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REVIEW ARTICLE

Toxic effects of chlorine gas and potential treatments: a literature review

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ABSTRACT

Chlorine gas is one of the highly produced chemicals in the USA and around the world. Chlorine gas has several uses in water purification, sanitation, and industrial applications; however, it is a toxic inhalation hazard agent. Inhalation of chlorine gas, based on the concentration and duration of the exposure, causes a spectrum of symptoms, including but not limited to lacrimation, rhinorrhea, bronchospasm, cough, dyspnea, acute lung injury, death, and survivors develop signs of pulmonary fibrosis and reactive airway disease. Despite the use of chlorine gas as a chemical warfare agent since World War I and its known potential as an industrial hazard, there is no specific antidote. The resurgence of the use of chlorine gas as a chemical warfare agent in recent years has brought speculation of its use as weapons of mass destruction. Therefore, developing antidotes for chlorine gas-induced lung injuries remains the need of the hour. While some of the pre-clinical studies have made substantial progress in the understanding of chlorine gas-induced pulmonary pathophysiology and identifying potential medical countermeasure(s), yet none of the drug candidates are approved by the U.S. Food and Drug Administration (FDA). In this review, we summarized pathophysiology of chlorine gas-induced pulmonary injuries, pre-clinical animal models, development of a pipeline of potential medical countermeasures under FDA animal rule, and future directions for the development of antidotes for chlorine gas-induced lung injuries.

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Chlorine gas

Chlorine (Cl_2) is a yellowish-green gas with the strong odor of bleach at room temperature. At temperatures below –30 °F/–34 °C or at higher pressure, chlorine is a clear to amber-colored liquid. Chlorine is one of the most highly produced and used chemicals around the world, and is among the top 10 chemicals produced in the USA. Chlorine is used for water treatment, bleaching of paper, industrial manufacture of several chemicals, and for many other purposes (https://www.chlorineinstitute.org/stewardship/chlorine/chlorine-applications/, accessed 14 January 2019). In the hospital setting, especially in developing countries, bleach plays an important role in sanitation, particularly to kill viral contaminations and prevent nosocomial infections.

Accidental chlorine exposures are frequently encountered in domestic and occupational environments. The immediate signs and symptoms of chlorine exposure include eye redness and lacrimation, nose and throat irritation, cough, suffocation, the sensation of choking, and dyspnea. Although inhalation exposures are the most common, ocular and cutaneous exposures also cause deleterious effects. Exposure to the skin leads to burning, blistering, and frostbite-like injuries.

Chlorine gas as a chemical threat agent

Chlorine gas has been used as a chemical weapon since World War I. More than 150 tons of chlorine gas was released by German troops against Allied Forces from approximately 6000 gas cylinders on 22 April 1915, in Ieper, Belgium. This attack killed up to 5000 and caused injuries on both sides (https://www.un.org/press/en/2015/sc12001.doc.htm, accessed on 16 February 17). Soldiers described the smell of chlorine gas as a distinct mix of pepper and pineapple.

Since then, there have been several reports on the use of chlorine gas in Iran, Iraq, and Syria. During Operation Iraqi Freedom in 2007, insurgents in Iraq repeatedly detonated chlorine tanker trucks outfitted with explosives, resulting in multiple deaths and casualties (Jones et al. 2010). The UN-supported OPCW fact-finding missions proved that Syria used chlorine gas allegedly on the civilian population (https://www.opcw.org/news/article/opcw-fact-finding-mission-compelling-confirmation-that-chlorine-gas-used-as-weapon-in-syria/ accessed on 14 October 16; https://www.bbc.com/news/world-middle-east-43697084 accessed on 30 October 18). Schneider and Lutkefend's public policy report 'Nowhere to Hide: The Logic of Chemical Weapons Use in Syria' concluded that Assad's regime used improvised chlorine munitions, and accounted for at least 89% of all chemical attacks during the Syrian war (Schneider and Lütkefend 2019). The chlorine munitions were deployed using either barrel or lob bombs.

To estimate the gravity of Cl_2, Homeland Security predicted that if a tank of highly compressed chlorine gas exploded upwind of a populated area with approximately

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700 000 individuals, at least 5% (35 000) would be exposed to lethal doses of Cl_2, and half of those victims would die of respiratory failure. An additional 15% (105 000) would be hospitalized, and 64% would seek medical treatment at local hospitals (http://www.globalsecurity.org/security/library/report/2004/hsc-planning-scenarios-jul04_08.htm accessed on 13 October 2016). Similar predictions of fatalities with chlorine gas release under less extreme conditions were modeled elsewhere (Barrett 2009). As Cl_2 is easy to manufacture with salt and water (http://www.eurochlor.org/the-chlorine-universe/how-is-chlorine-produced.aspx, accessed on 19 November 2018) and easy to deploy as a potential chemical weapon of mass destruction (WMD) (Jones et al. 2010), developing a countermeasure to treat lung injuries caused by chlorine remains one of the highest priorities of the Biomedical Advanced Research and Development Authority (BARDA) Chemical Medical Countermeasures Program.

Chlorine gas in transportation and occupational incidents

Accidental exposure to chlorine gas in domestic and occupation settings is another big concern. Chlorine gas has been released accidentally in the USA in several incidents. It is estimated that chlorine accounts for 84% of the total toxic industrial hazards transported every year (http://www.ipd.anl.gov/anlpubs/2001/01/38251.pdf, accessed 22 February 17) (Branscomb et al. 2010). Thirty-six percent of chlorine accidents have led to emergency room visits (Kales et al. 1997). The 2005 train derailment in Graniteville, SC was considered one of the largest transportation incidents in the USA. Nine people died and 200 were admitted to the hospital for inhalation pulmonary injuries (CDC 2005; Balte et al. 2013; Mackie et al. 2014). Based on the hazardous release of chlorine gas in the Graniteville train derailment, detailed epidemiologic modeling of chlorine plumes was described (Jani et al. 2016). Other occupational and transportation incidents of a massive chlorine leak were in Tacoma, WA on 12 February 2007 and Las Vegas, NV on 29 August 2007. Although no fatalities were reported in either of these incidents, the seventh largest container port at Tacoma, WA was closed due to the chlorine gas leak.

