

Norman, S.A., Berlin, J.A., Soper, K.A., Middendorf, B.F. & Stolley, P.D. (1996) Authors' response: Cancer incidence in a group of workers potentially exposed to ethylene oxide. *Int. J. Epidemiol.*, **25**, 686

Nygren, J. Cedervall, B., Eriksson, S., Dušinská, M. & Kolman, A. (1994) Induction of DNA strand breaks by ethylene oxide in human diploid fibroblasts. *Environ. mol. Mutag*., **24**, 161–167

O'Neil, M.J., ed. (2006) *Merck Index*, 14th Ed., Whitehouse Station, NJ, Merck, p. 651

Occupational Safety and Health Administration (2005) *Regulatory Review of the Occupational Safety and Health Administration's Ethylene Oxide Standard* (29 CFR 1910.1047), Washington DC

Olsen, G.W., Lacy, S.E., Bodner, K.M., Chau, M., Arceneaux, T.G., Cartmill, J.B., Ramlow, J.M. & Boswell, J.M. (1997) Mortality from pancreatic and lymphopoietic cancer among workers in ethylene and propylene chlorohydrin production. *Occup. environ. Med.*, **54**, 592–598

Ong, T., Bi, H.-K., Xing, S., Stewart, J. & Moorman, W. (1993) Induction of sister chromatid exchange in spleen and bone marrow cells of rats exposed by inhalation to different dose rates of ethylene oxide. *Environ. mol. Mutag*., **22**, 147–151

Oser, J.L., Crandall, M., Phillips, R. & Marlow, D. (1978a) *Indepth Industrial Hygiene Report of Ethylene Oxide Exposure at Union Carbide Corporation, Institute, West Virginia* (PB82-114786), Springfield, VA, National Technical Information Service

Oser, J.L., Crandall, M. & Rinsky, R. (1978b) *Industrial Hygiene Survey of Dow Chemical Company, Plaquemine, Louisiana* (PB81-229924), Springfield, VA, National Technical Information Service

Oser, J.L., Young, M., Boyle, T. & Marlow, D. (1979) *Indepth Industrial Hygiene Report of Ethylene Oxide Exposure at Union Carbide Corporation, South Charleston, West Virginia* (PB82-110024), Springfield, VA, National Technical Information Service

Osterman-Golkar, S. & Bergmark, E. (1988) Occupational exposure to ethylene oxide. Relation between in vivo dose and exposure dose. *Scand. J. Work Environ. Health*, **14**, 372–377

Osterman-Golkar, S., Farmer, P.B., Segerbäck, D., Bailey, E., Calleman, C.J., Svensson, K. & Ehrenberg, L. (1983) Dosimetry of ethylene oxide in the rat by quantitation of alkylated histidine in hemoglobin. *Teratog. Carcinog. Mutag*., **3**, 395–405

Ott, M.G., Teta, M.J. & Greenberg, H.L. (1989) Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. *Am. J. ind. Med.*, **16**, 631–643

Pauwels, W. & Veulemans, H. (1998) Comparison of ethylene, propylene, and styrene 7,8-oxide in vitro adduct formation on N-terminal valine in human haemoglobin and on N-7-guanine in human DNA. *Mutat. Res.*, **418**, 21–33

Pedersen-Bjergaard, J. & Rowley, J.D. (1994) The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. *Blood*, **83**, 2780–2786

Pedersen-Bjergaard, J., Christiansen, D.H., Desta, F. & Andersen, M.K. (2006) Alternative genetic pathways and cooperating genetic abnormalities in the pathogenesis of therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia*, **20**, 1943–1949

Pero, R.W., Widegren, B., Högstedt, B. & Mitelman, F. (1981) In vivo and in vitro ethylene oxide exposure of human lymphocytes assessed by chemical stimulation of unscheduled DNA synthesis. *Mutat. Res.*, **83**, 271–289

Pero, R.W., Bryngelsson, T., Widegren, B., Högstedt, B. & Welinder, H. (1982) A reduced capacity for unscheduled DNA synthesis in lymphocytes from individuals exposed to propylene oxide and ethylene oxide. *Mutat. Res.*, **104**, 193–200

