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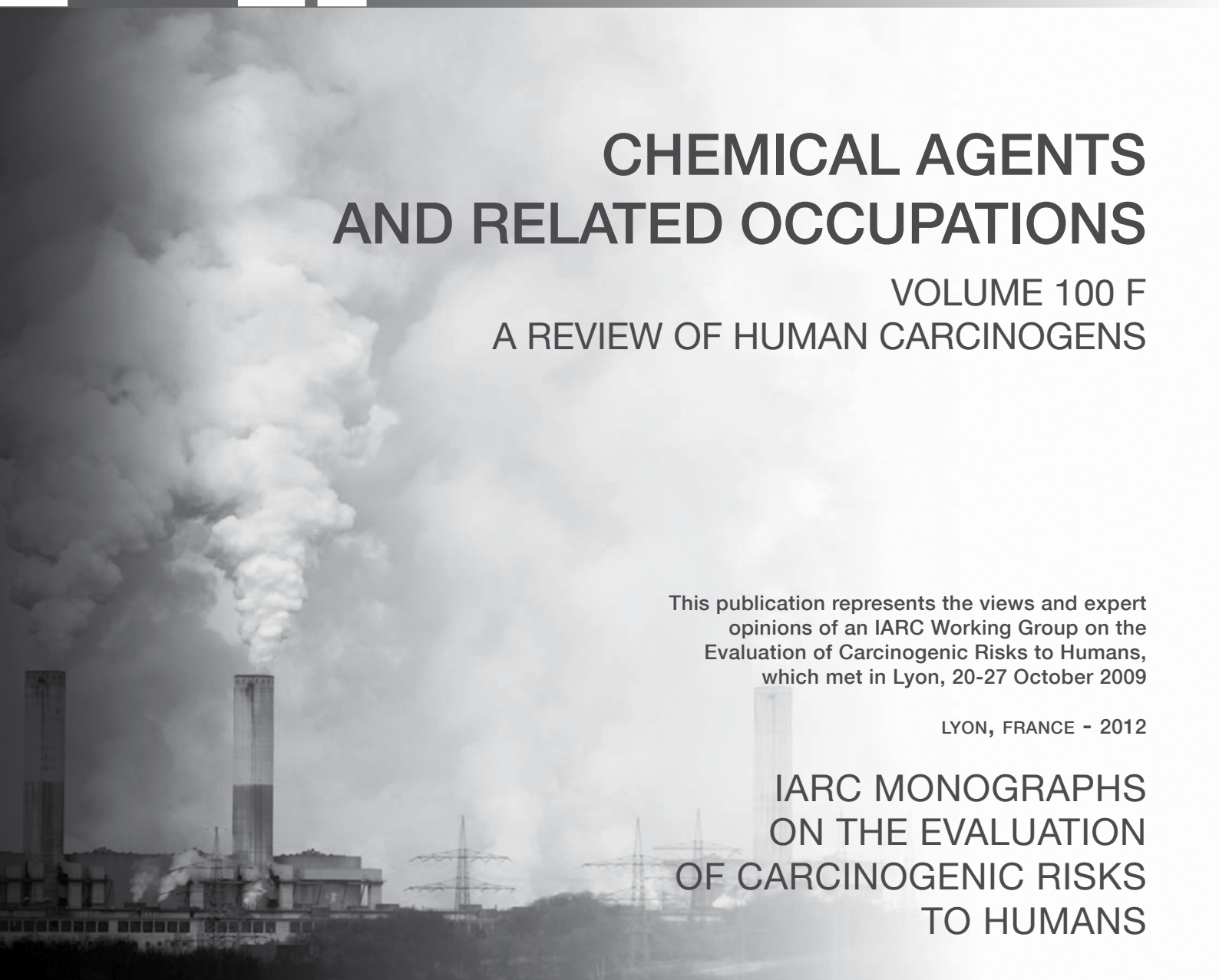

CHEMICAL AGENTS AND RELATED OCCUPATIONS

VOLUME 100 F
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 20-27 October 2009

LYON, FRANCE - 2012

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

SULFUR MUSTARD

Sulfur mustard, also known as mustard gas, was considered by previous IARC Working Groups in 1975 and 1987 (IARC, 1975, 1987a). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 505-60-2
Chem. Abstr. Serv. Name:
1,1′-Thiobis(2-chloroethane)
Synonyms: Sulfur mustard, mustard gas
Description: Colourless, oily liquid; forms prisms on cooling (O'Neill, 2006)
Melting-point: 13–14 °C (O'Neill, 2006)
Vapour pressure: 0.90 mm Hg at 30 °C (O'Neill, 2006)
Solubility: Very sparingly soluble in water; soluble in fat solvents and other common organic solvents; high lipid solubility (O'Neill, 2006)
Octanol-water partition coefficient: log K_{ow}, 2.41 (HSDB, 2009)

$$Cl—CH_2—CH_2—S—CH_2—CH_2—Cl$$

$C_4H_8Cl_2S$
Relative molecular mass: 159.1

1.2 Uses

Vesicants or blistering agents were among the first chemicals that were applied as lethal/tactical weapons during World War I. Mustard agents, also known as sulfur mustard or mustard gas, were the most widely used (WHO, 1970).