Clinical presentation of chlorine gas-induced lung injuries

Chlorine gas causes a spectrum of clinical symptoms depending on the concentration of chlorine gas, duration of exposure, minute ventilation of the victims, and individual victim characteristics such as age, sex, physical characteristics, preexisting diseases, and cigarette smoking (Das and Blanc 1993; White and Martin 2010; Runkle et al. 2013). In 2001, Winder summarized the clinical manifestations of chlorine gas on the pulmonary system (Winder 2001; Evans 2005; White and Martin 2010). Although symptoms can vary widely in humans, low exposure (1–30 ppm) for up to one-hour results in mild to moderate mucous membrane irritation, whereas higher exposure (30 ppm and slightly above) causes chest pain, dyspnea, and cough. Acute pulmonary edema typically develops at 40–60 ppm. Concentrations above 400 ppm are usually fatal over 30 minutes, whereas levels above 1000 ppm are typically fatal within minutes (White and Martin 2010). Mortality rates vary depending on the level of exposure but typically range from 1% to 2% (Van Sickle et al. 2009). When a death occurs within a few hours of exposure, the cause is primarily attributed to pulmonary edema and hemorrhage. The National Institute for Occupational Safety and Health (NIOSH) suggests that a chlorine concentration of 10 ppm is considered dangerous to health or life. Although symptoms of moderate exposure to Cl_2 gas resolve, several epidemiologic studies on victims of the chlorine gas leak in the Graniteville, SC train derailment incident show long-term respiratory problems (Runkle et al. 2013; Abara et al. 2014; Mackie et al. 2014). Govier and Coulson systematically reviewed civilian exposure to chlorine gas, and reported clinical features (in the order of decreasing incidence rate) of cough, dyspnea, sore throat, eye irritation, excessive sputum or hemoptysis, wheeze, nausea or vomiting, headache, and non-cardiogenic pulmonary edema/acute respiratory distress syndrome (ARDS) (Govier and Coulson 2018). Underlying comorbidities such as respiratory syncytial virus infection potentiate Cl_2-induced lung injury (Song et al. 2015). Although no prospective studies were conducted to evaluate the influence of cigarette smoking on outcomes after exposure to chlorine gas, cigarette smokers will be at higher risk for severe injuries from chlorine gas inhalation.

Mechanism of chlorine toxicity

The anatomical localization of toxicity of an irritant gas depends on the solubility of the gas in water. Unlike ammonia (highly water-soluble) and phosgene (limited water solubility), which cause upper and lower respiratory problems, respectively, chlorine gas has intermediate water solubility, which can lead to both upper and lower respiratory damage (Evans 2005; Summerhill et al. 2017). Few studies have hypothesized that chlorine gas is a highly water-soluble gas, upper airways are the major site of absorption, and most of the gas is scrubbed in the nasal mucosa (Wolf et al. 1995; Nodelman and Ultman 1999).

Chlorine gas reacts with mucus on the epithelial lining to form hydrochloric acid and hypochlorous acid (HOCl). Hypochlorite is an oxidative product of chlorine gas that plays an important role in mediating the effects of chlorine gas. Hydrochloric acid is 30-fold less toxic than chlorine gas. Indeed, the acidic nature of chlorine gas is not primarily responsible for its effects but rather its strong oxidative properties (Morris et al. 2005).

$$Cl_2 + H_2O \rightleftarrows HCl + HOCl$$
$$2HOCl \rightleftarrows 2HCl + O_2$$

In vitro studies have shown that HOCl forms reactive intermediates that can nitrate, chlorinate, and dimerize aromatic amino acids by reacting with nitrite (NO_2^-, the auto-oxidation product of nitric oxide (NO)). Previous publications have shown schemes of some of the potential reactions that form

reactive oxygen and nitrogen species upon inhalation of chlorine (Evans 2005; White and Martin 2010).

Recent studies have also shown mitochondrial dysfunction following chlorine exposure. Mitochondria are an important source of reactive oxygen species (ROS), which may be elevated after cell injury. The ROS released from affected mitochondria may injure unaffected mitochondria, causing both acute and delayed effects (Murphy 2009; Lee et al. 2012). Carlisle et al. reviewed mitochondrial dysfunction as a pathologic basis for cardiopulmonary effects of chlorine gas inhalation (Carlisle et al. 2016). Under normal homeostasis, healthy mitochondria produce ROS that mediate cell signaling. Chlorine exposure causes mitochondrial dysfunction resulting in the production of excessive amounts of ROS that subsequently become a major contributor of cellular and organ injury leading to mitochondrial DNA damage, formation of mitochondrial permeability transition (MPT) pores, and cell death. Damaged mitochondria are typically scavenged by lysosomal–mitophagy systems to bring back to normal homeostasis. Protective role of modulation of mitophagy in chlorine gas-induced mitochondrial dysfunction was tested with trehalose or rapamycin, an activator of autophagy and 3-methyladenine (3-MA), an inhibitor of autophagy. Upregulation of mitophagy mitigated chlorine gas-induced toxicity through prevention of mitochondrial oxidative damage (Jurkuvenaite et al. 2015; Carlisle et al. 2016).

Pathophysiology of chlorine gas-induced lung injuries

Acute lung injury (ALI): Hydrochloric acid and HOCl form when chlorine gas reacts with mucus, and cause inflammation of the upper and lower airways, leading to bronchospasm, cough, and dyspnea. The acids and free radicals generated disrupt cell membranes and proteins, leading to profound injury to the epithelium, endothelium, and capillaries. Chlorine gas exposure causes overexpression of inducible nitric oxide synthase (iNOS) in the vasculature, which in turn, mediates inflammation and the generation of oxidative stress (Honavar et al. 2011). In mouse models of ALI caused by chlorine gas, desquamation of epithelia, infiltration of leucocytes, hemorrhage, edema, atelectasis, emphysema, and necrosis occurred (Balakrishna et al. 2014). Cl_2 exposure also increases expression of pro-inflammatory markers and endogenous mediators of inflammation. Studies showed recruitment of inflammatory neutrophils and macrophages, and production of reactive oxygen and nitrogen species following chlorine exposure (White and Martin 2010; Balakrishna et al. 2014). Inflammatory mediators such as CXC chemokines including IL-8 (CXCL8), MIP-2 (CXCL2), and KC (CXCL1) serve as chemoattractants for recruitment of neutrophils. The recruited inflammatory neutrophils contribute to the generation of myeloperoxidase (MPO) (Balakrishna et al. 2014). MPO catalyzes the formation of reactive oxygen intermediates, including HOCl which may further augment chlorine injury. MPO has been demonstrated to be a local mediator of tissue damage and implicated in various inflammatory diseases. More recently, MPO became an important therapeutic target in the treatment of inflammatory conditions (Hickey 2011; Aratani 2018). Balakrishna et al. showed that chlorine gas-induced cellular inflammatory response was accompanied by dramatic increase in levels of key pro-inflammatory factors such as KC (CXCL1), GCSF, IL-6, and VEGF in serum and BALF. Chlorine gas also caused significant vascular damage as evidenced by the high levels of vascular damage markers in BALF, including serum amyloid P (SAP) component, fibrinogen, adiponectin, and soluble vascular cell adhesion molecule 1 (sVCAM-1) (Balakrishna et al. 2014).

After chlorine gas exposure, mice exhibit reactive airway dysfunction syndrome, protein leakage, edema, and alveolar damage leading to hypoxia. Most victims of mild to moderate exposure to chlorine inhalation recover from acute symptoms within a few days. However, it is difficult to predict the prognosis of chlorine-induced pulmonary injuries as some victims may develop chronic respiratory problems such as reactive airway disease (RAD).