Picut, C.A., Aoyama, H., Holder, J.W., Gold, L.S., Maronpot, R.R. & Dixon, D. (2003) Bromoethane, chloroethane and ethylene oxide induced uterine neoplasms in B6C3F1 mice from 2-year NTP inhalation bioassays: Pathology and incidence data revisited. *Exp. Toxicol. Pathol.*, **55**, 1–9

Poirier, V. & Papadopoulo, D. (1982) Chromosomal aberrations induced by ethylene oxide in a human amniotic cell line *in vitro*. *Mutat. Res.*, **104**, 255–260

Popp, W., Wahrenholz, C., Przygoda, H., Brauksiepe, A., Goch, S., Műller, G., Schell, C. & Norporth, K. (1994) DNA–protein cross-links and sister chromatid exchange frequencies in lymphocytes and hydroxyethyl mercapturic acid in urine of ethylene oxide-exposed hospital workers. *Int. Arch. occup. environ. Health*, **66**, 325–332

Potter, D., Blair, D., Davies, R., Watson, W.P. & Wright, A.S. (1989) The relationships between alkylation of haemoglobin and DNA in Fischer 344 rats exposed to [^{14}C]ethylene oxide. *Arch. Toxicol.*, **Suppl. 13**, 254–257

Preston, R.J. (1999) Cytogenetic effects of ethylene oxide, with an emphasis on population monitoring. *Crit. Rev. Toxicol.*, **29**, 263–282

Preston, R.J. & Abernethy, D.J. (1993) Studies on the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation. In: Sorsa, M., Peltonen, K., Vainio, N. & Hemminki, K, eds, *Butadiene and Styrene: Assessment of Health Hazards* (IARC Scientific Publications No. 127), Lyon, IARC, pp. 225–233

Puskar, M.A. & Hecker, L.H. (1989) Field validation of passive dosimeters for the determination of employee exposures to ethylene oxide in hospital product sterilization facilities. *Am. ind. Hyg. Assoc. J.*, **50**, 30–36

Puskar, M.A., Nowak, J.L. & Hecker, L.H. (1990) Generation of ethylene oxide permissible exposure limit data with on-site sample analysis using the EO Self-Scan™ passive monitor. *Am. ind. Hyg. Assoc. J.*, **51**, 273–279

Puskar, M.A., Szopinski, F.G. & Hecker, L.H. (1991) Development and validation of a protocol for field validation of passive dosimeters for ethylene oxide excursion limit monitoring. *Am. ind. Hyg. Assoc. J.*, **52**, 145–150

Rebsdat, S. & Mayer, D. (2005) Ethylene oxide. In: *Ullmann's Encyclopedia of Industrial Chemistry*, 7th Ed., Weinheim, Wiley–VCH Publishers (on line)

Recio, L., Donner, M., Abernethy, D., Pluta, L., Steen, A.-M., Wong, B.A., James, A. & Preston, R.J. (2004) In vivo mutagenicity and mutation spectrum in the bone marrow and testes of B6C3F1 lacI transgenic mice following inhalation exposure to ethylene oxide. *Mutagenesis*, **19**, 215–222

Ribeiro, L.R., Rabello-Gay, M.N., Salvadori, D.M.F., Pereira, C.A.B. & Beçak, W. (1987a) Cytogenetic effects of inhaled ethylene oxide in somatic and germ cells of mice. *Arch. Toxicol.*, **59**, 332–335

Ribeiro, L.R., Salvadori, D.M.F., Pereira, C.A.B. & Beçak, W. (1987b) Activity of ethylene oxide in the mouse sperm morphology test. *Arch. Toxicol.*, **60**, 331–333

Ribeiro, L.R., Salvadori, D.M.F., Rios, A.C.C., Costa, S.L., Tates, A.D., Törnqvist, M. & Natarajan, A.T. (1994) Biological monitoring of workers occupationally exposed to ethylene oxide. *Mutat. Res.*, **313**, 81–87

Richmond, G.W., Abrahams, R.H., Nemenzo, J.H. & Hine, C.H. (1985) An evaluation of possible effects on health following exposure to ethylene oxide. *Arch. environ. Health*, **40**, 20–25

Russell, I.B., Cumming, R.B. & Hunsicker, P.R. (1984) Specific-locus mutation rates in the mouse following inhalation of ethylene oxide and application of the results to estimation of human genetic risk. *Mutat. Res.*, **129**, 381–388