Mustard gas was first used during World War I during the battle of Flanders, near Ypres, Belgium, in July 1917 (the French name for mustard gas is Ypérite). It was then used in 1918 and again in Ethiopia in 1936. During World War II, mustard gas was the major chemical warfare agent; it was produced and stockpiled by many countries and is probably still the most distributed chemical warfare agent in the world (Szinicz, 2005). Mustard gas has more recently been used in the Egypt-Yemen conflict (1963–67) and in the war between Iraq and the Islamic Republic of Iran in 1984 (ATSDR, 2003; WHO, 2004).

On April 29, 1997, the Chemical Weapons Convention took effect. This Convention banned the development, production, acquisition, stockpiling, and transfer (direct or indirect), of chemical weapons. It prohibits the use of chemical weapons, the engagement in any military preparations aimed at using chemical weapons

and the encouragement, induction, or assistance with such activities. Each participating/signing state is commited to take measures to destroy their own chemical weapons and production facilities and to not use riot-control agents as a method of warfare. To oversee compliance with the Chemical Weapons Convention, the Organization for Prohibition of Chemical Weapons was created. It is based in The Hague, the Netherlands (Szinicz, 2005).

Sulfur mustard has been used as an antineoplastic agent without success, because of its high toxicity. A similar product, nitrogen mustard, has been successfully employed as an anticancer agent (IARC, 1975; Saladi *et al.*, 2005). Mustard gas/sulfur mustard has provided a useful model in biological studies on the mode of action of alkylating agents (IARC, 1975). It has also been used medicinally to control hyper-proliferation of psoriatic keratinocytes (ATSDR, 2003).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to mustard gas may occur in the following activities or industrial sectors: storage and destruction of mustard gas; construction work on military bases where mustard gas was previously released and remained as a contaminant in the soil or in excavated munitions dumps; activities in research laboratories where workers do not take the necessary precautions to prevent exposure; during fishing, when lumps of mustard gas are inadvertently caught in areas where it was historically dumped in the sea; and during armed conflicts, when it is used as a chemical warfare agent (ATSDR, 2003).

Methods currently available for detection of exposure to several chemical warfare agents, including mustard gas, have been reviewed (Noort *et al.*, 2002; Riches *et al.*, 2007; Black, 2008). These include analyses of metabolites in urine and blood, DNA adducts, and protein adducts.

1.3.2 Non-occupational exposure

Non-occupational exposure to mustard gas may occur around sites where the agent was released during warfare (e.g. Belgium, Morocco, Ethiopia, China, Iraq, and the Islamic Republic of Iran), where munitions are buried or where contaminated soils containing mustard gas are disturbed during excavation activities (ATSDR, 2003). The average and maximum atmospheric concentrations that are likely to have occurred under war conditions in areas where mustard gas-containing grenades or artillery shells were dropped, have been estimated at 3 and 5 ppm, respectively (Thorpe, 1974).

Environmental exposure may result from mustard gas/sulfur mustard vapour being carried over long distances by the wind and from local contamination of water (WHO, 2004). Although mustard gas/sulfur mustard is a reactive substance that hydrolyses rapidly upon contact with water, the oily liquid may persist in the environment for many years, or even decades. For example, there are sites where mustard gas originating from the First and Second World Wars still poses a threat to human health and the environment. The environmental fate of mustard gas/sulfur mustard has been discussed (Munro *et al.*, 1999; Ashmore & Nathanail, 2008).

In this *Monograph* the term mustard gas will be used in connection with its military use. In other cases, the agent will be termed sulfur mustard.

2. Cancer in Humans

The carcinogenic hazards of mustard gas were previously evaluated in *IARC Monograph* Volume 9 and in Supplement 7 (IARC, 1975, 1987a). Mustard gas causes respiratory cancers. Human data on the health effects of mustard gas are from battlefield exposures and accidents (single exposures), and from long-term exposures

in chemical factories. Epidemiological studies in humans point at a causal association between exposure to mustard gas and an excess risk for respiratory cancers.

