IJMS
Vol 34, No 3, September 2009

Review Article

Sulphur Mustard Poisoning and Its Complications in Iranian Veterans

Mahdi Balali-Mood[1], Beeta Balali-Mood[2]

Abstract

Sulphur mustard is a chemical warfare agent, which was largely used during the World War One and in Iraq-Iran conflict. It may also be used as a chemical terrorism agent. Therefore, medical professions should have sufficient knowledge and be prepared for medical intervention of any such chemical attack.

Sulphur mustard exerts direct toxic effects on the eyes, skin, and respiratory tract, with subsequent systemic actions on the nervous, immunologic, hematologic, digestive, and reproductive systems. It is an alkylating agent that affects DNA synthesis and thus, delayed complications have been considered since the World War One. Cases of malignancies in the target organs particularly in hematopoietic, respiratory, and digestive systems were reported. Common delayed respiratory complications include chronic bronchitis, bronchiectasis, frequent bronchopneumonia, and pulmonary fibrosis, all of which tend to deteriorate with time. Severe dry skin, delayed keratitis, and reduction of natural killer cells with subsequent increased risk of infections and malignancies are also among the most distressing long-term consequences of sulphur mustard intoxication. However, despite extensive research that has been conducted on Iranian veterans during the past decades, major gaps continue to remain in the sulphur mustard literature. Immunological and neurological dysfunctions and the relationship between exposure to sulphur mustard and mutagenicity, carcinogenicity, and teratogenicity are important fields that require further studies, particularly on Iranian veterans with chronic health problems caused by sulphur mustard poisoning. There is also a paucity of information on the medical management of acute and delayed toxic effects of sulphur mustard poisoning, a subject that greatly challenges the medical professions.

Iran J Med Sci 2009; 34(3): 155-171.

Keywords ● Chemical warfare agents ● sulphur mustard ● mustard gas ● poisoning ● complications ● toxic effects ● Iranian ● veterans

[1]Medical Toxicology Research Center,
Imam Reza Hospital,
Mashhad University of Medical Sciences,
Mashhad, Iran.
[2]Department of Chemistry,
Imperial College London,
London, UK.

Correspondence:
Mahdi Balali-Mood MD, PhD,
Medical Toxicology Research Center,
Imam Reza Hospital,
Medical School,
Mashhad University of Medical Sciences,
Mashhad, Iran.
Tel: +98 511 8819301
+98 511 8598973
Fax: +98 511 8813714
+98 511 8525315
Email: BalalimoodM@mums.ac.ir
mbalalimood@ hotmail.com
Received: 10 August 2008
Revised: 17 September 2008
Accepted: 9 October 2008

Introduction

The present article has been prepared by utilizing the experience of the first author and the literature review. PubMed was searched using various names for sulphur mustard. The search included human exposure, poisoning, and complications of sulphur mustard since 1983, when the Iraqi army started chemical war gas attacks on the Iranian troops. The first author's collections of literature on sulphur

mustard included the books, monographs and old articles were used as well. We aimed to describe the basic chemistry and toxicology of sulphur mustard as well as its clinical effects and long term complications. Special attention was paid to studies on the Iranian veterans with history of exposure to sulphur mustard during the Iran-Iraq war. Case reports and unpublished data were excluded to reach higher evidence-based information. Therefore, the headings and subheadings were chosen based on the above objectives. The present article is not a meta analysis or critical review on sulphur mustard. We have tried to prepare a descriptive text on sulphur mustard with special reference to the Iranian patients for the medical and health care professionals.

Ethical Consideration

All studies performed by the authors and presented in this review followed the standard ethics. They were all confirmed by the Medical Research Ethics Committee of Mashhad University of Medical Sciences and were carried out after obtaining written informed consents from the patients.

Chemistry

Sulphur mustard is a bis (2-chloroethyl) sulphide, which was first synthesized in 1822 by Despretz. Its chemical structure is shown in figure 1.

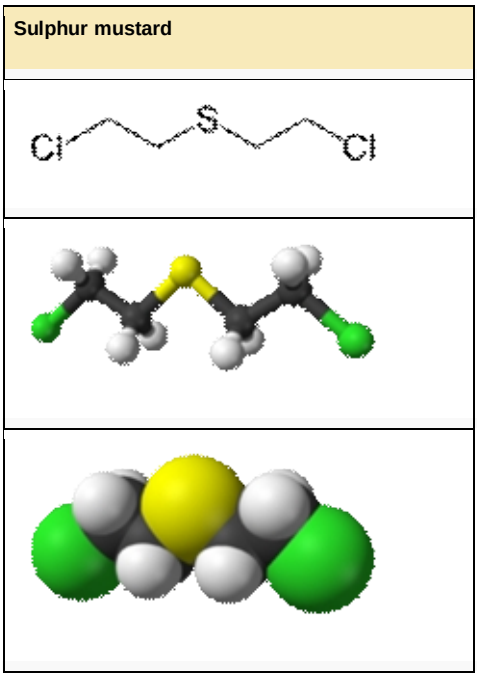

Figure 1: Chemical structure of sulphur mustard

Various Names of Sulphur Mustard

Sulphur mustard is also known as yperite (Ypres was the place of its first military use in Belgium), Lost (acronym of the German chemists Lommel and Steinkopf who investigated the military use of this chemical), and yellow cross that comes from the German shells which were marked with a yellow cross, means: “skin damaging agent”.[1]

Sulphur mustard is also known as HS ("Hun Stuff") or Levinstein mustard. Undistilled sulphur mustard contains 20–30% impurities. Distilled sulphur mustard that is approximately 96% pure was military coded as HD. The term "mustard gas" usually refers to this variety of sulphur mustard.[2]

Sulphur Mustard Synthesis

In 1886, Victor Meyer was the first to prepare pure sulphur mustard by the reaction of thiodiglycol with phosphorus trichloride.

$$3(HO\text{-}CH_2CH_2)_2S + 2PCl_3 \rightarrow 3(Cl\text{-}CH_2CH_2)_2S + 2P(OH)_3$$

Thiodiglycol was prepared by the reaction of 2-chloroethanol with potassium sulphide. Thiodiglycol can also be prepared by the American process, in which ethylene oxide reacts with hydrogen sulphide.

In the Meyer-Clarke method, concentrated hydrochloric acid (HCl) instead of PCl_3 is used as the chlorinating agent:

$$(HO\text{-}CH_2CH_2)_2S + 2HCl \rightarrow (Cl\text{-}CH_2CH_2)_2S + 2H_2O$$

Sulphur mustard was produced for use as a chemical warfare agent by what is known as the Levinstein process – the reaction of ethylene with sulphur dichloride before the World War One (WWI).[3]

$$CH_2CH_2 + SCl_2 \rightarrow (Cl\text{-}CH_2CH_2)_2S$$

Based upon the information in the media after the Iraq- Iran war, it seems that sulphur mustard produced in Iraq by the above thiodiglycol reactions.

Brief Physical and Chemical Properties

Sulphur mustard is an oily liquid, which is colourless if pure, but normally ranges from pale yellow to dark brown. Slight garlic or horseradish type odour as the Iranian veterans also described. Its density is 1.27 g/ml, melting point of 14.4 °C and boiling point of 217 °C. Sulphur mustard is only 0.05% soluble in water.[1,2]

Sulphur mustard is generally considered as a "persistent" chemical agent because of its low volatility. In cool weather there is little vapor; however, mustard's evaporation increases as the temperature increases. At higher temperatures, such as those in the Middle East during the hot season (38°C to 49°C), mustard vapor becomes a major hazard.[1-3]

Historical Uses

Sulphur mustard has been the most widely used chemical warfare agent in the past century. It was first employed extensively in WWI between 1914 and 1918. Despite the Geneva Protocol in 1925 prohibits the use of chemical warfare agents, sulphur mustard was used by Italian troops in Ethiopia (1935–36), and by Egyptian forces in Yemen (1963–67). The greatest military use of sulphur mustard was by the Iraqi army against Iranian soldiers and even civilians in Sardasht and Halabjah between 1983 and 1988, resulting in over 100,000 chemical casualties.[4,5]

Although there have been no substantiated reports of the use of sulphur mustard by terrorist groups, the simplicity of its chemical synthesis does offer the potential for use by terrorists.

Types of Exposure

According to the situation involved, several types of exposure including single, multiple, secondary, subclinical, and chronic may occur.