Persistent effects of chlorine gas: A recent review summarizes the persistent effects of chlorine inhalation on pulmonary health (Hoyle and Svendsen 2016). The database from the 2005 chlorine gas incident in Graniteville, SC is the largest of any study on long-term pulmonary and cardiac effects of chlorine gas exposure in humans (Abara et al. 2014). Clark et al. analyzed spirometry records from 1807 mill workers (7332 observations) from 4 years before to 18 months after the Graniteville Cl_2 disaster (Clark et al. 2016), and found significant reductions in lung function immediately after the chlorine incident. In the second year, there was a modest improvement in lung function; however, the proportion of mill workers who experienced an accelerated annual decline in FEV1 (forced expiratory volume in one second) increased significantly in 18 months after the chlorine incident. A prospective study in victims of accidental acute exposure to chlorine gas in Bangkok also revealed persistent effects (Chierakul et al. 2013).

Most of the pre-clinical studies on long-term effects of chlorine gas-induced pulmonary injuries have been conducted in rodents due to feasibility and cost-effectiveness. Common long-term outcomes of chlorine gas exposure are fibrosis and airway remodeling (Musah et al. 2012; Mo et al. 2013, 2015; Hoyle and Svendsen 2016), reactive airways dysfunction syndrome (RADS) (Jonasson, Koch, et al. 2013), loss of basal cells, and pathologic features similar to bronchiolitis obliterans (O'Koren et al. 2013; Musah et al. 2017). Mice that developed airway fibrosis had elevated baseline airway resistance and airway hyperreactivity in the methacholine (Mech) airway challenge test (Mo et al. 2013).

Cardiovascular effects of chlorine gas

Chlorine gas exposure often has cardiovascular consequences also (Kose et al. 2009; Van Sickle et al. 2009; Carlisle et al. 2016). Epidemiologic data and pre-clinical studies suggest that cardiac effects from chlorine gas exposure may last for many years. These effects include decreased myocardial contractile force, reduced systemic and diastolic blood pressure, failure of biventricular function, and even death (Zaky et al.

2015; Carlisle et al. 2016). In rats exposed to 500 ppm chlorine gas for 30 minutes, lactate levels increased in the coronary sinus, which suggests anaerobic metabolism in cardiac tissue (Zaky et al. 2015). Victims of the Graniteville, SC train derailment incident who died within four hours after chlorine gas exposure had cardiomegaly (Van Sickle et al. 2009). Of these victims, 87% had preexisting cardiomegaly, which suggests that cardiac patients are at higher risk for death (Van Sickle et al. 2009). Four of the 71 victims hospitalized had cardiomegaly on chest X-ray. In hospitalized patients, tachycardia and hypertension were observed. The rate of hospitalization for cardiovascular disease including high blood pressure, doubled in residents of Graniteville, SC from 2005 to 2012. In rat models of chlorine gas inhalation, Ahmad et al. showed mitochondrial injury as a mediator of cardiac toxicity (Ahmad et al. 2015), and a case study in an elderly patient exposed to chlorine gas reported myocardial infarction, acute ischemic stroke, and hyperglycemia (Kose et al. 2009).

Effects of chlorine gas on the central nervous system

Chlorine gas inhalation also affects the central nervous system (Kilburn 2000). Twenty-two study subjects who had been exposed to chlorine gas in occupational or domestic situations underwent a battery of neurophysiologic and cognitive evaluations. Balance, simple and choice reaction times, color discrimination, visual field performance, hearing, grip strength, adverse mood scores, trail making, and verbal recall were impaired (Kilburn 2000). Small hemorrhagic lesions in the white matter of the brain have been reported after chlorine gas inhalation (Leube and Kreiter 1971; Evans 2005). In a rat study, acute chlorine inhalation caused seizures and worsening of neuromuscular scores, which might be attributed to hypoxemia (Okponyia et al. 2018).

Effects of chlorine gas on other organs and systemic effects

While pulmonary injuries from chlorine exposure are common, dermal and ocular injuries can also occur. Skin exposure results in irritation, severe pain, chemical burns, or blisters, based on the severity of the exposure. Irritation, conjunctivitis, or corneal defects can occur after ocular exposures to chlorine gas. Although uncommon, some chromosomal changes have been reported in the literature. For example, studies in sheep showed that chlorine gas in drinking water can cause chromosomal aberrations (Sutiakova et al. 2004). Although such chromosomal aberrations have not been studied prospectively or retrospectively in humans or in pre-clinical animal models after accidental or chemical threat exposures, they are possible. Finally, chronic exposure to chlorine gas in the form of hypochlorite and chloramine in drinking water causes leukemia and lymphomas in rats (Soffritti et al. 1997). Prospective long-term studies are warranted if such effects are also seen in humans consuming public water supply which is generally bleached, particularly in developing countries.

Chlorine exposure and increased susceptibility to other diseases

Since chlorine gas damages the epithelial lining and disrupts the alveolar–capillary barrier, pulmonary tissue becomes susceptible to other diseases under acute and chronic conditions. Several studies have shown increased susceptibility to invasive lung fungal infections and respiratory syncytial virus, and to acquiring asthmatic phenotypes (Hox et al. 2011; Gessner et al. 2013; Song et al. 2015; Johansson et al. 2017; de Genaro et al. 2018).

In vitro *models of chlorine gas inhalation*

Studying the effects of chlorine gas *in vitro* aids in the initial screening of therapeutic drug candidates. However, a few such *in vitro* studies are referenced in the literature. Bessac et al. used HEK293T cells transfected with transient receptor potential (TRP) ion channels and cultured dorsal root ganglia for *in vitro* pharmacological functional assays (Bessac et al. 2008). When NaOCl-containing physiological buffer was added to cultured neurons, a robust increase in intracellular calcium was noted. Cultured neurons from trigeminal ganglia and nodose ganglia also showed robust responses to NaOCl. The neuronal NaOCl-activated calcium influx pathway was further examined using whole-cell patch-clamp electrophysiology. More interestingly, calcium influx induced by NaOCl was completely absent in neurons cultured from *Trpa1–/–* mice. These *in vitro* findings were further confirmed with *in vivo* mouse studies. Wild type and *Trpa1–/–* mice were exposed to NaOCl aerosol in barometric plethysmography, and found that TRPA1 plays a key role in chlorine gas-induced lung injury.