In an early study, the 1930–52 mortality records of 1267 war pensioners who had suffered from mustard gas-poisoning during World War I in the years 1917–18 were analysed and compared with records of 1421 pensioners who had chronic bronchitis but were never exposed to mustard gas, and with those of 1114 pensioners who were wounded in the war but not exposed to mustard gas (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-25-Table2.1.pdf). Mortality from cancer of the lung and pleura was increased in the first two groups (in both, 29 observed deaths, 14 expected), but not in the third (13 deaths observed, 16 expected). There were no significant differences with respect to cancers at other sites. Almost all mustard gas-exposed subjects also had chronic bronchitis (Case & Lea, 1955).

In a similar study, mortality records (1919–55) were examined of 2718 American soldiers exposed to mustard gas during 1917–18, of 1855 soldiers who had pneumonia but were not exposed to mustard gas, and of 2578 wounded soldiers without mustard gas-poisoning or pneumonia. Differences in mortality were seen only in the second decade (1930–39) of the follow-up. Deaths from all respiratory cancers (observed/expected), calculated from US mortality rates, showed a ratio of 39/26 (1.47) for the mustard gas-exposed soldiers (Beebe, 1960). A further study added another ten years of follow-up, but did not alter the initial conclusion: the relative risk of death from lung cancer among the exposed was 1.3 compared with the controls (95%CI: 0.9–1.9) (Norman, 1975).

In a Japanese factory producing mustard gas in the period 1929–45 – with large-scale production of 450 tonnes/month during 1937–44 – concentrations at the workplace were 50–70 mg/m^3. The first report of a cancer case in this plant appeared in 1952: a death from bronchial cancer of a 30-year old man who had been occupationally exposed to mustard gas for 16 months from 1941 (Yamada *et al.*, 1953). Further expansion and follow-up of the plant cohort were reported during the following decade (Yamada *et al.*, 1957; Yamada, 1963). In an extended study over the period 1952–67, observed numbers of deaths were compared with those expected on the basis of mortality rates in the Japanese population (Wada *et al.*, 1968). Of 495 workers who had manufactured mustard gas, 33 had died from cancers of the respiratory tract, compared with 0.9 expected. Of 960 male employees not engaged in the production, only three were known to have died since 1952 from respiratory tract cancers, compared with 1.8 expected. Although there was evidence of preferential reporting of deaths in the mustard gas-exposed group, the excess of respiratory tract cancers was substantial. There was evidence of a dose–response relationship between exposure to mustard gas and subsequent development of respiratory cancer (Nishimoto *et al.*, 1983, 1988; Yamakido *et al.*, 1996).

Another study considered workers in Germany engaged in production, testing and destruction of mustard gas and nitrogen mustard, mainly during the period 1935–45. The factory employed 878 workers, of whom 402 had worked in close contact with mustard gas, nitrogen mustard or with a mixture of the two. In addition, there had been limited exposure in the factory to bromoacetone, phosgene, chloropicrine and organic arsenicals. Among 271 workers exposed to mustard gas or nitrogen mustard and followed-up for compensation of occupational disease and mortality during 1951–74 there were 85 deaths, 32 of which were due to cancer. Twenty-six were lost to follow-up. Compared with Lower-Saxony mortality rates, a significant excess was found for bronchial carcinomas (11 deaths observed, five expected) (Weiss & Weiss, 1975).

In a follow-up of British workers involved in mustard gas-production during World War II, a

statistically significant increase in risk for cancer of the lung and pleura (RR 1.6, $0.05 < P < 0.10$) and of the larynx and trachea (three deaths, RR 7.5, $P < 0.02$) were identified among 502 individuals (Manning *et al.*, 1981).

From a cohort of 2498 men and 1032 women who had been involved in the manufacture of mustard gas in Cheshire, United Kingdom, during World War II, 3354 workers (95%) were traced for mortality until the end of 1984. Between April 1938 and November 1944 the factory had produced 24000 tonnes of mustard gas (none of this material was in fact used). Gas escaped on several occasions and several hundred individuals, mainly in the processing plants, had suffered blistering on the arms and acute effects on the eyes and respiratory tract caused by small amounts of mustard gas. Compared with national death rates for lung cancer, a highly significant excess was observed (200 obs., 138.4 exp. $P < 0.001$). In addition, large and highly significant excesses were reported for deaths from cancers of the larynx (11 observed, four expected, $P = 0.003$), pharynx (15 obs., 2.73 exp, $P < 0.001$), and all other buccal cavity and upper respiratory sites combined (lip, tongue, salivary gland, mouth, nose) (12 obs., 4.29 exp., $P = 0.002$). The risks for cancers of the lung and pharynx were significantly related to duration of employment. Significant excess mortality was also observed for cancers of the oesophagus (20 obs., 10.72 exp.) and stomach (70 obs., 49.6 exp.), but these excesses showed no consistent relation with time since first exposure, or with duration of exposure (Easton *et al.*, 1988).