Most human cases of sulphur mustard poisoning have occurred during armed conflicts and most accidents were a single exposure.[1-5] Multiple low sulphur mustard exposure occurred occupationally and during the WWI and in the Iran-Iraq conflict.[4,5] First aid workers, nursing and medical staff without proper personal physical protection who were looking after sulphur mustard casualties in the field clinics and hospitals during the Iraq-Iran war, have become intoxicated. Some of them are now suffering from the delayed toxic effects of sulphur mustard and have disabilities of 5 to 25%.[4]

Low level sulphur mustard exposure with or without symptoms, but with delayed or long-term health effects has been described in details.[6-9] Subclinical exposure to sulphur mustard in some Iranian combatants induced delayed toxic effects. Seventy seven patients, who were present in a contaminated area and had no acute symptoms or signs at the time of exposure, are now suffering from respiratory disorders such as bronchiectasis and bronchiolitis obliterans.[10]

Chronic sulphur mustard exposure is usually occupational. Some factory workers in Japan and UK have been reported to have sulphur mustard poisoning and malignancies caused by sulphur mustard.[11,12]

Routes of Exposure

Inhalation is the major route of exposure, which induces respiratory and systemic toxicity after absorption across the lung surface.[3,4,13] However, sulphur mustard is a vesicant or blistering agent that has direct toxic effects on the skin, producing erythema, blistering, epidermolysis, and necrosis. It is a lipid soluble compound and thus can be readily absorbed across the skin.[4,14] Its vesicant properties were first noted by Guthrie in 1860.[1]

The eyes are the most sensitive organs to sulphur mustard. This marked susceptibility is attributable to the aqueous–mucous surface of the cornea and conjunctiva as well as the high turnover rate and intense metabolic activity of the corneal epithelial cells.[15,16]

Sulphur mustard may also enter the body by oral ingestion. We observed a few Iranian combatants during the war who had ingested food contaminated by sulphur mustard and that subsequently became intoxicated. They experienced nausea, vomiting, hematemesis, abdominal pain and dyspnea. Sulphur mustard may also be absorbed through the lower gastrointestinal tract.[4] Injection is a very rare route of sulphur mustard intoxication and has not been reported in human beings.

Toxicity

Exposure to very high doses of sulphur mustard in the field may induce convulsions and death in less than one hour.[3,4,17] Such observations have not been reported during the Iraq-Iran war. Acute toxic effects generally appear after a variable period of latency depending on the dose, mode of exposure, environmental temperature, and probably the individual's characteristics.[2,4,17-19]

Subacute exposure occurred during the Iran-Iraq war and in the workers in sulphur mustard munitions factories. However, this type of exposure may present as a mild acute intoxication or a complication in the respiratory tract or even as malignancy.[20,21]

Delayed toxic effects of sulphur mustard have been documented. The first report of delayed toxic effects in Iranian veterans was reported in 1986.[22] Several articles on the delayed toxic effects and complications of sulphur mustard in Iranian veterans have been published since then.[8,10,13,14,16,18,22]

Several studies suggest that workers who were chemically exposed to mustard agents in British and Japanese munitions factories developed chronic respiratory effects. In a cohort mortality study of 3500 workers at a manufacturing plant in England, a statistically significant increase in the number of deaths caused by influenza, pneumonia, bronchitis, and asthma were reported. This was present even among those with less than 3 years of employment at the plant and so was not related to the duration of employment.[21]

A 25-year follow-up study of workers exposed to sulphur mustard in a Japanese production plant revealed that more highly exposed workers had more chronic bronchitis and a slightly lower ratio of forced expiratory volume at one second to the forced vital capacity (FEV1/FVC) than either the less-exposed or an unexposed group of their co-workers.[12] In another study, Brown reported a large number of employees, who worked at the Huntsville Arsenal in Alabama. They were continuously exposed to the gas over long periods of time and developed bronchiectasis with progressive emphysema and narrow attenuated bronchioles.[11]

Toxicodynamics

The monofunctional mustards have one alkylating site and, therefore, can attack and break the DNA at specific nucleotides. Although sulphur mustard reacts with RNA, proteins, and phospholipids, the consensus view is that a DNA alkylate plays an important role in delayed toxic effects.[23,24] The major alkylating site of nucleic acids of mammalian origin is the nitrogen residue of guanine.[25] Cell death from DNA cross-linking is delayed until the cell replicates its DNA or undergoes division. At higher cellular exposures, however, mechanisms other than DNA cross-linking become important and produce more rapid cell death. The acute damage to the cornea, other mucous membranes, and skin seen following exposure to sulphur mustard is probably generated by one or more of these mechanisms. One mechanism that may be involved in acute damage is nicotinamide adenine dinucleotide (NAD) depletion. Other potential mechanisms of cell death are related to rapid inactivation of sulfhydryl containing proteins and peptides, such as glutathione. These sulfhydryl compounds are critical in maintaining the appropriate oxidation-reduction state of cellular components. Glutathione is also important in reducing reactive oxygen species in the cell and preventing peroxidation and loss of membrane integrity.[26,27]

Toxicokinetics

Sulphur mustard is absorbed by inhalation, through the skin, or via the anterior surface of the eye. It may also be absorbed through the gastrointestinal tract following consumption of contaminated food. When delivered as liquid or vapour, the skin plays a very important role as a port of entry for sulphur mustard. It undergoes hydrolysis producing half -mustard and thiodiglycol, which is the major metabolite and is excreted in urine.

From the total mustard that penetrates, only 10–20% is fixed to macromolecules in skin. The remaining 80–90% is rapidly transported away from the site of absorption by the circulation.[28-30] In terminally ill patients with cancer, 80–90% of the radioactivity of the injected ^{14}C labelled sulphur mustard disappeared after several minutes from the blood and was excreted mainly in the urine within 24 hours.[31]

Sulphur mustard is eliminated in a two-compartment model. Its distribution is quick with a long terminal half life (5.56 min and 3.59 hours, respectively). Its volume of distribution at steady state (*V*dss) is 74.4 L.[32] Whole body autographic studies with ^{35}S labelled sulphur mustard have shown that elevated radioactivity was detected in the nasal region, followed by the kidneys, liver, and intestines at all times studied after percutaneous or intravenous administration.[33] In human beings, unhydrolysed sulphur mustard can be present in brain and fat depots even days after exposure.[34] Only limited data are available on biotransformation of sulphur mustard in man. Two studies in rats revealed that conjugation with glutathione was more important than hydrolysis.[31,35] More recent investigations demonstrated that 60% of the dose was excreted in the 24-hour urine.[32]

Toxic Effects on Cells

Toxic effects of sulphur mustard on cell metabolism in different organs may cause severe dysfunctions leading to malignancies. Sulphur mustard is classified as a carcinogen by the International Agency for Cancer Research. There is no evidence for the mutagenicity of sulphur mustard and no evidence of teratogenicity was found in rats treated with different doses of sulphur mustard.[36]

Human studies indicate a causal association between occupational exposure to sulphur mustard and the excessive occurrence of respiratory cancer, skin cancer, and possibly leukemia.[37] A significant excess of death (33 cases against 0.9 expected) caused by respiratory cancer was found among former workers of the Japanese poison gas factory that operated

from 1929 to 1945.[38] Similarly, highly significant excess in the cancers of the larynx, pharynx, and other parts of upper respiratory tract was observed in former employees of a British plant that manufactured sulphur mustard. A moderate, but still significantly higher mortality than normal population was also observed resulted from lung cancer.[39] Gastric cancer, basal cell carcinoma, Bowen's disease, Bowen's carcinoma, and skin spinocellular have all been reported following occupational exposure to mustard gas.[40,41]

A few studies are available regarding the reproductive effects of sulphur mustard. Intravenous injection of sulphur mustard in male mice results in damage to the testes, with inhibition of spermatogenesis.[42] Nevertheless, the damage is usually transient, because testicular recovery is observed in 2 weeks, with the formation of mature sperms 4 weeks after exposure. A two-generation study of rats indicated that exposure to sulphur mustard at levels of 0.03, 0.1, and 0.4 mg/kg/day did not have any adverse effects on the reproductive performance or the fertility of male or female rats throughout two consecutive generations, except for an altered sex ratio in the 0.4 mg/kg group.[36]

Toxic Effects on the Organs

Acute toxic effects of sulphur mustard on the eyes, respiratory tract, and the skin are more prominent. Eyes are the most sensitive organs to sulphur mustard. The first symptoms of exposure to sulphur mustard are usually those of the eyes.[4,18-20,43] Next to the eye lesions, the greatest discomfort produced by mustard gas results from irritation and toxicity of the respiratory system. Respiratory effects occur in a dose dependent manner from the nasal mucosa to the terminal bronchioles.[18,20]

Direct toxic effects of sulphur mustard on the skin are the main apparent effects that lead it to be called a vesicant or blistering agent. A German and an Iranian medical toxicologist (the first author) classified the cutaneous mustard gas lesions which described under the clinical manifestations.[44]

Gastrointestinal effects following exposure to sulphur mustard have been documented in some studies. Destruction of the mucosa and shedding of the epithelial elements, however, begin days after exposure, resulting in loss of large volumes of fluid and electrolytes.[42,45] Acute gastroduodenitis with hemorrhagic erosions, acute desquamative enteritis, and severe hemorrhagic necrotic colitis that were reported in veterans of WWI were not observed in Iranian veterans.[46]

Extremely heavy exposure to sulphur mustard can cause central nervous system excitation leading to convulsions in animals.[17] Balali-Mood and Navaeian reported convulsions in six Iranian veterans who were admitted to hospital during the early stages of their intoxication.[20] Most casualties from WWI and the Iran-Iraq conflict, however, revealed mild and very non-specific neurological effects such as headache, anxiety, fear of the future, restlessness, confusion, and lethargy.[20,46] A frequent long-term complication in patients exposed to sulphur mustard is delayed neuropathic symptoms, which are sparsely represented in most previous studies.[8,47]