Ahmad et al. studied the deleterious effects of chlorine gas inhalation using differentiated air/liquid interface cultures of human airway epithelial basal cells and rat cardiomyocytes (Ahmad et al. 2014). Loss of membrane integrity, caspase release, and apoptotic cell death were described in both tissue types after exposure to chlorine gas. Jurkuvenaite et al. exposed NCIH441 cells (human lung adenocarcinoma epithelial cells) to chlorine gas at 100 ppm for 15 minutes and found decreased cellular bioenergetics and mitochondrial membrane potential. Treatment with mitochondrial redox modulator, MitoQ, mitigated bioenergetics defects associated with MitoSOX signaling. It was also found that significant increase in autophagy at six hours post-exposure to chlorine gas to NCI-H441 cells, which suggested improvement of mitochondrial function. These *in vitro* findings were successfully translated to an *in vivo* mouse model. Autophagy activator, trehalose, decreased leukocyte counts in BALF after chronic administration in drinking water whereas acute administration decreased alveolar permeability in mouse chlorine model (Jurkuvenaite et al. 2015). *In vitro* studies involving other toxic inhalation agents have informed studies on chlorine gas-induced injury in *in vitro* systems (Andres et al. 2016). Organoid technology became an explosion of interest in recent times for studying several tissue systems, including lung. Although lung organoid models of chlorine

inhalation have been not studied, these cultures may be helpful for simulating injury and for rapid screening of potential therapeutic drug candidates.

Animal models of chlorine gas-induced acute lung injury and chronic effects

Rodent models are often used to study chlorine gas-induced acute and chronic cardiopulmonary effects and for initial screening of potential therapeutic drug candidates. However, several features of human anatomy and physiology are not common to rodent models, which accounts in part, for the failure of many clinical trials and for failure in higher mammalian pre-clinical models. Non-rodents such as dogs, sheep, rabbits, and pigs have been used to study injuries from chlorine gas inhalation; however, most animal models have limitations. Within mouse species alone, there are several strain-specific differences (Leikauf et al. 2010, 2012; Mo et al. 2013; O'Koren et al. 2013). The disparities in injury and response to treatment in inbred strains of mice result from histologic differences. Differences in injury phenotype of persistent effects of chlorine gas inhalation are more pronounced between mouse strains (Mo et al. 2013). Non-rodent models have other limitations such as the high cost of animals and maintenance, and difficulty in characterizing persistent effects of chlorine gas inhalation due to regulatory and animal welfare concerns.

Although the U.S. Food and Drug Administration (FDA) animal rule gives provision for approval of potential medical countermeasures based on data from a single pre-clinical animal model, regulatory reviewers look for therapeutic data in at least two animal models, including a higher mammalian species close to humans (Park and Mitchel 2016).

With the support from NIH CounterACT program (https://www.ninds.nih.gov/Current-Research/Trans-Agency-Activities/CounterACT, accessed 22 August 19), a trans-NIH initiative in translational research to develop medical countermeasures against potential and existing chemical threat agents, the repertoire of literature on animal models of chlorine inhalation in the last one decade revealed in-depth pathophysiology and potential medical countermeasures. No single animal model represents all features of human ALI. However, animal models of different species at different chlorine concentrations and times of exposure have characterized individual features of ALI (Matute-Bello et al. 2008, 2011; Wang et al. 2008; Ballard-Croft et al. 2012; Balakrishna et al. 2014). The clinical features of ALI are acute onset, diffuse bilateral alveolar injury, acute oxidative phase, and repair over an extended period with fibrosis (Morris et al. 2005; Mo et al. 2013; O'Koren et al. 2013; Balakrishna et al. 2014). The respiratory features of ALI are severe hypoxemia, V/Q mismatch, decreased compliance, and decreased alveolar fluid clearance (Wang, Zhang, et al. 2002; Wang et al. 2004; Balakrishna et al. 2014). Biologic changes observed in ALI include disruption of epithelial and endothelial permeability, protease activation, coagulopathies, and increased production of pro-inflammatory cytokines in lung tissue, plasma, and bronchoalveolar lavage fluid (BALF) (Gessner et al. 2013; Balakrishna et al. 2014; Zarogiannis et al. 2014; Song et al. 2015). Histopathologic changes observed in ALI include infiltration of neutrophils, intra-alveolar coagulation and fibrin deposits, and injury of the alveolar epithelium (Musah et al. 2012; Balakrishna et al. 2014; Mo et al. 2015).

The following representative animal models have been widely described in the literature.

Mouse: Mice are widely used in biomedical research due to their availability, ease of handling, availability of the complete mouse genome, easy genetic manipulation, and fast reproduction rate. Also, many conditions in humans can be modeled in mice due to their resemblance in genetic, biologic, and behavioral characteristics. Several inbred strains of mice have been used to study chlorine gas-induced ALI and its long-term effects. Table 1 shows representative mouse strains, chlorine exposure conditions, study/observation period, and therapeutic agents tested. Whole-body and nose-only chlorine gas exposure manifolds were tested. Appreciable lung injury by chlorine gas exposure depends on the type of exposure, the concentration of chlorine gas, time of exposure, and mouse strain.

Whole-body exposure to chlorine gas in mouse models have been widely studied in various strains (Song et al. 2011; Zarogiannis et al. 2011; Balakrishna et al. 2014; Zarogiannis et al. 2014; Wigenstam et al. 2015) whereas some studies utilized nose-only exposure manifolds (McGovern et al. 2010, 2011; O'Koren et al. 2013; McGovern et al. 2015; Hamamoto et al. 2017). C57BL/6 mice that were exposed to 400 ppm Cl_2 for 30 minutes produced several features of chemical lung injury, including airway hyperreactivity and increase in lung elastance, protein leakage, infiltration of neutrophils and macrophages into lungs, increased production of oxidative mediators and inflammatory chemokines and cytokines (Balakrishna et al. 2014). The same combination of chlorine concentration and time of exposure resulted in a similar injury phenotype (Yadav et al. 2010; Song et al. 2011). The characteristic long-term effects of chlorine exposure in mouse models are obliterans bronchiolitis, airway fibrosis, repair and remodeling (Tuck et al. 2008; Musah et al. 2012; Mo et al. 2013; O'Koren et al. 2013). The role of basal cells in airway repair and regeneration was studied (Musah et al. 2012; O'Koren et al. 2013).

Compared to higher mammalian models, studying the time-course effects of chlorine insults on the lungs is more feasible in mice (Tuck et al. 2008). The main caveat with mouse models is strain differences in the response to chlorine exposure within the same species and careful consideration should be given to mouse strain being tested for characterizing the injury. Leikauf et al. studied extensive functional genomics in 16 inbred strains of the mouse (Leikauf et al. 2010). Mouse strain differences in ALI and airway fibrosis were studied in C57BL/6, FBV/N, and A/J strains (Tian et al. 2008; Mo et al. 2013). Another limitation of mouse models is variability in persistent effects of chlorine inhalation in very high acute concentrations.

Rats: Second only to mouse models, rats are frequently used to study chlorine-induced lung injuries. In an acute Cl_2 inhalation study, rats (adult male Sprague-Dawley rats,

Table 1. Representative mouse models of chlorine gas inhalation.