Rusyn, I., Asakura, S., Li, Y., Kosyk, O., Koc, H., Nakamura, J., Upton, P.B. & Swenberg, J.A. (2005) Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair gene in rat brain, spleen, and liver. *DNA Repair*, **4**, 1099–1110

Saalo, A., Soosaar, A., Vuorela, R. & Kauppinen, T. (2006) [ASA 2004], Helsinki, Finnish Institute of Occupational Health. [available at: http://www.ttl.fi/NR/rdonlyres/5A54A452-7350-4255-8DF3-AF632D9D2775/0/ASA_2004.pdf] (in Finnish)

Sadtler Research Laboratories (1991) *Sadtler Standard Spectra. 1981–1991 Supplementary Index*, Philadelphia, PA

Sangster, J. (1989) Octanol–water partition coefficients of simple organic compounds. *J. phys. chem. Ref. Data*, **18**, 1144

Sarto, F., Cominato, I., Pinton, A.M., Brovedani, P.G., Faccioli, C.M., Bianchi, V. & Levis, A.G. (1984a) Workers exposed to ethylene oxide have increased incidence of sister chromatid exchange. In: Berlin, A., Draper, M., Hemminki, K. & Vainio, H., eds, *Monitoring Human Exposure to Carcinogenic and Mutagenic Agents* (IARC Scientific Publications No. 59), Lyon, IARC, pp. 413–419

Sarto, F., Cominato, I., Pinton, A.M., Brovedani, P.G., Faccioli, C.M., Bianchi, V. & Levis, A.G. (1984b) Cytogenetic damage in workers exposed to ethylene oxide. *Mutat. Res.*, **138**, 185–195

Sarto, F., Clonfero, E., Bartolucci, G.B., Franceschi, C., Chiricolo, M. & Levis, A.G. (1987) Sister chromatid exchanges and DNA repair capability in sanitary workers exposed to ethylene oxide: evaluation of the dose–effect relationship. *Am. J. ind. Med.*, **12**, 625–637

Sarto, F., Tomanin, R., Giacomelli, L., Iannini, G. & Cupiraggi, A.R. (1990) The micronucleus assay in human exfoliated cells of the nose and mouth: Application to occupational exposures to chromic acid and ethylene oxide. *Mutat. Res.*, **244**, 345–351

Sarto, F., Törnqvist, M.A., Tomanin, R., Bartolucci, G.B., Osterman-Golkar, S.M. & Ehrenberg, L. (1991) Studies of biological and chemical monitoring of low-level exposure to ethylene oxide. *Scand. J. Work Environ. Health*, **17**, 60–64

Scheick, C., Spiteller, G., & Dasenbrock, C. (1997) Thiodiacetic acid—a metabolite of ethylene oxide. *Z. Naturforsch.*, **52C**, 70–76

Schettgen, T., Broding, H.C., Angerer, J. & Drexler, H. (2002) Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. *Toxicol. Lett.*, **134**, 65–70

Schulte, P.A., Boeniger, M., Walker, J.T., Schober, S.E., Pereira, M.A., Gulati, D.K., Wojciechowski, J.P., Garza, A., Froelich, R., Strauss, G., Halperin, W.E., Herrick, R. & Griffith, J. (1992) Biologic markers in hospital workers exposed to low levels of ethylene oxide. *Mutat. Res.*, **278**, 237–251

Schulte, P.A., Walker, J.T., Boeniger, M.F., Tsuchiya, Y. & Halperin, W.E. (1995) Molecular, cytogenetic, and hematologic effects of ethylene oxide on female hospital workers. *J. occup. med. Health*, **37**, 313–320

Sega, G.A. & Generoso, E.E. (1988) Measurement of DNA breakage in spermiogenic germ-cell stages of mice exposed to ethylene oxide, using an alkaline elution procedure. *Mutat. Res.*, **197**, 93–99

Sega, G.A., Generoso, E.E. & Brimer, P.A. (1988) Inhalation exposure-rate of ethylene oxide affects the level of DNA breakage and unscheduled DNA synthesis in spermiogenic stages of the mouse. *Mutat. Res.*, **209**, 177–180