Hemato-Immunological Effects

Sulphur mustard as an alkylating agent is particularly toxic to rapidly proliferating cells such as lymphoid and bone marrow cells. Leukocytosis is common within the first few days after exposure. White blood cell counts then begin to drop on the 3rd and 4th days after exposure and reach their minimum level around the 9th day. This leukopenia is followed by a decrease in megakaryocytes and finally in the erythropoietic series.[48-50] Bone marrow biopsies have shown hypocellular marrow and atrophy involving all the elements.[50] If cytopenia is not marked and there are still remaining stem cells, recovery will take place as the patient recovers.[49,50-53] The bone marrow studies reveal a severe decrease in cellularity and fat replacement, and nuclear changes, such as budding, double nuclear, and karyorrhexis in erythrocyte precursors. The toxic effects of sulphur mustard on the haematopoietic system are dose dependent and it is concluded that it causes aplastic or ineffective hematopoiesis.[52] Severe leukopenia, however, is an ominous sign, leading to secondary infections and higher mortality rates in these patients. Victims of sulphur mustard poisoning with white blood cell counts of 200 cells/mL or less die during their initial admissions.[48]

Sulphur mustard poisoning could result in impairment of both humoral and cellular immune functions.[54-56] Along with the appearance of clinical disorders, both C3 and C4 titres rise, followed by a gradual decrease over 1 year. Most of the patients with exposure to sulphur mustard had increased levels of IgG and IgM during the first weeks and up to 6th month after exposure.[56]

Depression of cell-mediated immunity has been observed in Iranian veterans up to 3 years after exposure.[55] Natural killer (NK) cells, which are known to be one of the most important components of the cellular immunity, have been found to be significantly lower in patients with

severe respiratory complications 10 years after exposure.[56] In a controlled study, the number of NK cells was still significantly lower 16 to 20 years after exposure.[8,51]

Acute Clinical Manifestations

The first contact with sulphur mustard is not painful and only a garlic or sulphur odour can be noticed. Normally, a symptom-free interval is observed for several hours. The duration of this interval correlates inversely with the absorbed dose of sulphur mustard.

Target Organs

Respiratory tract, eyes, and skin are the three major target organs for the toxic effects of sulphur mustard. However, other organs including the central nervous system, digestive and cardiovascular systems are also affected. The frequency of acute clinical effects of sulphur mustard in different organs of 233 patients treated in Imam Reza hospital in Mashhad (northeast Iran) a few days after exposure in May 1984 is shown in figure 2.[20]

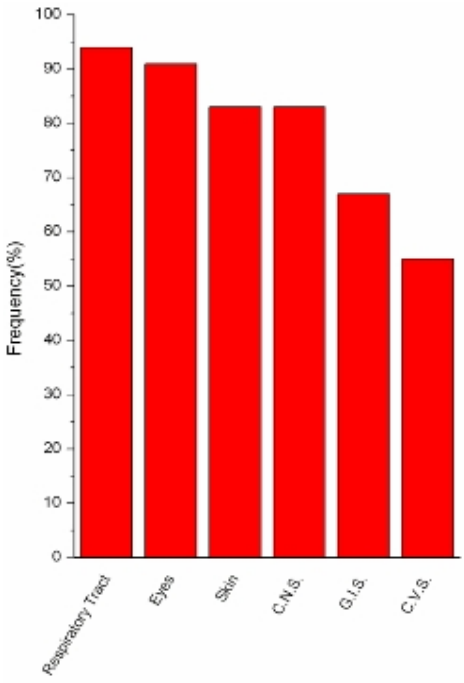

Figure 2: Frequency of acute clinical effects of sulphur mustard in different organs of 233 patients treated in northeast Iran a few days after exposure.

Eyes

Initial clinical symptoms and signs occur in the eyes within 1 hour after exposure, starting with a sensation of grittiness, a progressive soreness, and a bloodshot appearance, then proceeding to acute conjunctivitis. After several hours, the corneal epithelium begins to vesicate and slough, leading to severe pain, blepharospasm and decreased visual acuity. Gradual spontaneous recovery usually occurs after 48 hours, with full regeneration of the corneal epithelium within 4 to 5 days. Complete symptomatic recovery, however, may take 6 weeks or longer.[4,18,43,48]

Skin

Skin effects start with erythema a few hours after exposure with no itching or pain leading to blister formation and further lesions within hours and days later.[4,57] Different types of the cutaneous mustard gas lesions may occur. They have been classified as below:

- Erythematous form
- Pigmentary exfoliation
- Superficial vesicular to bullous form
- Bullous necrotization
- Deep necrotizing nonbullous form, and
- Allergic and toxic contact reactions of the skin

Different forms of the cutaneous lesions may be observed in one patient simultaneously. The pigmentary exfoliative form is often observed with severe lung damage.[44]

Respiratory Tract

Respiratory symptoms usually occur after the eyes, but earlier than the skin lesions. Dyspnea, coughing, and chest discomfort are the first symptoms progressing to acute rhinopharingotracheobronchitis. In severe cases, bronchopneumonia, adult respiratory distress syndrome, and even pulmonary emboli may develop causing mortality mostly in the second week after exposure.[4,58,59]

Neuropsychiatric Effects

Low dose exposure may result in headache, nausea, vomiting, and loss of appetite.[18,57]

Gastrointestinal Effects

Higher dose exposure may damage more severely the gastrointestinal tract.[57]

Immuno-Hematological Effects

Exposure to sulphur mustard affects the immune system and the bone marrow. This may result in immune suppression, leucopoenia, diarrhea, fever, weakness and, in very severe cases, excitation of the central nervous system with convulsions.[4,49,54-56] The maximum intensity of symptoms can be reached after days. An exposure to large doses of sulphur mustard can cause damage to the hematopoietic and the immune system.[49,50,52]

Toxicological Analyses

Environmental

Environmental identification of sulphur mustard in the field is of great medical importance to confirm the diagnosis and to evaluate and decontaminate those exposed. Decontamination of the environment also requires agent identification.

Identification of sulphur mustard in the air is now possible using portable detectors, special biosensors that are available in some advanced chemical defence laboratories. It is also possible to quantify sulphur mustard concentration in the air using a portable gas chromatograph-mass spectrometer.[60]

Sulphur mustard is a very stable compound, which can be identified in the soil many months, and even years, after exposure under particularly cold environmental conditions. Sulphur mustard and its hydrolysis products including half-mustard and thiodiglycol can be identified and quantified by the specific and sensitive analytical methods such as gas chromatography mass spectrometry.[60,61]

It is insoluble in water and its hydrolysis in the environment is very slow.

However, identification of sulphur mustard in water is possible in advanced environmental and chemical, biological, radiological, and nuclear (CBRN) defense laboratories by gas chromatography mass spectrometry.[57]

Patients

Alkylation products of sulphur mustard with DNA and proteins (e.g. hemoglobin and albumin), as well as its urinary metabolites have proven useful targets for the diagnosis of exposure to sulphur mustard in human beings. Urinary markers are readily accessible, although their rapid elimination, limits their use for retrospective detection. Adducts with macromolecules such as proteins, offer longer lasting biological markers for exposure to sulphur mustard, possibly up to several months.[62]

Blood

The primary site of DNA alkylation by sulphur mustard is the N7 position of deoxyguanosine residues.[62] Upon depurination of the resulting N7-(2- hydroxyethylthioethyl) 0-2´- oxyguanosine, N7-(2- hydroxyethylthioethyl) guanine (N7-HETE-Gua) is obtained. While gas chromatography-mass spectrometry (GC-MS) analysis has proved problematic, N7-HETE-Gua is conveniently analyzed using liquid chromatography-mass spectrometry (LC-MS).[61,62] The adduct can be detected in urine, and also after processing of skin and blood samples of animals exposed to sulphur mustard. An enzyme-linked immunosorbent assay (ELISA) has been successfully developed using monoclonal antibodies raised against N7-HETE guanosine- 5´- phosphate coupled to keyhole limpet hemocyanin.[63]

This method was applied to blood samples from two casualties of the Iran-Iraq War, collected 22 and 26 days following the alleged exposure to sulphur mustard.[64] The alkylation of proteins by sulphur mustard mainly occurs on carboxyl, amino, and sulfhydryl groups, and on the nitrogens of the imidazole ring of histidine. Definitive evidence of specific alkylation sites can be obtained by using modern MS techniques. While MS methods can be used to confirm the diagnosis under more sophisticated conditions, the ELISA approach has been mainly developed for use under field conditions. Haemoglobin and albumin are two abundant proteins in human blood that can be readily isolated for determination of sulphur mustard adducts.[4,63]

Urine

While the hydrolysis product of sulphur mustard, namely thiodiglycol, is only a minor metabolite, the sulfoxide derivative of thiodiglycol is abundantly present in the urine and can be reduced to thiodiglycol for GC-MS analysis.[65]

Unfortunately, both thiodiglycol and its sulfoxide are not unequivocal markers of poisoning in human beings and low concentrations are present in normal human urine.[65-70] The -lyase metabolites, which are derived from an initial reaction of sulphur mustard with glutathione, are unequivocal biomarkers and can be reduced to thioether derivatives for subsequent GC-MS analysis.[70] This method has been applied to urine samples from two human casualties accidentally exposed to the agent and from five Iranian casualties of chemical war attacks. The -lyase metabolites were detected in one sample collected 13 days after the alleged exposure to sulphur mustard.[70,71]

Blister Fluid

Analysis of the vesicle fluid for thiodiglycol may suspect exposure to sulphur mustard. The fluid contained in the vesicles is non-toxic, and presents no risk to the attending medical staff.[72]

Tissue

DNA adducts can be also detected in skin of people exposed as it was done after processing in animals exposed to sulphur mustard.[64] Organ tissues of the post-mortem samples taken from chemical Iranian martyrs revealed sulphur mustard with different concentrations in different organs.[33,34]

Post-Mortem

Toxicological analyses of the post-mortem samples (blood, urine, organ tissues) of an Iranian combatant who died in Belgian hospitals revealed sulphur mustard.[34] The detailed biological fate of sulphur mustard in Iranian chemically poisoned patients who admitted to British hospitals including post-mortems toxicological analyses has been reported.[73]

Delayed Toxic Effects

During the Iran–Iraq war, about 100,000 people suffered from exposure to sulphur mustard. Now after 20 years, about 40,000 veterans have complained of delayed effects that poisoning.[4,5,10,74] The first report of delayed toxic effects in Iranian veterans was reported in 1986.[22] The most prominent late clinical effects were observed in the respiratory tract (78%), nervous system (45%), skin (41%), and eyes (36%).[22,40] The delayed toxic effects on the eyes as chronic conjunctivitis and on the skin as hyperpigmentation in a patient 6 weeks after exposure to sulphur mustard is shown in figure 3.