A retrospective mortality follow-up study was conducted among 1545 Navy recruits who were stationed in Bainbridge, Maryland, USA. During 1944–45 they had voluntarily participated in mustard gas-chamber tests, to assess the quality of protective clothing and masks. Controls were 2663 Navy recruits who were stationed at the same location at the same time as the exposed, but had not participated in the tests. These groups were followed-up until 31 December 1995. Cause-specific mortality risks associated with mustard gas-exposure and the extent or duration of the exposure were examined by use of adjusted and unadjusted relative risk estimates. There was no excess of any cause-specific mortality associated with different levels of mustard gas-exposure among the veterans, although the concentrations had been sufficient to cause skin reactions, such as erythema and ulceration (Bullman & Kang, 2000). [The Working Group noted that levels of exposure were probably substantially lower than those in studies of production workers and World War I veterans.]

Several studies have consistently shown an increased risk for lung cancer among workers in mustard gas-production and among World War I veterans who had been exposed to mustard gas. Two studies among workers in mustard gas-production showed evidence of an exposure–response relationship with duration of employment. Two studies, both based on small numbers, reported an excess risk for laryngeal cancer. However, neither of these studies adjusted for potential confounders, such as tobacco smoking and alcoholic beverage consumption.

3. Cancer in Experimental Animals

Studies with experimental animals exposed to sulfur mustard were reviewed in *IARC Monograph* Volume 9 and in Supplement 7 (IARC, 1975, 1987a). It was concluded that there was *limited evidence* in experimental animals for the carcinogenicity of mustard gas (sulfur mustard). Furthermore, it was noted that some routes of administration, e.g. subcutaneous or intravascular injection, may have little relevance to common human exposures.

In an inhalation study with male and female strain-A mice, an increased incidence in lung tumours [not further specified] was observed in

Table 3.1 Carcinogenicity studies in experimental animals exposed to sulfur mustard

Species, strain (sex) Duration, Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, strain A (M, F) 4–11 mo Heston (1953a)	*Inhalation* (single 15-min exposure) in 8-L dessicator containing 0 (controls) or 0.01 mL sulfur mustard on absorbent paper. 40/group/sex	Lung tumours (in M+F combined): 4 mo after exposure: 6/32 (controls), 9/30 11 mo after exposure: 10/25 (controls), 20/29 4–11 mo after exposure: 21/77 (27%, controls), 33/67* (49%)	*$P < 0.01$	Purity NR Mice were 2–3 mo of age at start Lung tumours not further specified. Three exposed mice and no controls developed lymphocytic leukaemias, which the authors considered unrelated to exposure.
Mouse, C3H, C3Hf, and strain A (M, F) Animals held until dead, moribund, or the appearance of tumours Heston (1953b)	*Subcutaneous injection* of 0.5 mL of 0.05% sulfur mustard in olive oil, once/wk C3H: 32 M, 8 F (six injections) C3Hf: 40 M, 10 F (six injections) strain A: 16 M, 14 F (five injections) Controls: C3H: 32 M, 8 F (untreated) C3Hf: 40 M (untreated) strain A: 16 M, 14 F (olive oil, five injections)	Fibrosarcomas at injection site: 1/8 C3H (M), 0/8 C3H (F), 2/38 C3Hf (M), 2/9 C3Hf (F), 1/14 strain A (M), 0/12 strain A (F) Rhabdomyosarcoma: 1/24 C3H (M) No subcutaneous sarcomas occurred in controls. Mammary tumours: *Exposed*: 2/9 C3Hf (F), 8/8 C3H (F), 1/12 strain A (F) *Controls*: 2/100 C3Hf (F, see comments), 7/8 C3H (F), 0/14 strain A (F)	NR, [NS] (see comments)	Purity NR Authors noted that 2/9 C3Hf female mice with mammary tumours is a significant incidence, compared with 2/100 untreated female C3Hf mice from another study [$P < 0.05$]. [The Working Group considered that untreated mice are inadequate controls for subcutaneous injection.]
Mouse, strain A (M, F) 4 mo Heston (1950)	*Intravenous injection* (4 × , on alternate d) of 0.25 mL 1:10 saturated solution of sulfur mustard in water (0.06–0.07%). Study 1: 15/group/sex Study 2: 24/group/sex	Pulmonary tumours (in M+F combined): Exposed, study 1: 93% [14/15]* Controls, study 1: 61% [15/28] Exposed, study 2: 68% [32/47]** Controls, study 2: 13% [6/46]	NR *[$P < 0.05$] **[$P < 0.0001$]	Purity NR Mice were 2 mo of age at start Lung tumours not further specified. The authors stated that preparation of dosing solutions differed, resulting in a slightly lower dose for study 2.
Rat, Sprague-Dawley (M, F) 42 wk Sasser *et al.* (1996)	*Oral* (*gavage*) 0, 0.03, 0.1, 0.4 mg/kg bw sulfur mustard, 5 d/wk (for 13 wk before mating and throughout gestation, parturition, lactation, in a 42-wk two-generation study) 27 F/group/generation 20 M/group/generation	Fore-stomach papillomas: F_0 (M) 0/20, 0/20, 1/20, 2/20 F_0 (F) 0/27, 0/27, 3/27, 3/27 F_1 (M) 0/20, 0/20, 2/20, 2/20 F_1 (F) 0/27, 0/27, 2/27, 3/27	NR, [NS]	Purity, 97.3% Two-generation study