Mouse strain	Cl_2 concentration (ppm)	Time of exposure (min)	Whole-body/ nose only	Duration of study	Therapeutic agent tested	Remarks	References
C57BL/6	400	30	Whole-body	24 h	TRPV4 inhibitors (GSK2220691 and GSK2337429A)	TRPV4 inhibitors are in advanced testing	(Balakrishna et al. 2014)
C57BL/6	400	30	Whole body	24 h	N-acetyl cysteine; heparin	N-acetyl cysteine alone or in combination with dexamethasone or triptolide, reparixin and rolipram had no beneficial therapeutic effects; aerosolized heparin reduced protein levels and inflammatory cells in the BALF	(Zarogiannis et al. 2014; Wigenstam et al. 2015)
C57BL/6	600	45	Whole-body	24 h	Ascorbate and deferoxamine	Treatment reduced alveolar-capillary permeability, inflammation, epithelial sloughing, and lipid peroxidation	(Zarogiannis et al. 2011)
C57BL/6	600	45	Whole-body	24 h	Nitrite	Further tested in rabbits with positive outcomes	(Honavar et al. 2014, 2017)
A/J	240	60	Whole-body	8 weeks	–	Acute Cl_2 exposure may cause chronic abnormalities in the lungs despite rapid repair of injured epithelium	(Mo et al. 2015)
C57BL/6 and FVB/N	197–289	66	Whole-body	6–48 h	–	Both strains had similar injury pattern	(Tian et al. 2008; Mo et al. 2013)
C57BL/6	60	60	Whole-body	3–48 h	–	Impaired surfactant function, and altered BAL phospholipid and surfactant protein content	(Massa et al. 2014)
FVB/NJ and A/J	240	60	Whole-body	Multiple (days 1, 4, 7, 10, and 14)	–	Differential susceptibility of inbred mouse strains to chlorine-induced airway fibrosis	(Mo et al. 2013)
C57BL/6	240	60	Whole-body	2, 4, 7, and 10 days after exposure	–	Role of basal cells in tracheal epithelial repair and airway fibrosis was elucidated	(Musah et al. 2012)
FVB/N	228–270	60	Whole-body	24 h	Rolipram	Pulmonary edema and airway hyperreactivity were reduced with the treatment	(Chang et al. 2012)
FVB/N	240	60	Whole-body	6–48 h	Nine corticosteroids	Mometasone and budesonide were effective among the nine corticosteroids tested	(Chen et al. 2013)
C57BL/6	200–350	30	Nose only	6–12 days	–	Bronchiolitis obliterans-like pathological changes	(O'Koren et al. 2013)
BALB/c	100	5	Nose only	24–72 h	AEOL10150; dimethylthiourea; Montelukast	Role of neutrophils in Cl_2 gas-induced ALI was established; post-exposure treatment with AEOL10150 or dimethylthiourea, or pre-exposure treatment with Montelukast attenuated Cl_2 gas-induced ALI	(McGovern et al. 2010, 2011, 2015; Hamamoto et al. 2017)

300–340 g) were exposed to various concentrations of chlorine gas in a whole-body exposure chamber (Okponyia et al. 2018). The following lethal doses were defined within six hours after Cl_2 exposure: LD_{17} at 500 ppm for 30 minutes, LD_{58} at 600 ppm for 27 minutes, and LD_{75} at 600 ppm for 30 minutes. This massive exposure to chlorine gas resulted in severe acute respiratory failure, hypoxemia, decreased cardiac output, seizures, and neuromuscular abnormalities as evidenced by ataxia and hypotonia (Okponyia et al. 2018). In other acute studies, rats were exposed to 184 or 400 ppm Cl_2 for 30 minutes in whole-body exposure chambers (Leustik et al. 2008; Yadav et al. 2011). Within one hour after chlorine gas exposure, rats had arterial hypoxemia, respiratory acidosis, elevated albumin, IgG, and IgM in BALF, increased BALF surfactant surface tension, and injury to airways and pulmonary parenchyma. Lipid peroxidation, based on F_2-isoprostane levels in lung tissue, was elevated in chlorine-exposed rats. Further, the levels of endogenous antioxidants such as ascorbate and glutathione (GSH) were significantly decreased in lung tissues and BALF of chlorine-exposed rats (Leustik et al. 2008). These findings persisted until 24 hours after Cl_2 exposure. Another short-term study was conducted over 24 hours by exposing rats (adult male Wistar rats, 8–9 weeks old, 200–250 g) to 413 ppm chlorine gas for 30 minutes (Luo et al. 2014). The study concluded that the relationship between exhaled NO and CO_2 in exhaled breath analysis may serve to monitor inflammation and oxidative stress in response to any therapeutic administration (Luo et al. 2014).

In a long-term time-course study, Sprague-Dawley rats were exposed to 200 ppm chlorine gas for 15 minutes using a nose-only exposure system. At 24 hours after exposure, leukocyte counts and pro-inflammatory markers were elevated in BALF, and edema appeared in lungs and heart. At later time points (14, 28, and 90 days post-chlorine exposure), delayed inflammatory response was observed along with lung fibrosis, indicated by elevated pulmonary macrophages, TGF-β1 expression in BALF, and collagen deposits in lung tissue (determined with Masson's Trichrome and a spectrophotometric method, Sircol™ Collagen Assay kit for rats, Biocolor Ltd., Belfast, UK) (Wigenstam et al. 2016). Elevated pulmonary macrophages play a key role in the development of fibrosis through activation and production of pro-inflammatory cytokine, IL-1β (Rastrick and Birrell 2014). Demnati et al. exposed rats to 1500 ppm of chlorine gas for five minutes and then studied long-term effects of chlorine inhalation over a 3-month period after exposure. Parameters such as lung resistance, responsiveness to inhaled Mech, the airway epithelium and bronchoalveolar lavage (BAL) were assessed. Chlorine exposed rats became sensitive to Mech and in some rats, Mech hyperresponsiveness persisted for 3 months. Histopathology revealed epithelial flattening, necrosis, increase in smooth muscle mass and evidence of epithelial regeneration (Demnati, Fraser, Ghezzo, et al. 1998).

Rabbits: Rabbits have been used in multiple studies to confirm findings in rodent models in an incremental manner (Honavar et al. 2017; Musah et al. 2017), and New Zealand white rabbits are most commonly used. In one study of ALI, anesthetized rabbits were exposed to 800 ppm chlorine gas for 4 minutes under mechanical ventilation, and then extubated to recover from anesthesia. In corroboration with rodent studies, rabbits had hypoxemia, pulmonary edema, airway epithelial injury, inflammation, altered baseline lung mechanics, and airway hyperreactivity to inhaled Mech. Rabbits exposed to 400 ppm for eight minutes exhibited mild hypoxemia, increased area of pressure–volume loops, airway hyperreactivity, and pathology in small airways with lesions similar to bronchiolitis obliterans (Musah et al. 2017). New Zealand White rabbits exposed to 600 ppm chlorine gas for 45 minutes in a whole-body exposure chamber had elevated protein, total leucocyte, and neutrophil counts in BALF (Honavar et al. 2017). Several features of acute and persistent effects of chlorine gas inhalation observed in rodent studies were recapitulated in rabbits.