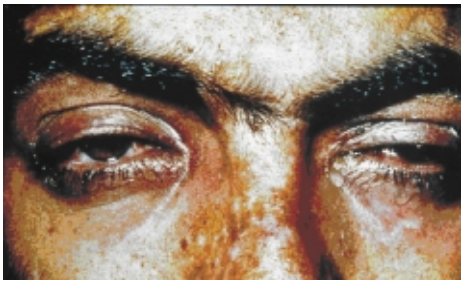

Figure 3: Chronic conjunctivitis and skin hyperpigmentation in a patient 6 weeks after exposure to sulphur mustard

Chronic

Chronic occupational exposure to sulphur mustard is discussed above. Most of the clinical symptoms and signs were observed in the respiratory tract, presenting as coughing with or without productions, dyspnea, wheezing, and bronchial rale. Chronic bronchitis, obstructive and restrictive lung diseases leading to chronic obstructive pulmonary diseases (COPD) and bronchiectasis have been reported.[12] Malignancies, particularly in the respiratory tract and hematopoietic system, may occur.[21]

Complications

It is very difficult to differentiate between the delayed toxic effects and complications of sulphur mustard poisoning. However, complications may be defined as the persistent, permanent, and life threatening delayed toxic effects of sulphur mustard. Long-term complications of sulphur mustard are discussed below.

Long-Term Complications

Information on the long-term effects of exposure to sulphur mustard comes from two major sources of investigations: Firstly, the studies of soldiers who were exposed to the agent on the battlefield and secondly the studies of workers who were employed in mustard gas factories (occupational exposure). While long-term effects after battlefield exposure are referred to as “late” or “delayed” complications, the term “chronic” seems to be more suitable for the complications caused by occupational exposure. It must also be emphasized that delayed effects generally occur months or years after a single or brief exposure and are not the same as chronic poisoning, which comes from continuous exposure to the poison over a relatively long period of time. The first report on the delayed toxic effects of sulphur mustard poisoning in 236 Iranian veterans revealed that the most common effects were on the respiratory tract (78%), CNS (45%), skin (41%), and the eyes (36%). These effects were recorded between 2 and 28 months after exposure.[22] Comparison of early (1 week after exposure) and late (2 years after exposure) toxic effects of sulphur mustard poisoning in 77 victims indicated that eye lesions do not change significantly, dermal complications tend to decrease, and respiratory complications generally deteriorate over the years.[6,8,13,16,74] In a study of 34,000 Iranians, 13 to 20 years after exposure to sulphur mustard, the most common complications were found in the lungs (42.5%), eyes (39%), and the skin (24.5%).[75] In a group of 40 severely intoxicated Iranian veterans in Mashhad, 16 to 20 years after their initial exposure, the most commonly affected organs were lungs (95%), peripheral nerves (75%), skin (72.5%), and the eyes (67.5%) as shown in table 1.[8]

Table 1*:* Frequency of delayed complications of sulphur mustard in different organs of 40 Iranian veterans in Mashhad (northeast Iran), 16-20 years after exposure.

Organs	Number of patients	Percentages
Respiratory tract	38	95
Peripheral nerves	30	75
Skin	29	72.5
Eyes	27	67.5

Respiratory

Respiratory complications are the greatest cause of long-term disability among people with exposure to sulphur mustard. A triad of cough, expectoration, and dyspnea has been found to be present in more than 80% of Iranian

veterans 3 years after their initial exposure.[6,40] Haemoptysis (mainly streaky), chest tightness, chest pain, and nocturnal dyspnea is also frequent. The main objective clinical findings are generalized wheezing (the most common sign), crackles, decreased lung sounds, clubbing, and cyanosis.[6,40,74]

Pulmonary function testing has revealed more obstructive patterns than restriction. About half of these obstructive spirometric parameters reverse in response to inhaled bronchodilators. FVC, FEV1, and FEV1/FVC (FEV1%) have all been found to be significantly lower in sulphur mustard intoxicated veterans in comparison with healthy non-exposed individuals and survivors of chemical war attack who had used a gas mask at the time of attack.[4,8,40,74] Abnormal spirometric findings in general, and restrictive patterns in particular, tend to increase over time.[4,8,74] A study was conducted on 77 victims who were present in a contaminated area and had no acute signs and symptoms at the time of exposure, showed late respiratory disorders. This study indicated that subclinical exposure to sulphur mustard could be responsible for the occurrence of delayed respiratory complications such as bronchiectasis and bronchiolitis obliterans.[10] Chest radiography in patients with late respiratory complications of sulphur mustard has been shown an increased bronchovascular markings, hyperinflation, bronchiectasis, pneumonic infiltration, and radiologic evidence of pulmonary hypertension.[8,74] However, such radiography is not sensitive enough for the detection of respiratory complications in these patients and high resolution computed tomography (HRCT) of the chest may be required as the diagnostic imaging procedure of choice.[8,76,77] A study of 197 Iranian veterans 10 years after a single heavy exposure to sulphur mustard revealed that a series of delayed destructive pulmonary sequelae were developed, such as chronic bronchitis (58%), asthma (10%), bronchiectasis (8%), large airway narrowing (9%), and pulmonary fibrosis (12%).[78] Each of these complications is described in more detail below.

1. Chronic bronchitis: Several studies have reported chronic bronchitis as the most common late complication of the respiratory system resulting from exposure to mustard gas.[8,10,40,78-82]

Hypoxemia and hypercapnia are commonly observed in moderate to severe cases, leading to cor pulmonale and respiratory failure in the final stages of the disease.[4,8,40,78] Infection of the respiratory tract, resulting in bronchopneumonia, is also a common problem often complicated by septicemia.[4,8,19]

2. Asthma: Airway hypersensitivity, manifested as typical attacks of breathlessness, wheezing, and nocturnal cough, as well as a reversible obstructive pattern on pulmonary function tests, have been reported between four weeks to 20 years after sulphur mustard inhalation. Patients with chronic bronchitis may also have some degree of bronchospasm, which does not respond to bronchodilators. Attacks of bronchospasm are characteristically triggered by respiratory infections, environmental allergens, and cold weather.[78,83-85] New techniques, such as impulse oscillometry, have been used for evaluation of airway dysfunction. However, it was found less sensitive than spirometry in spotting small airways obstructions. Impulse oscillometry is a good diagnostic method in the detection of pulmonary involvements in non-cooperative patients.[86]

3. Bronchiectasis: Direct effects of sulphur mustard on the bronchial wall mucosa and, more recurrent respiratory infections following inhalations of sulphur mustard are known to be responsible for the development of bronchiectasis. Both the severity and frequency of bronchiectatic lesions tend to increase over the long-term follow-ups, as evidenced by a study of 40 Iranian veterans with severe late complications of sulphur mustard poisoning.[8] These lesions usually begin bilaterally in the lower lobes and then progress toward the middle lobe and the lingula. In severe cases with extensive bronchiectatic lesions, pulmonary hypertension and ultimately cor pulmonale may occur.[8,87-90]

4. Large airway narrowing: Airway narrowing, resulted from scarring or granulation tissue, is a late sequel of acute injuries to the trachea and large bronchi, usually developing 2 years after exposure.[88-90] A study of 19 Iranian veterans with large airway narrowing caused by sulphur mustard, revealed stenosis in the trachea (seven patients), main bronchi (eight patients), and lobar bronchi (four patients).[88] In contrast to stenosis caused by prolonged intubations, there is no predilection in the right main bronchus.[85,88] The major problem in these patients is the recurrence of the lesion, which usually occurs 6 months after treatment.[89]

5. Pulmonary fibrosis: Late onset pulmonary fibrosis has been reported in several Iranian veterans with exposure to sulphur mustard.[85,89] The analysis of bronchoalveolar lavage fluid from patients with mustard gas inhalation showed that these patients have an ongoing local inflammatory process of the lower respiratory tract resulting in the development of pulmonary fibrosis years after the initial exposure. Histopathological examination of transbronchial lung biopsies of veterans exposed to sulphur mustard revealed variegated fibrosis, diffuse fibrosis, and an absence of fibrosis in 86%, 4%, and 10% of the

patients, respectively. Usual interstitial pneumonitis accounted for 97% of all cases of fibrosis.[78] In another study, electron microscopic examination of seven transbronchial lung biopsies was carried out in a WHO research center in Japan. Abnormal findings included: proliferation, desquamation, and degeneration of the bronchial epithelial cells, interstitial fibrosis or fibrosing alveolitis and an increased type I and type II alveolar epithelial cells as well as hyperplasia of ciliated and goblet cells.[90] Inflammation and fibrotic processes in the lung tissue of patients exposed to sulphur mustard may be progressive.[58] Diffusing capacity of the lung could be used as an objective monitor of the degree of fibrosis and also as a good predictor of prognosis.[78] A clinical review on the respiratory complications of sulphur mustard has been published in June 2007 in Iranian Journal of Medical Sciences.[74]