bw, body weight; d, day or days; F, female; M, male; min, minute or minutes; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

49% of the animals exposed to sulfur mustard, compared with 27% in controls (Heston, 1953a). Intravenous injection of sulfur mustard also increased the incidence in lung tumours [not further specified] in male and female strain A mice (Heston, 1950). When administered by subcutaneous injection to mice, sulfur mustard induced a few fibrosarcomas and one rhabdomyosarcoma at the injection site in males and females, and mammary tumours in females (Heston, 1953b). Oral administration of sulfur mustard induced fore-stomach papillomas in male and female rats (Sasser *et al.*, 1996; Table 3.1).

4. Other Relevant Data

Since its first use in 1917, there have been nearly 400 000 casualties among the victims of mustard gas-poisoning (Rall & Pechura, 1993). After a lethal dose, death usually occurs within 2–3 days of exposure and is related to respiratory tract injuries, in particular secondary bronchopneumonia (Papirmeister *et al.*, 1991).

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Sulfur mustard can be absorbed after inhalation or through dermal exposure from air and soil. It is a lipophilic substance that easily penetrates into the skin and mucosal surfaces (Drasch *et al.*, 1987; Somani & Babu, 1989), resulting in a high degree of bio-availability.

About 80% of non-occluded, topically applied sulfur mustard evaporates from human skin, while 20% penetrates the skin within ten min (Renshaw, 1946; Kehe *et al.*, 2000). A comparable result was found in studies of human foreskin grafted onto a-thymic mice (Papirmeister *et al.*, 1984a, b). Of the dose that penetrates the skin, 60% is bound in the epidermal and dermal tissue, mostly in the cornified layer, while 40% – i.e. 8% of the initially applied amount – passes rapidly into the blood stream (Cullumbine, 1946, 1947; Nagy *et al.*, 1946; Renshaw, 1946). The penetration rate of sulfur mustard into human skin was estimated to be 1–4 mg/cm^2/min (i.e. 6–25 µmol/cm^2/min), dependent on the temperature (Nagy *et al.*, 1946; Renshaw, 1946).

Elevated concentrations of thio-diglycol, the major hydrolysis product of mustard gas, were detected in human urine after exposure to mustard gas vapour and aerosol (Jakubowski *et al.*, 2000). Thio-diglycol was also found in the urine of people exposed to airborne mustard gas during the war between Iraq and the Islamic Republic of Iran (Wils *et al.*, 1985, 1988). A mustard gas-specific DNA adduct, viz. *N*7-(2-hydroxyethylthioethyl)-2′-deoxyguanosine, as well as adducts to albumin and haemoglobin have been detected in the blood of two victims of mustard gas-poisoning during the war between Iraq and the Islamic Republic of Iran (Benschop *et al.*, 1997; Noort *et al.*, 1999). Autopsy samples from an Iranian soldier who died seven days after inhalation and/or dermal exposure to mustard gas indicated the following organ-distribution pattern: brain > kidney > liver > spleen > lung (Drasch *et al.*, 1987).

4.1.2 Experimental animals

Analysis of blood samples from hairless guinea-pigs exposed nose-only to 300 mg/m^3 (46 ppm) sulfur mustard during eight min, showed that a peak concentration was reached within five min after exposure (Langenberg *et al.*, 1998). In rabbits and monkeys that had undergone tracheal cannulation and were then exposed to nominal chamber concentrations of 40, 100, and 500 mg/m^3 sulfur mustard, only 15% of the dose was recovered, indicating that 85% was absorbed through the nasal mucous membrane (Cameron *et al.*, 1946). The absorption of sulfur mustard through the cornea was demonstrated

in guinea-pigs (Klain *et al.*, 1991). Thirty min after a 5-µL single topical application of radio-labelled sulfur mustard to the cornea of guinea-pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, blood plasma, and muscle.