Sega, G.A., Brimer, P.A. & Generoso, E.E. (1991) Ethylene oxide inhalation at different exposure-rates affects binding levels in mouse germ cells and hemoglobin. Possible explanation for the effect. *Mutat. Res.*, **249**, 339–349

Segerbäck, D. (1990) Reaction products in hemoglobin and DNA after in vitro treatment with ethylene oxide and *N*-(2-hydroxyethyl)-*N*-nitrosourea. *Carcinogenesis*, **11**, 307–312

Segerbäck, D. (1994) DNA alkylation by ethylene oxide and some mono-substituted epoxides. In: Hemminki, K., Dipple, A., Shuker, D.E.G., Kadlubar, F.F., Segerbäck, D. & Bartsch, H., eds, *DNA Adducts: Identification and Biological Significance* (IARC Scientific Publications No. 125), Lyon, IARC, pp. 37–47

Shell Chemicals (2005) *MSDS Ethylene Oxide 28.3.2005* [available at http://shellchemicals.com/msds/1,1098,1136,00.html accessed 30/10/07]

Shore, R.E., Gardner, M.J. & Pannett, B. (1993) Ethylene oxide: An assessment of the epidemiological evidence on carcinogenicity. *Br. J. ind. Med.*, **50**, 971–997

Shults, R.A. & Seitz, T.A. (1992) *Health Hazard Evaluation Report, Valley Hospital, Palmer, Alaska* (Report No. HETA 91-293-2203), Cincinnati, OH, National Institute for Occupational Safety and Health

Sisk, S.C., Pluta, L.J., Meyer, K.G., Wong, B.C. & Recio, L. (1997) Assessment of the in vivo mutagenicity of ethylene oxide in the tissues of B6C3F1 *lacI* transgenic mice following inhalation exposure. *Mutat. Res.*, **391**, 153–164

van Sittert, N.J., de Jong, G., Clare, M.G., Davies, R., Dean, B.J., Wren, L.J. & Wright, A.S. (1985) Cytogenetic, immunological, and haematological effects in workers in an ethylene oxide manufacturing plant. *Br. J. ind. Med.*, **42**, 19–26

van Sittert, N.J., Beulink, G.D.J., van Vliet, E.W.N. & van der Waal, H. (1993) Monitoring occupational exposure to ethylene oxide by the determination of hemoglobin adducts. *Environ. Health Perspect.*, **99**, 217–220

van Sittert, N.J., Boogaard, P.J., Natarajan, A.T., Tates, A.D., Ehrenberg, L.G. & Törnqvist, M.A. (2000) Formation of DNA adducts and induction of mutagenic effects in rats following 4 weeks inhalation exposure to ethylene oxide as a basis for cancer risk assessment. *Mutat. Res.*, 447, 27–48

Smerhovsky, Z., Landa, K., Rössner, P., Brabec, M., Zudova, Z., Hola, N., Pokorna, Z., Mareckova, J. & Hurychova, D. (2001) Risk of cancer in an occupationally exposed cohort with increased level of chromosomal aberrations. *Environ. Health Perspect.*, **109**, 41–45

Snellings, W.M., Weil, C.S. & Maronpot, R.R. (1984) A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. *Toxicol. appl. Pharmacol.*, **75**, 105–117

Sobaszek, A., Hache, J.C., Frimat, P., Akakpo, V., Victoire, G. & Furon D. (1999) Working conditions and health effects of ethylene oxide exposure at hospital sterilization sites. *J. occup. environ. Med.*, **41**, 492–499

Solomon, J. (1999) Cyclic adducts and intermediates induced by simple epoxides. In: Singer, B. & Bartsch, H., eds, *Exocyclic Adducts in Mutagenesis and Carcinogenesis* (IARC Scientific Publications No. 150), Lyon, IARC, pp. 123–125

Sorsa, M., Ojajärvi, A. & Salomaa, S. (1990) Cytogenetic surveillance of workers exposed to genotoxic chemicals: Preliminary experiences from a prospective cancer study in a cytogenetic cohort. *Teratog. Carcinog. Mutag.*, **10**, 215–221

Star, E.G. (1980) [Mutagenic and cytotoxic effect of ethylene oxide on human cell cultures.] *Zbl. Bakt. Hyg. I. Abt. Orig. B*, **170**, 548–556 (in German)