Dermal

The occurrence and persistence of lesions after exposure to sulphur mustard are directly related to the duration and severity of exposure. Injury that results in erythema and edema without vesicle formation is almost always followed by a complete healing and no residual effects.[4,91] Blistering and necrotic wounds, however, cause permanent residual effects. The first report of delayed toxic effects of sulphur mustard poisoning two years after exposure, in 236 Iranian veterans, revealed late skin effects such as hyperpigmentation (34%), hypopigmentation (16%), and dermal scarring (8%).[22] The most common skin complaint among these patients was itching followed by a burning sensation and desquamation. These symptoms are basically caused by dryness of the skin and thus become worse in dry weather and after physical activity. A more recent study of 40 Iranian veterans, who were heavily exposed to the gas 16 to 20 years previously, revealed the most common cutaneous lesions as hyperpigmentation, erythematous papular rash, dry skin, multiple cherry angiomas, atrophy, hypopigmentation, and hypertrophy.[8] These lesions were found on the genital areas (48%), the back (48%), the front thorax and abdomen (44%), lower extremities (mainly inguinal) (44%), upper extremities (mainly auxiliary) (41%), and the head and neck (15%). Dry skin was more prominent in the extremities. Hyperpigmentation in some patients had the appearance of pigmented xerodermoid, which is a diffuse hyperpigmented area with superimposed macular hypo- and hyperpigmentations.[8,14]

In another study, the cutaneous lesions of 500 Iranian veterans exposed to sulphur mustard were compared with 500 of unexposed veterans. An association was found between the exposure and late skin lesions such as severe dry skin, hyper- and hypopigmentation, local hair loss, eczema, and chronic urticaria.[92] There was also a higher incidence of vitiligo, psoriasis, and discoid lupus erythematosus among the poisoned patients. This could be resulted from the immunological basis of these disorders and the fact that sulphur mustard has adverse long-term effects on the immune system. Previously injured sites have been reported to be sensitive to subsequent mechanical injury and showed recurrent blistering after mild injury.[92]

Histopathological examination of skin biopsies has revealed non-specific findings including epidermal atrophy, keratosis, and basal membrane hyperpigmentation. Non-specific fibrosis and melanophages have also been observed within the dermis.[8,14,92] Occupational exposure to sulphur mustard has been demonstrated to cause a variety of skin changes, including pigmentary disorders, skin ulcers, and cutaneous cancers.[93]

Ophthalmologic

In less than 1% of patients with battlefield exposure to sulphur mustard, a delayed type of ulcerative keratopathy may develop, leading to late-onset blindness.[94-98] The maximum delayed toxic effects usually occur 15 to 20 years after initial exposure, although latency periods as long as 40 years or as short as 6 years have also been reported.[16,98,99] Patients are usually symptom-free when delayed keratitis, characterized by photophobia, lacrimation, and failing vision develops.[97] In acute stages, the limbal region frequently presents a marbled appearance in which porcelain like areas of ischemia is surrounded by blood vessels of irregular diameter. Later, vascularized scars of the cornea are covered with crystal and cholesterol deposits, leading to a worsening of the opacification, recurrent ulcerations, and sometimes corneal perforation. Opacification of the cornea is seen predominantly in the lower and central portions, whereas the upper part is often protected by the eyelid.[97,99] Surprisingly, lesions even recur after corneal transplantation.[98] The exact pathogenesis of this condition is unknown, but degenerative processes and immune reactions against corneal proteins (collagen-mustard compound) have been suggested as the cause of long-term damage.[99] Unfortunately, there has been no report on any long-term studies on mustard gas workers to determine their ocular status after prolonged occupational exposure.

Psychiatric Complications

Casualties from WWI and from the Iran-Iraq conflict were noted to have long-term mood and anxiety disorders, as well as post-traumatic stress disorder (PTSD).[22,100] Debility, loss of vitality, impaired concentration, sensory hypersensitivity, diminished libido, weakened potency, neuralgic complaints, and disorders in autonomic regulation are the common manifestations. Neuropsychiatric evaluation of 1428 Iranian veterans, 3 to 9 years after exposure to sulphur mustard, revealed anxiety (15%), depression (46%), personality disorders (31%), convulsions (6%), and psychosis (3%).[38] Disorders of consciousness (27%), attention (54%), emotion (98%), behavior (80%), thought process (14%), and memory (80%) were studied in 70 patients, 3 to 5 years after exposure to sulphur mustard.[22] Depression and post-traumatic stress in Iranian survivors of chemical warfare, mostly sulphur mustard exposure, were also reported.[98,101] In another study, decreased libido and impotence were recorded in 52% and 9% of patients, respectively. Quite interestingly, 10% of the patients revealed an increased libido. Functional photophobia, functional aphonia, and effort syndrome have also been reported.[8]

Neuromuscular Conditions

Electromyography and nerve conduction velocity, on 40 Iranian veterans with severe late manifestations of sulphur mustard poisoning, revealed abnormalities in the peripheral nervous system of 77.5% of the patients. Disturbances in nerve conduction velocity were more common in sensory nerves compared with motor nerves and more prevalent in the lower extremities than in the upper extremities. Electromyographic recordings revealed a normal pattern in 24 (60%) patients, incomplete interference with normal amplitude in 6 (15%) patients, and incomplete interference with low amplitude in 10 (25%) patients. Disturbances in nerve conduction velocity and electromyography in both upper and lower extremities were mostly symmetric.[85]

Immunologic and hematopoietic myelosuppression is the most serious effect of sulphur mustard. Sulphur mustard can cause long term effects on the immune system in patients with severe intoxication. The impaired immunity is probably responsible for the increased risk of infections in these patients. Forty men with the mean age 43.8±9.8 years, who had confirmed as having sulphur mustard poisoning 16 to 20 years before the study, were investigated. Significant changes of haematological and immunological parameters in these patients compared with 25 controls are summarized in table 2.[51]

Mutagenicity

A two-generation study of rats indicated that mustard gas was not teratogenic.[37] There has been no other report on the teratogenicity of sulphur mustard and no clear evidence of this problem after human exposure has been made, although there have been some claims.

Carcinogenicity

Sulphur mustard is genotoxic because of its reactions with DNA, which is an important first step in carcinogenesis. Although most cells possess effective DNA repair mechanisms, these are not always effective in the case of sulphur mustard damage. Alkylation of O6-guanine by sulphur mustard seems to be critical. O6-ethylthioethylguanine is a poor substrate for the DNA repair enzyme O6-alkylguanine-DNA alkyltransferase.[102] Therefore, this O6-lesion may be the most important mutagenic lesion. However, only limited data are available on the specific mutations produced by sulphur mustard. Mutations in a tumor suppressor or an oncogene gene can favour a proliferate advantage of a clonal cell. Notably, alterations in the p53 tumor suppressor gene have been described in Japanese mustard gas workers.[103]

Table 2: Significant changes of haematological and immunological parameters in 40 patients with severe sulphur mustard intoxication compared with 25 controls.

	Patients Mean ± SD	Controls Mean ± SD	P value
WBC count 1000/ mm3	7.24 ± 1.90	5.79 ± 1.12	0.025
RBC count Million/mm3	5.46 ± 0.45	5.19 ± 0.28	0.035
Monocyte %	4.8 ± 1.6	3.9 ± 1.1	0.013
HCT %	48.3 ± 3.5	45.5 ± 1.9	0.047
IgM mg/dl	235.3 ± 84.8	136.8 ± 58.3	0.0001
C3 Micg/dl	109.8 ± 30.1	90.9 ± 14.8	0.03
CD3%	71.1 ± 8.6	65.6 ± 10.7	0.037
CD16+5 (NK cells)	11.6 ± 5.8	17.5 ± 9.6	0.006

WBC: white blood cell, RBC: red blood cell, HCT: hematocrit, SD: standard deviation

However, most of the lesions in this population were similar to smoking related mutations. Mutations in lymphocytes at the hypoxanthine phosphoribosyltransferase (hprt) gene locus have also been reported.[104]

Reproductive

The effects of exposure to sulphur mustard during pregnancy are unknown. Data addressing the productive toxicities of sulphur mustard in human models are both lacking and contradictory.[105]

Cardiotoxicity

There has been only one report on the cardiotoxicity of sulphur mustard in Iranian veterans.[106] Myocardial perfusion scans of 22 consecutive patients intoxicated with sulphur mustard (21 men and one woman, all < 44 years) more than 15 years before the study was compared with 14 controls. Pattern of myocardial perfusion in intoxicated patients was significantly different from normal controls resembling either coronary artery disease or mild cardiomyopathic changes.[106]

Management

Specific

No effective antidote has been developed for the treatment of sulphur mustard poisoning in human beings. Sodium thiosulfate and N-acetylcysteine have been considered, although the acute clinical efficacy of these agents has yet to be established. Sodium thiosulfate infusion (10%) may prevent the toxic manifestations of sulphur mustard, providing it is administered immediately after an exposure and no later than 30 minutes after exposure.[107,108]

In a recent study,[109] polymerase inhibitors, anti-inflammatory drugs, antioxidants, matrix metalloproteinase inhibitors, and probably regulators of DNA damage repair were identified as promising approaches to improve treatment. In another report,[110] the new ways of treatment were about N-acetyl cysteine for lung injury, poly (ADP-ribose) polymerase inhibitors, calmodulin antagonists and calcium chelators. Therapeutic effects of all these medications have not yet been confirmed in humans.