After six hours of cutaneous exposure with occlusion, > 90% of a topically applied dose of sulfur mustard was absorbed into rat skin (Hambrook *et al.*, 1993). Within 60 minutes of the application, the initial rate of uptake had increased linearly with the applied dose in the range of 3–605 µg/cm^2 (0.02–3.6 µmol/cm^2) and reached a maximum of approximately 7 µg/cm^2/min (0.042 µmol/cm^2/min) at a dosage of 955 µg/cm^2 (6 µmol/cm^2). The fraction of sulfur mustard retained in the skin ranged from 10–50% in different studies (Renshaw, 1946; Cullumbine, 1947; Hambrook *et al.*, 1992), while the remainder is absorbed systemically.

Exposure of experimental animals to sulfur mustard by intravenous or intra-peritoneal injection has been reviewed (ATSDR, 2003). These studies provide evidence about routes of exposure other than those involving he skin, the lung or the eyes. The concentration of radio-labelled sulfur mustard in rats four days after intravenous injection indicated the following distribution-pattern: kidney > lung > liver > spleen > brain (Maisonneuve *et al.*, 1994). The difference with the distribution in humans (see above) may be due to different measurement methods, inter-species differences, or variations in post-exposure time, but the route of exposure appears to be an important toxicokinetic factor as well.

The reactivity of sulfur mustard with a wide variety of cellular macromolecules is well documented (IARC, 1975, 1987b; ATSDR, 2003). The presence of two chlorine atoms makes it a strong bi-functional alkylating agent with a high chemical reactivity (Dacre & Goldman, 1996). The chlorine atom is typically released under formation of a carbonium ion, which then undergoes intra-molecular cyclization to create a highly reactive compound. Formation of the carbonium ion is facilitated in aqueous solution (Somani & Babu, 1989), which explains the sensitivity of mucosal tissues, such as the eye, to its effect (Solberg *et al.*, 1997).

The cyclic intermediate mentioned above reacts with and alkylates a variety of electron-rich structures in the cell, such as the guanine moieties in DNA (Dacre & Goldman, 1996) and the sulfhydryl (-SH) and amino (-NH2) groups of proteins and nucleic acids (Solberg *et al.*, 1997). Evidence of covalent binding to cellular DNA, RNA and proteins *in vivo* was obtained in mice injected intra-peritoneally with [^{35}S]-labelled sulfur mustard (IARC, 1987b). DNA is the most functionally sensitive cellular target of sulfur mustard (Crathorn & Roberts, 1966).

Sulfur mustard-specific DNA adducts have been found in the nasal epithelium, naso-pharynx, larynx, carina, lung, spleen, and bone marrow of guinea-pigs after nose-only exposure (Langenberg *et al.*, 1998). The evidence of sulfur mustard-induced DNA adducts in tissues (Somani & Babu, 1989; Fidder *et al.*, 1994, 1996a; van der Schans *et al.*, 1994; Niu *et al.*, 1996) and of sulfur mustard-derived metabolites in urine (Wils *et al.*, 1985, 1988; Jakubowski *et al.*, 2000) suggests the existence of other metabolic pathways, which may include direct alkylation reactions, reaction with glutathione, hydrolysis and oxidation.

4.2 Genetic and related effects

Exposure to sulfur mustard has long been known to produce DNA interstrand cross-links (Roberts *et al.*, 1971a, b; Shahin *et al.*, 2001), which were first noted in *E. coli* (Lawley & Brookes, 1965). When sulfur mustard reacts with DNA, one of the products comprises two guanines linked by a mustard molecule (Walker, 1971). This cross-link can arise from a pair of guanines in opposite strands of the DNA molecule: this interstrand cross-link inhibits cell division (Papirmeister,

1993). However, the cross-link can also arise in significant amounts between two neighbouring guanines in the same strand (Walker, 1971). Transcription, translation, enzyme catalysis and other cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA are much less sensitive to sulfur mustard.

Sulfur mustard induced dose-related inter-strand cross-links in the DNA of rat epidermal keratinocytes in primary mono-layer culture (Lin *et al.*, 1996a), affecting cell cycle and DNA synthesis (Lin *et al.*, 1996b). Similar results were seen in HeLa cells (Ball & Roberts, 1972) and in rat cutaneous keratinocytes (Ribeiro *et al.*, 1991). Sulfur mustard has also been shown to affect DNA mismatch-repair in African green monkey kidney cells (Fan & Bernstein, 1991).