Pigs: Pigs are often used in biomedical pulmonary translational research because their anatomy and physiology are similar to humans. A research team at the Center for Teaching and Research in Disaster Medicine and Traumatology, University of Linköping, Linköping, Sweden has used pig models extensively to study chlorine gas-induced pulmonary injuries and to screen potential therapeutic candidates (Gunnarsson et al. 1998; Wang, Abu-Zidan, et al. 2002; Wang, Zhang, et al. 2002; Wang et al. 2004, 2005, 2006). In fact, they were the first to use higher mammalian models to characterize chlorine gas-induced lung injuries at higher exposures. In their studies, anesthetized and mechanically ventilated pigs were exposed to several concentrations of chlorine gas over a range of exposure times. Pigs exposed to chlorine gas showed severe pulmonary dysfunction with an increased pulmonary vascular resistance index, a drop in arterial oxygenation, and reduced lung-thorax compliance. A few caveats of the studies include use of higher tidal volume (10–20 mL/kg), use of no positive end-expiratory pressure (PEEP), and shorter study periods. Gunnarsson et al. exposed anesthetized mechanically ventilated pigs to 100 L of 140 ppm for 10 minutes. A rapid drop in oxygen tension, biphasic decline in lung compliance, a significant reduction in cardiac output was observed. Histopathology revealed sloughing of bronchial epithelium and infiltration of leucocytes. However, no injury was noted in alveoli (Gunnarsson et al. 1998). A study was undertaken to prospectively evaluate the benefits of prone and supine positioning of pigs following exposure to 400 ppm of chlorine gas for 20 minutes in supine position (Wang, Abu-Zidan, et al. 2002). Exposure to chlorine gas resulted in a threefold increase in pulmonary vascular resistance index, a drop in arterial oxygenation and declined lung-thorax compliance. However, within five hours of evaluation post-chlorine exposure, prone positioning improved venous admixture (Qs/Qt), lung-thorax compliance, and oxygen delivery compared to supine positioning (Wang, Abu-Zidan, et al. 2002).

Sheep: Sheep have also been used to study chlorine gas-induced pulmonary injuries and to screen therapeutic agents (Batchinsky et al. 2006; Fukuda et al. 2016). Anesthetized female sheep were exposed to 60–500 ppm chlorine gas for 30 minutes under mechanical ventilation. The chlorine gas-induced pulmonary injury had features that resembled

smoke inhalation and ARDS resulting from systemic diseases. Logistic regression analysis suggested an LD_{50} at 280 ppm chlorine gas for 30 minutes. The study also revealed injury in smaller airways and alveoli (Batchinsky et al. 2006).

In another ovine model, sheep were exposed to 140 ppm chlorine gas at 25 breaths/min for 30 minutes via an endotracheal tube under anesthesia. Sheep were awakened, and conscious sheep were maintained on mechanical ventilators for 48 hours. All sheep survived until the end of the study period. The ratio of the partial pressure of oxygen to fraction of inhaled oxygen remained less than 228 mmHg until 24 hours after chlorine exposure. The pulmonary shunt fraction and lung wet-to-dry weight ratios were increased in chlorine-exposed sheep.

Primate models: There are no primate models of acute high chlorine gas exposure to mimic accidental or chlorine bomb deployment situations. Chronic effects of chlorine inhalation were studied in Rhesus monkeys (*Macaca mulatta*) exposed to 0.1–2.3 ppm chlorine gas for six hours per day, five days per week, for 1 year (Klonne et al. 1987). Exposure to 2.3 ppm caused upper respiratory pathology, while lower concentrations caused questionable clinical features. The study concluded that Rhesus monkeys are less sensitive to chlorine gas compared to rodents.

The current line of treatment of chlorine gas-induced lung injuries

There are no mechanism-based treatments for chlorine gas injury. Treatment of chlorine intoxication is largely supportive. At the site of exposure, pre-hospital support and stabilization involve removing victims from the source of Cl_2 exposure and giving supplemental oxygen. Administering inhaled beta-agonists to control bronchospasm may be considered. Standardized protocols for triaging victims may help to prioritize treatment plans.

At the hospital, specifically, humidified oxygen is appropriate for all victims along with inhaled bronchodilators (Güloğlu et al. 2002). However, a recent rodent study showed that supplemental oxygen improves survival rates but worsens cardiopulmonary function (Okponyia et al. 2018). Although some benefit from agents such as nebulized sodium bicarbonate and corticosteroids has been suggested, the evidence is anecdotal (Leustik et al. 2008; McGovern et al. 2011; Mackie et al. 2014). Decontaminating victims in the emergency department is critical. Importantly, over 95% of victims of the Graniteville, SC incident arrived at the hospital in private vehicles probably without any decontamination (Wenck et al. 2007).

Potential treatment targets and drug candidates in the research pipeline

Efforts are underway to develop medical countermeasures against chlorine gas-induced lung injuries with the support from NIH CounterACT program and Biomedical Advanced Research Development Authority (BARDA). To treat victims of chlorine exposure in a mass casualty situation, preference is given to stable formulations for intramuscular injections using auto-injectors for self-administration.

Transient receptor potential ion channel inhibitors: TRP ion channels are nonspecific cationic channels expressed ubiquitously on the plasma membrane of almost all mammalian cell types. These ion channels play an important role in normal homeostasis. In the past decade, these ion channels are extensively studied, particularly, for their role in normal homeostasis and different diseases. The role of TRP ion channels has been studied extensively in various animal models of lung injuries (Bessac et al. 2008; Brone et al. 2008; Buch et al. 2013; Balakrishna et al. 2014; Grace et al. 2014; Morty and Kuebler 2014; Achanta and Jordt 2017). Among these channels, the role of TRP vanilloid 4 (TRPV4) and TRP ankyrin repeat 1 (TRPA1) has been extensively studied *in vitro* and *in vivo* (Bautista et al. 2006; Bessac and Jordt 2008; Bessac et al. 2008; Balakrishna et al. 2014; Achanta and Jordt 2017; Summerhill et al. 2017).

TRPV4 ion channels are calcium-permeating ion channels that are abundant in vascular endothelium. Thus, when intracellular calcium levels increase, the TRPV4 response results in plasma extravasation, vascular leakage, and edema (Tiruppathi et al. 2006; Thorneloe et al. 2012). TRPV4 ion channels are also expressed at the alveolar septal barrier, which plays an important role in mediating the movement of intravascular fluid into the interstitial and alveolar air space of the lung. This cascade of events has been verified by preclinical *in vitro* and *in vivo* studies using selective TRPV4 blockers and genetic knock-out studies (Jian et al. 2008; Thorneloe et al. 2012). TRPV4 ion channels are also expressed in other tissues including airway smooth muscle, trachea, heart, liver, brain, placenta, and salivary glands.