No specific treatment has been described for the delayed toxic effects and complications of sulphur mustard in different target organs. However, recent clinical studies revealed that macrolides such as azithromycin and antioxidants may improve respiratory symptoms and pulmonary function. Interferon gamma could improve the pulmonary function of such patients with bronchiolitis.[4,74,85] Therapeutic effects of these compounds have yet to be confirmed.

Supportive

Supportive care focuses on the prevention and treatment of infection and reduction of pain. Given the range of chronic health effects of sulphur mustard, patients are best managed by multidisciplinary clinical teams of specialists. Financial, social, and cultural support together with health education to maintain a good life style is of great importance.[4] It was shown that the Iranian chemically injured veterans suffering from severe itching have a significantly poorer quality of life than do patients with milder symptoms.[111]

Protections

Apart from personal physical protection (special suit, mask with charcoal filter, proper gloves and boots), chemical protection using antioxidants and medications such as sodium thiosulfate and hexamethylenetetramine (HMT) are recommended. HMT has been shown to protect human lung cells against sulphur mustard. The ability of HMT to protect against the toxicity of sulphur mustard was investigated in the human upper respiratory tract cell lines BEAS-2B and RPMI 2650. Sulphur mustard was highly toxic to both cell lines, with LC50 values of 15-30 microM. HMT, at a concentration of 10 mM, was shown to protect the cell lines against the toxic effects of 20 microM and 40 microM of sulphur mustard. Results demonstrated that it was necessary for HMT to be in situ at the time of exposure to sulphur mustard for effective cytoprotection. No protection was seen when cells were treated with HMT following exposure to sulphur mustard, or where HMT was removed before exposure to the agent. It was thus suggested that HMT may be effective prophylaxis for exposure to sulphur mustard by inhalation.[112]

Conclusions and Recommendations

Sulphur mustard was used as an incapacitating warfare agent in the past century, particularly in the WWI and the Iraq-Iran conflict. It has proved to have long-lasting toxic effects. This may entice further use of the agent in future military and terrorist attacks. Sulphur mustard exerts its toxicity through a number of postulated pathogenic mechanisms including DNA alkylation, NAD depletion, and inactivation of glutathione. The eyes, skin, and respiratory system are the three major targets for direct toxic effects of sulphur mustard. When absorbed in large amounts, it can also damage rapidly the proliferating cells of the bone marrow, causes

severe suppression of the immune system, and systemic toxicities such as neurological and digestive disorders. Even more important is a wide range of chronic health effects including chronic bronchitis, bronchiectasis, frequent bronchopneumonia, and pulmonary fibrosis, all of which tend to deteriorate by time. Severe dry skin, delayed keratitis, and pathogenic status of cell mediated immunity with a subsequent increased risk of infections and even possible malignancies are also among the most distressing long-term consequences of sulphur mustard intoxication. However, because of major shortcoming in the sulphur mustard literature on cardiovascular complications, further studies on sulphur mustard intoxicated patients are required. Immunological and psychological dysfunctions and the relationship between exposure to sulphur mustard and carcinogenesis and teratogenesis are important fields that require further investigations.

There is also a paucity of information regarding medical management of acute and delayed toxic effects of such poisoning, a subject which greatly challenges health-care specialists.

It is hoped that by the advancement of the Organization for Prohibition of Chemical Weapons (OPCW), no chemical war agent be used in the future. However, chemical terrorism involving the use of sulphur mustard remains a major threat to human health globally. Thus, further studies on this subject are recommended.

Acknowledgement

Financial support by the Iranian chapter of the Academy of Sciences for Developing World (TWAS) at the International Centre for Scientific Cooperation, Ministry of Science and Technology of Islamic Republic of Iran for preparation of this manuscript is greatly appreciated.

Conflict of Interest: None declared

References

1 Pechura C M, Rall DP. Chemistry of Sulfur Mustard and Lewisite. In: Veterans at Risk: The Health Effects of Mustard Gas and Lewisite, Institute of Medicine, The National Academies Press, Washington D C, USA, 1993. p. 71-80.

2 Sidell F R, Takafuji E T, Franz D R. Vesicants. In: Zajtchuk R, Bellamy RF, eds. Medical aspects of chemical and biological warfare, Published by the Office of The Surgeon General at TMM Publications, Borden Institute, Walter Reed Army Medical Center, Washington, DC, USA, 1997. p. 197-228.

3 Prentiss A.M. Vesicant agents. IN: Chemicals in warfare: a treatise on chemical warfare. McGraw-Hill, New York, USA, 1937. p. 177-300.

4 Balali-Mood M, Hefazi M. The pharmacology, toxicology, and medical treatment of sulphur mustard poisoning. *Fundam Clin Pharmacol* 2005; 19: 297-315.

5 United Nations Security Council. Report of the mission dispatched by the Secretary General to investigate allegations of the use of chemical weapons in the conflict between the Islamic Republic of Iran and Iraq. S/19823 and S/19823/Addendum 1. United Nations, New York, USA. April 25, 1988.

6 Balali-Mood M, Hefazi M. Comparison of early and late toxic effects of sulfur mustard in Iranian veterans. *Basic Clin Pharmacol Toxicol* 2006; 99: 273-82.

7 Mandel M, Gibson WS. Clinical manifestations and treatment of mustard gas poisoning. *J Am Med Assoc* 1917; 69: 1970-1.

8 Balali-Mood M, Hefazi M, Mahmoudi M, et al. Long-term complications of sulphur mustard poisoning in severely intoxicated Iranian veterans. *Fundam Clin Pharmacol* 2005; 19: 713-21.

9 Hurst CG, Smith WJ. Chronic effects of acute low level exposure to the chemical warfare agent sulphur mustard. In: Somani S M and Romano J A (Eds) Chemical Warfare Agents: Toxicity at Low Levels, CRC Press, London, 2001. p. 245-60.

10 Ghanei M, Fathi H, Mohammad MM, et al. Long-term respiratory disorders of claimers with subclinical exposure to chemical warfare agents. *Inhal Toxicol* 2004; 16: 491-5.

11 Brown EC. Pulmonary effects following chronic exposure to HS vapor. In: Medical Division Report No. 187**.** U.S. Army Chemical Centre*,* Washington*,* DC*,* USA; 1949. p. 1-31*.*

12 Nishimoto Y, Burrows B, Miyanishi M, et al. Chronic obstructive lung disease in Japanese poison gas workers. *Am Rev Respir Dis* 1970; 102: 173-9.

13 Hefazi M, Attaran D, Mahmoudi M, Balali-Mood M. Late respiratory complications of mustard gas poisoning in Iranian veterans. *Inhal Toxicol* 2005, 17: 587-92.

14 Hefazi M, Maleki M, Mahmoudi M, Tabatabaee A, Balali-Mood M. Delayed complications of sulfur mustard poisoning in the skin and the immune system of Iranian veterans 16-20 years after exposure. *Int J Dermatol* 2006; 45: 1025-31.