Sulfur mustard has been shown to form DNA adducts *in vitro* (van der Schans *et al.*, 1994; Niu *et al.*, 1996; ATSDR, 2003). Upon incubation of double-stranded calf-thymus DNA or human blood with [^{35}S]-labelled sulfur mustard, the following adducts were identified: *N*7-[2-[(2-hydroxyethyl)thio]ethyl]-guanine, bis[2-(guanin-7-yl)ethyl]sulfide, *N*3-[2-[(2-hydroxyethyl)thio]ethyl]-adenine, and O^6-[2-[(2-hydroxyethyl)thio]ethyl]-guanine and its 2′-deoxyguanosine derivative (Fidder *et al.*, 1994). The primary site of DNA-alkylation by sulfur mustard is the *N*7 position of deoxy-guanosine (Balali-Mood & Hefazi, 2005). Upon depurination of the resulting *N*7-(2-hydroxyethyl)-2′-deoxyguanosine, the base adduct *N*7-(2-hydroxyethylthioethyl)-guanine (*N*7-HETE-Gua) is released. The toxic effects of sulfur mustard have been attributed to DNA adducts such as *N*7-hydroxyethylthioethyl-guanine, 3-hydroxyethylthioethyl adenine, and the cross-link, di-(2-guanin-7-yl-ethyl) sulphide (Saladi *et al.*, 2006). DNA extracted from human leukocytes and exposed to [^{14}C]-labelled sulfur mustard *in vitro* was shown to contain the adduct *N*7-(2-hydroxyethylthioethyl)guanine (Ludlum *et al.*, 1994). It has been demonstrated that alkyltransferase is inefficient in repairing O^6-ethylthioethylguanine, and the persistence of this adduct could have serious consequences (Ludlum *et al.*, 1986). Alkylation by sulfur mustard also affects transcriptional processes and may lead to truncated transcripts by impairing RNA polymerase via an alkylated promoter (Masta *et al.*, 1996). Analysis of truncated transcripts revealed that sulfur mustard preferentially alkylates the DNA-template strand at 5′-AA and 5′-GG sequences. Low doses of sulfur mustard can also inhibit cell division by cross-linking of complementary DNA strands, or cause mutagenesis by inducing errors in replication or repair (Papirmeister, 1993; ATSDR, 2003). It has been noted that cells in late G1-phase (post-mitotic) or early S-phase (DNA synthesis) are particularly sensitive to the effects of alkylation (Somani & Babu, 1989).

The ability of sulfur mustard to induce mutations has been demonstrated in numerous experimental systems (Fox & Scott, 1980). *TP53* mutations – predominantly G→A transitions – were detected in tumours of individuals exposed to mustard gas (Hosseini-Khalili *et al.*, 2009). Sulfur mustard has been shown to induce mutations in specific DNA regions (r-RNA-coding locus) (Fahmy & Fahmy, 1971; IARC, 1975).

Fishermen who were exposed to mustard gas from leaking shells picked up during fishing showed an increased incidence of sister chromatid exchange in the lymphocytes (Wulf *et al.*, 1985). Sulfur mustard induces chromosomal aberrations and DNA damage in rodent cells *in vitro* and mutations in mouse-lymphoma cells *in vitro* and *in vivo* (IARC, 1987b). *In vivo,* sulfur mustard has been shown to induce micronuclei in mouse bone-marrow (Ashby *et al.*, 1991). It also induced chromosome aberrations in cultured rat lymphosarcoma cell lines (Scott *et al.*, 1974). In a host-mediated assay in male BDF1 mice, with a murine leukaemia cell line (L_{517}8Y/Asn) as an indicator, sulfur mustard induced both

chromosome aberrations and reversed mutations to asparagine-186 independence, after single subcutaneous doses of 100 mg/kg bw. Similar results were obtained with the same cell line tested *in vitro* (Capizzi *et al.*, 1973). Dominant lethal mutations in adult male rats were induced after exposure to sulfur mustard at 0.1 mg/m^3 for 52 weeks (Rozmiarek *et al.*, 1973). Aneuploidy, heritable translocations, dominant lethal mutations and sex-linked recessive lethal mutations have been observed in *Drosophila* exposed to sulfur mustard. The substance is mutagenic to fungi and induces DNA damage in bacteria and yeast (Kircher & Brendel, 1983).