Balakrishna et al. used two structurally different novel TRPV4 inhibitors (GSK2220691 and GSK2337429A, tested in both intraperitoneal and intramuscular routes of administration) in mouse non-lethal models of chlorine gas- and hydrochloric acid-induced ALI. Mimicking chlorine gas exposure in the field, treatment was given post-exposure to the chlorine gas. Overall, administration of either inhibitor beginning immediately after chlorine gas exposure reduced pulmonary edema, improved pulmonary function and oxygenation, reduced airway hyperreactivity, and reduced BALF neutrophils, macrophages, and cytokines, suggesting reduced inflammation (Balakrishna et al. 2014). Studies in TRPV4 knock-out mice recapitulated these findings. A review of the role of TRPV4 inhibitors in pulmonary protection supported further development as a chemical medical countermeasure (Morty and Kuebler 2014). Indeed, a TRPV4 inhibitor is now one of two drug candidates that reached advanced development based on mechanism of action (https://www.phe.gov/Preparedness/news/Pages/gsk-chem.aspx, accessed 30 October 2018).

TRPA1 is the most divergent ion channels in the TRP family. Expressed primarily in sensory neurons, TRPA1 is activated by pro-inflammatory mediators and noxious environmental substances. Exogenous or endogenous TRPA1 agonists covalently modify cysteine residues within the

ankyrin repeat-rich N-terminus to activate the channel (Bessac and Jordt 2008; Achanta and Jordt 2017). Bessac et al. showed that hypochlorite, the oxidizing mediator of chlorine gas, activates the TRPA1 ion channel using *in vitro* and *in vivo* studies (Bessac et al. 2008). Hypochlorite activated Ca^{2+} influx and membrane current in an oxidant-sensitive subpopulation of chemosensory neurons. However, neurons cultured from mice lacking TRPA1 do not show these responses. TRPA1 channels were strongly activated by hypochlorite in primary sensory dorsal root ganglion neurons and heterologous cells. In plethysmography respiratory function tests, Trpa1(–/–) mice displayed profound deficiencies in hypochlorite- and hydrogen peroxide-induced respiratory depression, as well as decreased oxidant-induced pain behavior, compared to wild type mice (Bessac et al. 2008). TRPA1 ion channels are also implicated in other chemical threat agents-induced injuries (Bessac et al. 2009; Bessac and Jordt 2010; Rothenberg et al. 2016; Achanta et al. 2018). Targeting TRPA1 ion channel may benefit in mitigating several chemical threat agents-induced skin and lung injuries.

Antioxidants: N-acetyl cysteine is an antioxidant that reduces lung injury when administered in combination with corticosteroids after Cl_2 exposure. As a solo therapeutic agent, however, N-acetyl cysteine does not ameliorate pulmonary inflammation (Wigenstam et al. 2015). Other antioxidants, such as ascorbate and deferoxamine, have also been tested in rodent models. When administered after Cl_2 exposure, ascorbate and deferoxamine reduced mortality and decreased lung injury by modulating alveolar-capillary permeability, inflammation, epithelial sloughing, and lipid peroxidation (Zarogiannis et al. 2011) and reduced epithelial hyperplasia, mucus accumulation, and airway hyperreactivity (Fanucchi et al. 2012). In an ovine model of Cl_2 injury, intramuscular administration of R-107, a NO donor, peroxynitrite modulator, and superoxide scavenger, attenuated the severity of lung injury by inhibiting pulmonary shunt fraction and reducing pulmonary edema formation (Fukuda et al. 2016). Inhalation of chlorine gas causes increased oxidative stress, loss of NO signaling homeostasis, and intra/extra-pulmonary inflammation. R-107 is another drug that has reached advanced pre-clinical testing as a chemical medical countermeasure (https://www.phe.gov/Preparedness/news/Pages/gsk-chem.aspx, accessed 15 January 2019). The catalytic antioxidant Mn(III) tetrakis (*N*,*N*′-diethylimidazolium-2-yl) porphyrin, (AEOL10150 [AEOL]), efficiently scavenges peroxynitrite, inhibits lipid peroxidation, and exhibits superoxide dismutase (SOD)- and catalase-like activities (McGovern et al. 2011). In a mouse model of chlorine inhalation injury, AEOL rescued Cl_2-induced airway hyper-responsiveness, pulmonary inflammation, and oxidative stress, and promoted airway epithelial cell regeneration (McGovern et al. 2011).

Nitrites: Nitric oxide is a critical messenger of homeostasis in several physiologic functions including modulation of inflammation. Exposure to chlorine gas disrupts the formation of NO (Samal et al. 2010; Honavar et al. 2011). Recent studies in rodents and rabbits have shown that administration of nitrite after chlorine gas exposure mitigates pulmonary injury (Yadav et al. 2011; Honavar et al. 2014, 2017). Both enzymatic and non-enzymatic production of NO from nitrite has been documented. Nitrite is an anion that is reduced to NO and other NO-containing species in a hypoxic environment *in vivo*.

Corticosteroids: Nine corticosteroids were screened for their efficacy in rodent models of chlorine injury (Chen et al. 2013). Among the nine corticosteroids tested in the intraperitoneal route of administration, mometasone and budesonide were effective in mitigating certain injury parameters (Chen et al. 2013). Corticosteroids such as dexamethasone and mometasone were partially effective in mitigating ALI in rodent models in a dose-dependent manner (Demnati, Fraser, Martin, et al. 1998; Chen et al. 2013; Jonasson, Wigenstam, et al. 2013; Wigenstam et al. 2016). Among corticosteroids, budesonide has been studied extensively in rodent and swine models of chlorine gas-induced ALI (Wang, Zhang, et al. 2002; Wang et al. 2004; Chen et al. 2013). Budesonide is available as a pill, inhaler, nasal spray, and rectal suppository. Novel intramuscular preparations of budesonide with poly(lactic-co-glycolic acid) (PLGA) have been tested in rodent models (Hoyle et al. 2016). In a swine model of Cl_2-induced ALI (400 ppm for 20 minutes), aerosolized combination therapy with budesonide and terbutaline, a β2-adrenergic agonist, improved outcomes compared to single-agent therapies with either of these drug candidates (Wang et al. 2004). Wang et al. tested the therapeutic efficacy of corticosteroids such as inhaled budesonide and intravenous betamethasone in anesthetized and mechanically ventilated pigs exposed to 400 ppm of chlorine gas for 15 minutes. Treatment with corticosteroids improved arterial oxygen tension, decreased pulmonary vascular resistance and airway pressure compared to placebo-treated pigs (Wang et al. 2005). The therapeutic benefits of budesonide are also time-dependent. Swine that received budesonide immediately or within 30 minutes after Cl_2 exposure had therapeutic benefits but not when treated at 60 minutes after exposure (Wang, Zhang, et al. 2002).