Stayner, L., Steenland, K., Greife, A., Hornung, R., Hayes, R.B., Nowlin, S., Morawetz, J., Ringenburg, V., Elliot, L. & Halperin, W. (1993) Exposure–response analysis of cancer mortality in a cohort of workers exposed to ethylene oxide. *Am. J. Epidemiol*., **138**, 787–798

Steenland, K. & Stayner, L. (1993) An epidemiological study of workers potentially exposed to ethylene oxide. *Br. J. ind. Med.*, **50**, 1125–1126

Steenland, K., Stayner, L., Greife, A., Halperin, W., Hayes, R., Hornung, R. & Nowlin, S. (1991) Mortality among workers exposed to ethylene oxide. *New Engl. J. Med*., **324**, 1402–1407

Steenland, K., Whelan, E., Deddens, J., Stayner, L. & Ward, E. (2003) Ethylene oxide and breast cancer incidence in a cohort study of 7576 women (United States). *Cancer Causes Control*, **14**, 531–539

Steenland, K., Stayner, L. & Deddens, J. (2004) Mortality analyses in a cohort of 18 235 ethylene oxide exposed workers: Follow up extended from 1987 to 1998. *Occup. environ. Med*., **61,** 2–7

Steger, J. (1989) *Analytical Method Evaluation for Measuring Ethylene Oxide Emissions from Commercial Dilute-acid Hydrolytic Control Units* (EPA Report No. EPA-600/3-89-016; US NTIS PB89-155253), Research Triangle Park, NC, US Environmental Protection Agency, Office of Research and Development

Stolley, P.D., Soper, K.A., Galloway, S.M., Nichols, W.W., Norman, S.A. & Wolman, S.R. (1984) Sister-chromatid exchanges in association with occupational exposure to ethylene oxide. *Mutat. Res.*, **129**, 89–102

Swaen, G.M.H., Slangen, J.M.M., Ott, M.G., Kusters, E., Van Den Langenbergh, G., Arends, J.W. & Zober, A. (1996) Investigation of a cluster of ten cases of Hodgkin's disease in an occupational setting. *Int. Arch. occup. environ. Health*, **68**, 224–228

Swenberg, J.A., Fedtke, N., Fennell, T.R. & Walker, V.E. (1990) Relationships between carcinogen exposure, DNA adducts and carcinogenesis. In: Clayson, D.B., Munro, I.C., Shubik, P. & Swenberg, J.A. eds, *Progress in Predictive Toxicology*, Amsterdam, Elsevier Scientific Publications, pp. 161–184

Szopinski, F.G., Puskar, M.A. & Hecker, L.H. (1991) Field validation of three passive dosimeters for excursion limit monitoring of ethylene oxide. *Am. ind. Hyg. Assoc. J*., **52**, 151–157

Sociale Zaken & Werkgelegenheid (2006) [Modification of the rules concerning work conditions.] *Off. State J*., **28 December, 252**, 23 (in Dutch)

Takeshita, M., Chang, C.-N., Johnson, F., Will, S. & Grollman, A.P. (1987) Oligodeoxynucleotides containing synthetic abasic sites. *J. biol. Chem*., **262**, 10171–10179

Tan, E.-L., Cumming, R.B. & Hsie, A.W. (1981) Mutagenicity and cytotoxicity of ethylene oxide in the CHO/HGPRT system. *Environ. Mutag*., **3**, 683–686

Tardif, R., Goyal, R., Brodeur, J., & Gérin, M. (1987) Species differences in the urinary disposition of some metabolites of ethylene oxide. *Fundam. appl. Toxicol*., **9**, 448–453

Tates, A.D., Grummt, T., Törnqvist, M., Farmer, P.B., van Dam, F.J., van Mossel, H., Schoemaker, H.M., Osterman-Golkar, S., Uebel, C., Tang, Y.S., Zwinderman, A.H., Natarajan, A.T. & Ehrenberg, L. (1991) Biological and chemical monitoring of occupational exposure to ethylene oxide. *Mutat. Res.*, **250**, 483–497

Tates, A.D., Boogaard, P.J., Darroudi, F., Natarajan, A.T., Caubo, M.E. & van Sittert, N.J. (1995) Biological effect monitoring in industrial workers following incidental exposure to high concentrations of ethylene oxide. *Mutat. Res.*, **329**, 63–77