15 Pickard HL. Ocular action of dichloroethyl sulfide (mustard gas). *Am J Ophthalmol* 1919; 3: 136.
16 Etezad-Razavi M, Mahmoudi M, Hefazi M, Balali-Mood M. Delayed ocular complications of mustard gas poisoning and the relationship with respiratory and cutaneous complications. *Clin Experiment Ophthalmol* 2006; 34: 342-6.
17 Anslow WP, Houk CR. Systemic pharmacology and pathology of sulfur and nitrogen mustards. In: Anslow WP, Houk CR, eds. Chemical Warfare Agents and Related Chemical Problems. Washington, DC, USA: National Defense Research Committee; 1946. p. 440-78.
18 World Health Organization. Health aspects of chemical and biological weapons. World Health Organization, Geneva, Switzerland, 1970.
19 Balali-Mood M, Farhoodi M, Panjvani FK. Report of three fatal cases of war gas poisoning. In: Heyndrickx A, ed. Proceedings of the Second World Congress on New Compounds in Biological and Chemical Warfare. Ghent, Belgium: Rijksuniversiteit, 1986. p. 475-82.
20 Balali-Mood M, Navaeian A. Clinical and paraclinical findings in 233 patients with sulfur mustard poisoning. In: Heyndrickx A, ed. Proceedings of the Second World Congress on New Compounds in Biological and Chemical Warfare. Ghent, Belgium: Rijksuniversiteit, 1986, p. 464-73.
21 Easton DF, Peto J, Doll R. Cancers of the respiratory tract in mustard gas workers. *Br J Ind Med* 1988; 45: 652-9.
22 Balali-Mood M. First Report of Delayed toxic effects of Yperite poisoning in Iranian fighters, Proc. Int Assoc Foren Toxicol 1986. p. 474-9.
23 Walker IG. Intrastrand bifunctional alkylation of DNA in mammalian cells treated with mustard gas. *Can J Biochem* 1971; 49: 332-6.
24 Ball CR, Roberts JJ. Estimation of interstrand DNA crosslinking resulting from mustard gas alkylation of HeLa cells. *Chem Biol Interact* 1972; 4: 297-303.
25 Wheeler GP. Studies related to the mechanisms of action of cytotoxic alkylating agents: a review. *Cancer Res* 1962; 22: 651-88.
26 Rankin PW, Jacobson MK, Mitchell VR, Busbee DL. Reduction of nicotinamide adenine dinucleotide levels by ultimate carcinogens in human lymphocytes. *Cancer Res* 1980; 40: 1803-7.
27 Eklöw L, Moldéus P, Orrenius S. Oxidation of glutathione during hydroperoxide metabolism: a study using isolated hepatocytes and glutathione reductase inhibitor 1, 3-bis(2-chloroethyl)-1-nitrosurea. *Eur J Biochem* 1984; 138: 459-63.
28 Cullumbine H. Medical aspects of mustard gas poisoning. *Nature* 1947; 159: 151-3.
29 Langenberg JP, van der Schans GP, Spruit H.E, et al. Toxicokinetics of sulphur mustard and its DNA-adducts. *Drug Chem Toxicol* 1998, 21: 131-47.
30 Chilcott RP, Jenner J, Carrick W, et al. Human skin absorption of Bis-2-(chloroethyl) sulphide (sulphur mustard) in vitro. *J Appl Toxicol* 2000; 20: 349-55.
31 Davison C, Rozman RS, Smith PK**.** Metabolism of bis-beta-chloroethyl sulfide (sulfur mustard gas). *Biochem Pharmaco1* 1961; 7: 65-74.
32 Kehe K, Szinicz L. Medical aspects of sulphur mustard poisoning. *Toxicology* 2005; 214: 198-209.
33 Clemedson CJ, Kristoffersson H, Soerbo B, Ullberg S. Whole body autoradiographic studies of the distribution of sulphur 35-labelled mustard gas in mice. *Acta Radiol Ther Phys Biol* 1963; 1: 314-20.
34 Drasch G, Kretschmer G, Kauert L, von Meyer L. Concentration of mustard gas [bis(2-chloroethyl)sulfide] in the tissues of a victim of a vesicant exposure. *J Forensic Sci* 1987; 32: 1788-93.
35 Roberts JJ, Warwick GP. Studies of the mode of action of alkylating agents. VI. The metabolism of bis-2-chlorethylsulphide (mustard gas) and related compounds. *Biochem Pharmacol* 1963; 12: 1329-34.
36 Sasser LB, Cushing JA, Dacre JC. Two-generation reproduction study of sulfur mustard in rats. *Reprod Toxicol* 1996; 10: 311-9.
37 Pechuta CM, Rall DP. Relationship of mustard agent and Lewisite exposure to carcinogenesis. In: Pechuta CM, Rall DP, eds. Veterans at Risk, the Health Effects of Mustard Gas and Lewisite. Washington DC, USA: National Academy Press; 1993. p. 81-111.
38 Wada S, Miyanishi M, Nishimoto Y**,** et al. Mustard gas: a cause of respiratory neoplasia in man. *Lancet* 1968; 2: 1161-3.
39 Manning KP, Skegg DC, Stell PM, Doll R. Cancer of the larynx and other occupational hazards of mustard gas workers. *Clin Otolaryngol* 1981; 6: 165-70.
40 Balali-Mood M. Evaluation of late toxic effects of sulfur mustard poisoning in 1428 Iranian veterans. The Seminar on Late Complications of Chemical Warfare Agents in Iranian Veterans. Tehran, Iran: Veteran Foundation; 1992. p. 15-37.

41 Inada S, Hiragun K, Seo K, Yamura T. Multiple Bowen's disease observed in former workers of a poison gas factory in Japan with special references to mustard gas exposure. *J Dermatol* 1978; 5: 49-60.
42 Graef I, Karnofsky DA, Jaeger V, et al. The clinical and pathologic effects of the nitrogen and sulfur mustards in laboratory animals. *Am J Pathol* 1948; 24: 1-47.
43 Giraud H. The first symptoms of intoxication from mustard gas. *J Med Chir Prat* 1917; 88: 890-4.
44 Helm UK, Balali-Mood M. Cutaneous lesions produced by sulfur mustard. The First International Medical Congress on Chemical Warfare Agents in Iran. June 13-6, Mashhad, Iran, Mashhad University of Medical Sciences, 1988. p. 90.
45 Papirmeister B, Feister AJ, Robinson SI. Medical Defense against Mustard Gas: Toxic Mechanisms and Pharmacological Implications. Boca Raton, USA: CRC Press; 1991.
46 Canelli AF. Contrbuto alla conoscenza dell' intossicazione acuta da "Yperite" ed in particolare del suo reperto anatomo-patologico. *Rivista Ospedaliera Italiana* 1918; 8: 2-7.
47 Thomsen AB, Erkisen Y, Smidt-Nielsen K. Chronic neuropathic symptoms after exposure to mustard gas: a long-term investigation. *J Am Acad Dermatol* 1988, 39: 187-90.
48 Willems JL. Clinical management of mustard gas casualties. *Ann Med Mil Belg* 1989; 3: S1-S61.
49 Balali-Mood M, Tabarestani M, Farhoodi M, Panjvani FK. Study of clinical and laboratory findings of sulfur mustard in 329 war victims. *Med J I R* 1991; 34: 7-15.
50 Tabarestani M, Balali-Mood M, Farhoodi M. Hematological findings of sulfur mustard poisoning in Iranian combatants. *Med J Islam Repub Iran* 1990; 4: 185-90.
51 Mahmoudi M, Hefazi M, Rastin M, Balali-Mood M. Long-term hematological and immunological complications of sulfur mustard poisoning in Iranian veterans. *Int Immunopharmacol* 2005; 5: 1479-85.
52 Tabarestani M, Farhoudi M, Balali-Mood M. Stem cell and erythroid precursors disorders in three patients with sulfur mustard poisoning. The First International Medical Congress on Chemical Warfare Agents in Iran. June 13-6 Mashhad, Iran, Mashhad University of Medical Sciences, 1988 .p. 10.
53 Krumbhaar EB, Krumbhaar HD. The blood and bone marrow in yellow cross gas (mustard gas) poisoning. *J Med Res* 1919; 40: 497-506.
54 Dayhimi I, Bahar K, Eliasy H. The effect of sulfur mustard gas (SMG) on the immune system. The First International Medical Congress on Chemical Warfare Agents in Iran. June 13-6, Mashhad, Iran: Mashhad University of Medical Sciences; 1988. p. 12.
55 Zandieh T, Marzaban S, Tarabadi F, Ansari H. Defects of cell-mediated immunity in mustard gas injury after years. (Abstract). *Scand J Immunol* 1990; 32: 423.
56 Ghotbi L, Hassan Z. The immunostatus of natural killer cells in people exposed to sulphur mustard. *Int Immunopharmacol* 2002; 2: 981-5.
57 Dacre J.C., Goldman M. Toxicology and pharmacology of the chemical warfare agent sulphur mustard. *Pharmaco Rev* 1996; 48: 289-326.
58 Aghanouri R, Ghanei M, Aslani J, et al. Fibrogenic cytokine levels in bronchoalveolar lavage aspirates 15 years after exposure to sulfur mustard. *Am J Physio Lung Cell Mo Physiol* 2004; 287: L1160-4.
59 WHO. Health Aspects of Chemical and Biological warfare Agents, world Health Organization, December 2003.
60 Heyndrickx A. Analytical methods for detection and determination of sulphur mustard in environmental and biologicalsamples. In: Heyndrickx B, ed. Proceedings of the Second World Congress on New Compounds in Biological and Chemical Warfare. Ghent, Belgium, Rijksuniversiteit; 1986. p. 112-28.
61 Noort D, Benschop HP, Black RM. Biomonitoring of exposure to chemical warfare agents: a review. *Toxicol Appl P*harmacol 2002; 184: 116-26.
62 Fidder A, Moes GWH, Scheffer AG, et al. Synthesis, characterization, and quantitation of the major adducts formed between sulfur mustard and DNA of calf thymus and human blood. *Chem Res Toxicol* 1994; 7: 199-204.
63 van der Schans GP, Scheffer AG, Mars-Groenendijk RH, et al. Immunochemical detection of adducts of sulfur mustard to DNA of calf thymus and human white blood cells. *Chem Res Toxicol* 1994; 7: 408-13.
64 Benschop HP, van der Schans GP, Noort D, et al. Verification of exposure to sulfur mustard in two casualties of the Iran- Iraq conflict. *J Anal Toxicol* 1997; 21: 249 -51.
65 Black RM, Brewsterk K, Clarke RJ, et al. Biological fate of sulfur mustard, 1,1'-thiobis(2-chloroethane): isolation and identification of urinary metabolites following intraperitoneal administration to rat. *Xenobiotica* 1992, 22: 405-18.