Sulfur mustard appears to preferentially damage the cells that are the most actively regenerating after injury, such as basal cells located above the dermal papillae in the skin (Papirmeister *et al.*, 1991), and epithelial secretory cells in the trachea (Calvet *et al.*, 1996). In the cell, DNA and proteins are the main targets of alkylation by sulfur mustard; it is not unexpected, therefore, that the most severe lesions affect cells with the strongest proliferative and metabolic capacity. Impairment of the DNA-polymerase function has also been proposed. In particular, impairment of the replicative fidelity of DNA during the S-phase could contribute to mitotic and chromosomal effects (Bignold, 2006). Recently, both base-excision repair and nucleotide-excision repair were identified as repair pathways that are activated after exposure of human lymphoblastoid cell lines to the sulfur-mustard surrogate 2-chloroethyl-ethylsulphide (Jowsey *et al.*, 2009)

Several studies have shown that sulfur mustard applied topically on the skin can diffuse and produce biochemical alterations consistent with free-radical-mediated oxidative stress, including increased lipid peroxidation and antioxidant enzyme activities, depletion of glutathione content in the eye, kidney, brain, lungs, and liver of rats and mice (Arroyo *et al.*, 2000). Sulfur mustard undergoes nucleophilic substitution reactions to form a sulfonium ring (Yang *et al.*, 1992) that, in the presence of oxygen, first generates a non-toxic, reactive sulfoxide intermediate. Extensive oxidation leads to toxic sulfone species (Arroyo *et al.*, 2000).

Besides genotoxic mechanisms responsible for the acute and delayed effects of sulfur mustard, other mechanisms may be responsible for sulfur mustard-induced vesication, since acute skin injury develops much earlier than would be expected from genotoxic effects alone. Also, tissue injury does not develop when low, therapeutically effective doses of sulfur mustard are used to control the hyper-proliferation of psoriatic keratinocytes. While the mechanisms underlying the toxicity of sulfur mustard are currently not fully understood, one hypothesis to explain its cytotoxicity involves poly(ADP-ribose) polymerase (PARP). It has been proposed that sulfur mustard alkylates DNA, which causes DNA strand breaks whose accumulation can cause activation of the nuclear repair-enzyme PARP. This causes cellular depletion of nicotinamide adenine dinucleotide, which decreases glycolysis and leads to protease release and cellular injury. Dermal-epidermal separation and blister formation may involve the fragmentation of anchoring filaments by protease released from moribund or dead cells (Papirmeister, 1993). Treatment of HeLa cells with sulfur mustard produces a rapid stimulation of PARP activity, followed by a decline in nicotinamide-adenine-dinucleotide levels two hours later (Clark & Smith, 1993). The hypothesis is almost fully confirmed in a study in which PARP inhibitors prevent the sulfur mustard-induced losses of adenosine triphosphate, nicotinamide-adenine-dinucleotide and viability in human peripheral blood cells (Meier & Kelly, 1993). Several other studies provide partial support for this hypothesis and suggest that additional pathways may be involved.

Sulfur mustard was found to inhibit antioxidant enzyme activities in blood cells and other tissues of rats, after topical application; the treatment could impair cyto-protective

defence mechanisms (Husain *et al.*, 1996). Enzyme activities were measured 24 hours after dermal treatment with 98 mg/mg (0.5 LD50) of sulfur mustard. Superoxide dismutase activity decreased significantly in white blood cells (70%), in platelets (65%), in spleen (72%) and in brain (29%) while it was not significantly altered in red blood cells, liver, and kidney. Catalase activity decreased significantly in white (54%) and red blood (23%) cells and in spleen (51%), while the activity in platelets, liver, kidney, and brain was not significantly altered. Glutathione peroxidase activity, as a consequence of glutathione and nicotinamide-adenine-dinucleotide-phosphate depletion, decreased significantly in white blood cells (42%), spleen (43%), and liver (22%). Glutathione levels in red blood cells, platelets, kidney, and brain were within 10% of control values.

4.3 Synthesis

Data from a variety of sources all strongly support a genotoxic mechanism underlying the carcinogenic action of mustard gas/sulfur mustard, mainly based on the observation that this chemical is a bi-functional alkylating agent (IARC, 1987b). It was the first chemical reported to induce mutations and chromosome rearrangements in *Drosophila melanogaster* (Auerbach & Robson, 1947; ATSDR, 2003). The direct reaction of this substance with DNA likely initiates a cascade of genetic events that lead to cancer. There is evidence to support DNA-alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and induction of chromosome-type and chromatid-type aberrations (ATSDR, 2003). Some of these changes are observed in nasal tissue, which is consistent with the nasal tissue being a target organ for this chemical. In addition, production of reactive oxygen species and cytotoxicity, other reported contributors to the mechanism of action, could act complementary to DNA alkylation.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of mustard gas. Mustard gas causes cancer of the lung.

Also, a positive association has been observed between mustard gas and cancer of the larynx,

There is *limited evidence* in experimental animals for the carcinogenicity of sulfur mustard.

There is *strong evidence* that the carcinogenicity of sulfur mustard operates by a genotoxic mechanism of action that involves DNA alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutations, and induction of chromosome-type and chromatid-type aberrations.

Sulfur mustard is *carcinogenic to humans (Group 1)*.

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