Tates, A.D., van Dam, F.J., Natarajan, A.T., van Teylingen, C.M.M., de Zwart, F.A., Zwinderman, A.H., van Sittert, N.J., Nilsen, A., Nilsern, O.G., Zahlsen, K., Magnusson, A.-L. & Törnqvist, M. (1999) Measurement of HPRT mutations in splenic lymphocytes and haemoglobin adducts in erythrocytes of Lewis rats exposed to ethylene oxide. *Mutat. Res.*, **431**, 397–415

Tavares, R., Ramos, P., Palminha, J., Bispo, M.A., Paz, I., Bras, A., Rueff, J., Farmer, P.B. & Bailey, E. (1994) Transplacental exposure to genotoxins. Evaluation in haemoglobin of hydroxyethylvaline adduct levels in smoking and non-smoking mothers and their newborns. *Carcinogenesis*, **15**, 1271–1274

Teta, M.J., Benson, L.O. & Vitale, J.N. (1993) Mortality study of ethylene oxide workers in chemical manufacturing: A 10 year update. *Br. J. ind. Med.*, **50**, 704–709

Teta, M.J., Sielken, R.L., Jr & Valdez-Flores, C. (1999) Ethylene oxide cancer risk assessment based on epidemiological data: Application of revised regulatory guidelines. *Risk Anal.*, **19**, 1135–1155

Thier, R. & Bolt, H.M. (2000) Carcinogenicity and genotoxicity of ethylene oxide: New aspects and recent advances. *Crit. Rev. Toxicol.*, **30**, 595–608

Thier, R., Balkenhol, H., Lewalter, J., Selinski, S., Dommermuth, A. & Bolt, H.M. (2001) Influence of polymorphisms of the human glutathione transferases and cytochrome P450 2E1 enzyme on the metabolism and toxicity of ethylene oxide and acrylonitrile. *Mutat. Res.*, **482**, 41–46

Thiess, A.M., Schwegler, H., Fleig, I. & Stocker, W.G. (1981) Mutagenicity study of workers exposed to alkylene oxides (ethylene oxide/propylene oxide) and derivatives. *J. occup. Med.*, **23**, 343–347

Tomkins, D.J., Haines, T., Lawrence, M. & Rosa, N. (1993) A study of sister chromatid exchange and somatic cell mutation in hospital workers exposed to ethylene oxide. *Environ. Health Perspect.*, **101** (Suppl. 3), 159–164

Tompa, A., Major, J. & Jakab, M.G. (1999) Is breast cancer cluster influenced by environmental and occupational factors among hospital nurses in Hungary? *Pathol. oncol. Res.*, **5**, 117–121

Tucker, S.P. & Arnold, J.E. (1984) *Evaluation of OSHA Method No. 30 for Ethylene Oxide in Air with 400-mg/200-mg Charcoal Tubes* (NIOSH Report No. 84/02/00; US NTIS PB84-242049), Cincinnati, OH, National Institute for Occupational Safety and Health

Tucker, J.D., Xu, J., Stewart, J., Baciu, P.C., & Ong, T.M. (1986) Detection of sister chromatid exchanges induced by volatile genotoxicants. *Teratog. Carcinog. Mutag.*, **6**, 15–21

US Census (1980) [http://www2.census.gov/prod2/decennial/documents/1980a_vsC-01.pdf]

Van Duuren, B.L., Orris, L. & Nelson, N. (1965) Carcinogenicity of epoxides, lactones, and peroxy compounds. Part II. *J. natl Cancer Inst.*, **35**, 707–717

Vergnes, J.S. & Pritts, I.M. (1994) Effects of ethylene on micronucleus formation in the bone marrow of rats and mice following four week of inhalation exposure. *Mutat. Res.*, **324**, 87–91

Vogel, E. & Natarajan, A.T. (1982) The relation between reaction kinetics and mutagenic action of mono-functional alkylating agents in higher eukaryotic systems: Interspecies comparisons. In: Hollaender, A. & De Serres, F.J. eds, *Chemical Mutagens VII*, New York, Plenum, pp. 295–336