66 Fidder A, Noort D, de Jong LP, et al. N7-(2-hydroxyethylthioethyl)-guanine: a novel urinary metabolite following exposure to sulphur mustard. *Arch Toxicol* 1996; 70: 854-5.
67 Black RM, Read RW. Biological fate of sulphur mustard, 1,1'-thiobis(2-chloroethane): identification of beta-lyase metabolites and hydrolysis products in human urine. *Xenobiotica* 1995; 25: 167-73.
68 Wills ERJ, Hulst AJ, De Jong AL, et al. Analysis of thiodiglycol in urine of victims of an alleged attack with mustard gas. *J Anal Toxicol* 1985; 9: 254-7.
69 Wills ER, Hulst AJ, van Laar J. Analysis of thiodiglycol in urine of victims of an alleged attack with mustard gas, part II. *J Anal Toxicol* 1988; 12: 15-9.
70 Black RM, Read RW. Detection of trace levels of thiodiglycol in blood, plasma, and urine using gas chromatography electron capture negative ion chemical ionization mass spectrometry. *J Chromatogr* 1988; 449: 261-70.
71 Black RM, Read RW. Improved methodology for the detection and quantitation of Urinary metabolites of sulfur mustard using gas chromatography tandem mass spectrometry. *J Chromatogr Biomed Appl* 1995; 665: 97-105.
72 Sulzberger MB, Katz JH. The absence of skin irritants in the contents of vesicles. *US Navy Med Bull* 1943, 43: 1258-62.
73 Black RM, Clarke RJ, Harrison JM, Read RW. Biological fate of sulphur mustard: identification of valine and histidine adducts in haemoglobin from casualties of sulphur mustard poisoning. *Xenobiotica* 1997; 27: 499-512.
74 Ghanei M, Adibi I. Clinical review of mustard lung. *Iran J Med Sci* 2007; 32: 58-65.
75 Khateri Sh, Ghanei M, Keshavarz S, et al. Incidence of lung, eye and skin lesions as late complications in 34,000 Iranian with wartime exposure to mustard agent. *J Occup Environ Med* 2003; 45: 1136-43.
76 Bagheri MH, Hosseini SK, Mostafavi SH, Alavi SA. High-resolution CT in chronic pulmonary changes after mustard gas exposure. *Acta Radiol* 2003; 44: 241-5.
77 Bakhtavar K, Sedighi N, Moradi Z. Inspiratory and Expiratory High-Resolution Computed Tomography (HRCT) in Patients with Chemical Warfare Agents Exposure. *Inhal Toxicol* 2008; 20: 507-11.
78 Emad A, Rezaian GR. The diversity of effects of sulfur mustard gas inhalation on respiratory system 10 years after a single heavy exposure: analysis of 197 cases. *Chest* 1997; 112: 734-8.
79 Ghanei M, Hosseini A R, Arabbaferani Z, Shahkarami E. Evaluation of chronic cough in chemical chronic bronchitis patients. *Environ Toxicol Pharmacol* 2005; 20: 6-10.
80 Ghanei M, Khedmat H, Mardi F, Hosseini A. Distal esophagitis in patients with mustard-gas induced chronic cough. *Dis Esophagus* 2006; 19: 285-8.
81 Ghanei M, Mokhtari M, Mohammad MM, Aslani J. Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. *Eur J Radiol* 2004; 52: 164-9.
82 Ghanei M, Moqadam FA, Mohammad MM, Aslani J. Tracheobronchomalacia and air trapping after mustard gas exposure. *Am J Respir Crit Care Med* 2006; 173: 304-9.
83 WHO. Chemical agents. In: Perry- Robinson JP, ed. Public Health Response to Biological and Chemical Weapons. 2nd ed. Geneva, Switzerland: World Health Organization; 2004. p. 164-701.
84 Bijani KH, Moghadamnia AA. Long-term effects of chemical weapons on respiratory Tract in the Iran-Iraq war victims living in Babol (North of Iran). *Ecotoxicol Environ Safe* 2002; 53: 422-4.
85 Balali-Mood M, Hefazi M, Mahmoudi M, et al. Evaluation of delayed toxic effects of Sulfur mustard poisoning in severely intoxicated Iranian veterans. *J Med CB R Def*. 2005: 3. Available from: URL: http://www.J Med Chem Def. org/issue_0301/Balali-Mood_0405.html.
86 Ghanei M. Darvishzadeh F, Alaeddini I, et al. Accuracy of Impulse Oscillometry in Airway Dysfunction. *Iran J Med Sci* 2007; 32: 205-210.
87 Hosseini K, Bagheri MH, Alavi S. Development of bronchiectasis, a late sequel of mustard gas exposure. *Iran J Med Sci* 1998; 23: 81-4.
88 Ghanei M, Akhlaghpoor A, Mohammad MM, Aslani J. Tracheobronchial stenosis following mustard gas inhalation. *Inhal Toxicol* 2004; 16: 845-9.
89 Aslani J. Late respiratory complications of sulfur mustard. In: Cheraghali AM, ed. Prevention and Treatment of Complications of Chemical Warfare Agents. Tehran, Iran: Chemical Warfare Research Center; 2000. p. 76-9.
90 Sohrabpour H. Evaluation of late toxic effects of sulfur mustard poisoning with electron microscopy of lung biopsies. [dissertation]. Shaheed Beheshti University of Medical Sciences: Tehran, Iran; 1992.
91 Warthin AS, Weller CV. Diagnosis and treatment of lesions due to vesicants.

Br Med J 1944; 2: 109 -12.
92 Fekri AR, Janghorbani M. Late cutaneous complications in Iranian veterans. The Seminar on Late Complications of Chemical Warfare Agents in Iranian Veterans. Tehran, Iran: Veteran Foundation; 1992. p. 57-89.
93 Khehr NW. Late manifestations in former mustard gas workers with special consideration of the cutaneous findings. *Zeitschrift fur Hautkrankheiten* 1984; 59: 1161-70.
94 Blodi FC. Mustard gas keratopathy. *Int Ophthalmol Clin*. 1971; 2: 1-13.
95 English F, Bennett Y. The challenge of mustard gas keratopathy. *Med J Aust* 1990, 152: 55-6.
96 Hughes WF. Mustard gas injuries to the eyes. *Arch Ophthalmol* 1942; 27: 582- 601.
97 Pleyer U, Sherif Z, Baatz H, Hartmann C. Delayed mustard gas keratopathy: clinical findings and confocal microscopy. *Am J Ophthalmol* 1999; 128: 506-7.
98 Javadi MA, Kazemi-Moghadam M. Ocular effects of sulfur mustard poisoning. In: Cheraghali AM, ed. Prevention and Treatment of Complications of Chemical Warfare Agents. Tehran, Iran: Chemical Warfare Research Center; 2000. p. 82-101.
99 Solberg Y, Alcalay M, Belkin M. Ocular injury by mustard gas. *Surv Ophthalmol* 1997; 41: 461-6.
100 Tabatabaee SM. Study of psychiatric complications of poisoning with chemical warfare agents. The First International Medical Congress on Chemical Warfare Agents in Iran. June 13-6, Mashhad, Iran: Mashhad University of Medical Sciences, 1988. p. 66.
101 Hashemian F, Khoshnood K, Desai MM, et al. Anxiety, Depression and posttraumatic stress in Iranian survivors of chemical warfare. *JAMA* 2006; 296: 560-6.
102 Ludlum DB, Kent S, Mehta JR. Formation of O6- ethylthioethylguanine in DNA by reaction with the sulfur mustard, chloroethyl sulfide, and its apparent lack of repair by O6-alkylguanine-DNA alkyltransferase. *Carcinogenesis* 1986; 7: 1203-6.
103 Takeshima Y, Inai K, Bennett WP, et al. p53 mutations in lung cancers from Japanese mustard gas workers. *Carcinogenesis* 1994; 15: 2075-9.
104 Yanagida J, Hozawa S, Ishioka S, et al. Somatic mutation in peripheral lymphocytes of former workers at the Okunojima poison gas factory. *Jpn J Cancer Res* 1988; 79: 1276-83.
105 Azizi F, Keshavarz A, Roshanzamir F, Nafarabadi M. Reproductive function in men following exposure to chemical warfare with sulfur mustard. *Medicine and war* 1995; 11: 34-44.
106 Gholamrezanezhad A, Saghari M, Vakili A, et al. Myocardial perfusion abnormalities in chemical warfare patients intoxicated with mustard gas. *Int J Cardiovasc Imaging* 2007; 23: 197-205.
107 Callaway S, Pearce KA. Protection against systemic poisoning by mustard gas, di(2-chloroethyl) sulphide, by sodium thiosulphate and thiocit in the albino rat. *Br J Pharmacol Chemother* 1958; 13: 395-8.
108 Connors TA, Jeney A, Jones M. Reduction of the toxicity of 'radiomimetic'alkylating agents in rats by thiol pretreatment. The mechanism of the protective action of thiosulfate. *Biochem Pharmacol* 1964; 13: 1545-50.
109 Kehe K, Balszuweit F, Emmler J, et al. Sulfur mustard research-strategies for the development of improved medical therapy. *Eplasty* 2008; 8: e32.
110 Mérat S, Perez JP, Rüttimann M, et al. Acute poisoning by chemical warfare agent: sulfur mustard. *Ann Fr Anesth Reanim* 2003; 22: 108-18.
111 Panahi Y, Davoudi SM, Sadr SB, et al. Impact of pruritus on quality of life in sulfur mustard-exposed Iranian veterans. *Int J Dermatol* 2008; 47: 557-61.
112 Andrew DJ, Lindsay CD. Protection of human upper respiratory tract cell lines against sulphur mustard toxicity by hexamethylenetetramine (HMT). *Hum Exp Toxicol* 1998; 17: 373-9.