Walker, V.E. & Skopek, T.R. (1993) A mouse model for the study of in vivo mutational spectra: Sequence specificity of ethylene oxide at the *hprt* locus. *Mutat. Res.*, **288**, 151–162

Walker, V.E., Fennell, T.R., Boucheron, J.A., Fedtke, N., Ciroussel, F. & Swenberg, J.A. (1990) Macromolecular adducts of ethylene oxide: A literature review and a time-course study on the formation of 7-(2-hydroxyethyl)guanine following exposures of rats by inhalation. *Mutat. Res.*, **233**, 151–164

Walker, V.E., MacNeela, J.P., Swenberg, J.A., Turner, M.J. & Fennell, T.R. (1992a) Molecular dosimetry of ethylene oxide: Formation and persistence of N-(2-hydroxyethyl)valine in hemoglobin following repeated exposures of rats and mice. *Cancer Res.*, **52**, 4320–4327

Walker, V.E., Fennell, T.R., Upton, P.B., Skopek, T.R., Prevost, V., Shuker, D.E.G. & Swenberg, J.A. (1992b) Molecular dosimetry of ethylene oxide: Formation and persistence of 7-(2-hydroxyethyl)guanine in DNA following repeated exposures of rats and mice. *Cancer Res.*, **52**, 4328–4334

Walker, V.E., Fennell, T.R., Upton, P.B., MacNeela, J.P. & Swenberg, J.A. (1993) Molecular dosimetry of DNA and hemoglobin adducts in mice and rats exposed to ethylene oxide. *Environ. Health Perspect.*, **99**, 11–17

Walker, V.E., Sisk, S.C., Upton, P.B., Wong, B.A. & Recio, L. (1997a) In vivo mutagenicity of ethylene oxide at the hprt locus in T-lymphocytes of B6C3F1 lacI transgenic mice following inhalation exposure. *Mutat. Res.*, **392**, 211–222

Walker, V.E., Meng, Q. & Clement, N.L. (1997b) Spectra of mutations in *hprt* exon 3 of T-cells from F344 rats and *lacI* transgenic and nontransgenic B6C3F1 mice exposed by inhalation to ethylene oxide. *Environ. mol. Mutag.*, **29** (Suppl. 28), 54

Walker, V.E., Jones, I.M., Crippen, T.L., Meng, Q., Walker, D.M., Bauer, M.J., Reilly, A.A., Tates, A.D., Nakamura, J., Upton, P.B. & Skopek, T.R. (1999) Relationships between exposure, cell loss and proliferation, and manifestation of *HPRT* mutant T-cells following treatment of preweanling, weanling, and adult male mice with *N*-ethyl-*N*-nitrosourea. *Mutat. Res.*, **431**, 371–388

Walker, V.E., Wu, K.-Y., Upton, P.B., Ranasinghe, A., Scheller, N., Cho, M.-H., Vergnes, J.S., Skopek, T.R. & Swenberg, J.A. (2000) Biomarkers of exposure and effect as indicators of potential carcinogenic risk arising from *in vivo* metabolism of ethylene to ethylene oxide. *Carcinogenesis*, **21**, 1661–1669

Weast, R.C. & Astle, M.J. (1985) *CRC Handbook of Data on Organic Compounds*, Vol. I, Boca Raton, FL, CRC Press, p. 627

WHO (2003) *Ethylene Oxide* (Concise International Chemical Assessment Document 54), Geneva, World Health Organization [available at http://www.inchem,org/documents/cicads/cidads/cicad54.htm]

Wojcik-O'Neill, K.M. & Ello, M. (1991) Equivalency of hydrochloric acid and distilled water extraction media for determining residual ethylene oxide in medical devices. *J. pharm. Sci.*, **80**, 783–784

Wolfs, P., Dutrieux, M., Scailteur, V., Haxhe, J.-J., Zumofen, M. & Lauwerys, R. (1983) [Monitoring of workers exposed to ethylene oxide in a plant distributing sterilizing gases and in units for sterilizing medical equipment.] *Arch. Mal. prof.*, **44**, 321–328 (in French)

Wong, O. & Trent, L.S. (1993) An epidemiological study of workers potentially exposed to ethylene oxide. *Br. J. ind. Med.*, **50**, 308–316

Wu, K.-Y., Ranasinghe, A., Upton, P.B., Walker, V.E. & Swenberg, J.A. (1999a) Molecular dosimetry of endogenous and ethylene oxide-induced N7-(2-hydroxyethyl)guanine formation in tissues of rodents. *Carcinogenesis*, **30**, 1787–1792

Wu, K.-Y., Scheller, N., Ranasinghe, A., Yen, T.-Y., Sangaiah, R., Giese, R. & Swenberg, J.A. (1999b) A gas chromatography/electron capture/negative chemical ionization high-resolution mass spectrometry method for analysis of endogenous and exogenous N7-(2-hydroxyethyl)guanine in rodents and its potential for human biological monitoring. *Chem. Res. Toxicol.*, **12**, 722–729

Wu, K.Y., Chiang, S.Y., Huang, T.H., Tseng, Y.S., Chen, Y.L., Kuo, H.W. & Hsieh, C.L. (2004) Formation of N-(2-hydroxyethyl)valine in human hemoglobin-effect of lifestyle factors. *Mutat. Res.*, **559**, 73–82

Yager, J.W. (1982) Sister chromatid exchanges induced in rabbit lymphocytes by ethylene oxide after inhalation exposure. *Environ. Mutag.*, **4**, 121–134

Yager, J.W. (1987) Effect of concentration-time parameters on sister-chromatid exchanges induced in rabbit lymphocytes by ethylene oxide inhalation. *Mutat. Res.*, **182**, 343–352

Yager, J.W. & Benz, R.D. (1982) Sister chromatid exchanges induced in rabbit lymphocytes by ethylene oxide after inhalation exposure. *Environ. Mutag.*, **4**, 121–134

Yager, J.W., Hines, C.J. & Spear, R.C. (1983) Exposure to ethylene oxide at work increases sister chromatid exchanges in human peripheral lymphocytes. *Science*, **219**, 1221–1223

Yong, L.C., Schulte, P.A., Wiencke, J.K., Boeniger, M.F., Connally, L.B., Walker, J.T., Whelan, E.A. & Ward, E.M. (2001) Hemoglobin adducts and sister chromatid exchanges in hospital workers exposed to ethylene oxide: Effects of glutathione S-transferase T1 and M1 genotypes. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 539–550

Yong, L.C., Schulte, P.A., Kao, C.-Y., Giese, R.W., Boeniger, M.F., Strauss, G.H.S., Petersen, M.R. & Wiencke, J.K. (2007) DNA adducts in granulocytes of hospital workers exposed to ethylene oxide. *Am. J. ind. Med.*, **50**, 293–302

Zamora, P.O., Benson, J.M., Li, A.P. & Brooks, A.L. (1983) Evaluation of an exposure system using cells grown on collagen gels for detecting highly volatile mutagens in the CHO/HGPRT mutation assay. *Environ. Mutag.*, **5**, 795–801

Zarbl, H. (2006) Cellular oncogenes and carcinogenesis. *In:* Warshawsky, D. & Landolph, J.R., Jr, eds, *Molecular Carcinogenesis and the Molecular Biology of Cancer*, New York, Taylor and Francis, pp. 103–129

Zhang, W., Johnson, F., Grollman, A.P. & Shibutani, S. (1995) Miscoding by the exocyclic and related DNA adducts 3,N^4-etheno-2′-deoxycytidine, 3,N^4-ethano-2′-deoxycytidine, and 3-(2-hydroxyethyl)-2′-deoxyuridine. *Chem. Res. Toxicol.*, **8**, 157–163

Zhao, C. & Hemminki, K. (2002) The *in vivo* levels of DNA alkylation products in human lymphocytes are not age dependent: An assay of 7-methyl- and 7-(2-hydroxyethyl)-guanine DNA adducts. *Carcinogenesis*, **23**, 307–310

Zhao, C., Tyndyk, M., Eide, I. & Hemminki, K. (1999) Endogenous and background DNA adducts by methylating and 2-hydroxyethylating agents. *Mutat. Res.*, **424**, 117–125

Zhong, B.-Z., Gu, Z.-W., Whong, W.-Z., Wallace, W.E. & Ong, T.-M. (1992) Comparative study of micronucleus assay and chromosomal aberration analysis in V79 cells exposed to ethylene oxide. *Teratog. Carcinog. Mutag.*, **11**, 227–233