Oxygen therapy: Continuous supplemental oxygen therapy after chlorine gas exposure has been controversial as excess oxygen can generate ROS, which may enhance toxicity. In an effort to stabilize cardiopulmonary physiology, supplemental oxygen is commonly administered to patients. However, a recent study in rats showed that continuous supplemental oxygen therapy is detrimental in treating Cl_2-induced ALI (Okponyia et al. 2018). The study found that chlorine inhalation causes severe respiratory, cardiac, and neuromuscular abnormalities. Supplemental oxygen therapy (0.8 FiO_2) improved survival rate and prevented seizure-related mortality, but it worsened the severity of respiratory physiology compared to control animals (0.21 FiO_2, that is, ambient room air), and did not improve respiratory, cardiac, or neuromuscular abnormalities. The study concluded that oxygen therapy can improve short-term survival, but that continuous supplemental oxygen therapy should be given with caution (Okponyia et al. 2018).

Heparin: In a rodent model of Cl_2-induced ALI, a cascade of coagulation abnormalities was activated systemically and within alveoli. Mice exposed to chlorine gas had increased

intra-alveolar hypercoagulation, measured by clotting time, clot formation time, and D-dimers compared to control mice exposed to room air. Further, BALF from Cl_2-exposed mice had higher levels of thrombin–antithrombin complexes. When mice were treated with aerosolized heparin after chlorine gas exposure, protein levels, and inflammatory cells were decreased in BALF (Zarogiannis et al. 2014).

Sodium bicarbonate: Sodium bicarbonate is a nonspecific antidote for treating acid–base abnormalities. Nebulized sodium bicarbonate was used as a single therapeutic agent or as an adjunct therapy in pulmonary injuries resulting from chlorine gas inhalation. Inhaled sodium bicarbonate neutralizes the hydrochloric acid that is formed when chlorine gas reacts with water in the respiratory tract (Vajner and Lung 2013).

Trehalose: When NCI-H441 cells (human lung adenocarcinoma epithelial cells) were exposed to 100 ppm chlorine gas for 15 minutes, cellular bioenergetic function and mitochondrial membrane potential decreased within one hour. However, at six hours after Cl_2 exposure, a significant increase in autophagy was observed, which was associated with improved mitochondrial function. When NCI-H441 cells were pretreated with trehalose (an autophagy activator), the bioenergetic function was improved. When mice were exposed to 400 ppm chlorine gas for 30 minutes, administration of 2% trehalose orally or via nebulization resulted in mixed outcomes that were dependent on the timing of treatment (Jurkuvenaite et al. 2015).

Increasing cyclic AMP levels: Hoyle et al. described the mechanisms by which cAMP regulates cellular processes affecting lung injury and discussed therapeutic benefits of investigating the drugs that enhance cAMP levels as potential medical countermeasures for chlorine gas-induced lung injuries (Hoyle 2010). Potential beneficial effects of increased cyclic AMP levels include inhibition of pulmonary edema, inflammation, and airway hyperreactivity. Beta(2)-adrenergic agonists and phosphodiesterase inhibitors increase cAMP levels through stimulation of cAMP synthesis and inhibition of cAMP degradation, respectively. The type 4 phosphodiesterase inhibitor, rolipram, was investigated as a rescue treatment for chlorine gas-induced lung injury (Chang et al. 2012).

Biomarkers

There are no validated biomarkers for diagnosing victims of chlorine exposure or to establish chlorine gas leak/attack. A very limited number of biomarkers for detecting chlorine exposure in humans or in animal models are in pre-clinical stages of validation. L-α-Phosphatidylglycerol chlorohydrins have been identified as potential biomarkers for chlorine gas exposure (Hemstrom et al. 2016). In that study, Hemstrom et al. used mass spectrometry analytical approaches to detect chlorinated biomolecules in BALF of mice. Chlorine reacts with pulmonary surfactant lipids, unsaturated phosphatidylglycerol, and phosphatidylcholine to form chlorohydrins. These chlorohydrins could be detected until 72 hours after chlorine gas exposure. These chlorinated biomolecules were not found in control mice or in humans with chronic respiratory diseases. Using a flow cytometry approach, Robaszkiewicz et al. showed that hypochlorite forms 3-chlorinated tyrosine residues via oxidation reactions in human cell lines (Robaszkiewicz et al. 2011). In a rat model of chlorine gas-induced injury, by-products of chlorine reactions, such as chlorotyrosine and chloramine, were elevated in plasma (Ahmad et al. 2015). In a mouse model of chlorine gas-induced lung injury, impaired surfactant function and altered BALF phospholipid and surfactant protein levels were noted (Massa et al. 2014).

Lung tissue and surfactant are enriched with plasmalogens. Chlorinated lipids (Cl-lipids) are produced when chlorine gas reacts with plasmalogen. In rodent models of chlorine gas-induced ALI, Cl-lipids, such as 2-chloropalmitaldehyde (2-Cl-Pald) and 2-chlorostearaldehyde (2-Cl-Sald), and their oxidized products free and esterified 2-chloropalmitic acid and 2-chlorostearic acid were detected in the lungs and plasma. The highest concentrations of Cl-lipids were observed immediately after chlorine gas exposure. Cl-lipid levels remained several folds higher at 24 hours post Cl_2 exposure compared to baseline values, but decreased over the first 72 hours after exposure. Glutathione adducts of 2-Cl-Pald and 2-Cl-Sald, as well as 3-chlorotyrosine levels, also increased in chlorine gas-exposed animals (Spickett 2007; Ford et al. 2016). All of these biomarkers were tested in rodent models and it would be interesting to validate these findings in higher mammalian models close to humans or in actual samples of victims of chlorine gas exposure. Future efforts should also establish a relationship between a biomarker and the progression of the disease. In order to identify chlorine gas leak/attack, it would be worthwhile to assess chlorine exposure from a forensic point of view near sites of chlorine gas bomb deployment.

Conclusions/recommendations

While chlorine gas offers several benefits, it is a potential chemical threat that is easy to manufacture and easily accessible. Despite knowing its deleterious effects on the pulmonary system for more than a century, there is no effective antidote due to the complexity of the pathophysiology and clinical outcomes following exposure. Recent preclinical studies have identified several targets for treatment, and some are in advanced testing. Currently, the potential medical countermeasures against chlorine gas are tested as a solo treatment. However, the approach for treating exposed patients must be multidimensional, and would include acid-base regulation, oxygen supplementation, mechanical ventilation, and administration of the potential drug candidate. Future studies should focus on combination therapies to address various pathophysiologic events that happen immediately after exposure, in order to stabilize the patient and restore the architecture of the pulmonary system. In addition to the development of potential medical countermeasures against chlorine gas-induced lung injuries, identifying biomarkers for chlorine gas injury that serves as a surrogate marker of chlorine gas exposure and disease progression fills the knowledge gap.

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References

Abara W, Wilson S, Vena J, Sanders L, Bevington T, Culley JM, Annang L, Dalemarre L, Svendsen E. 2014. Engaging a chemical disaster community: lessons from Graniteville. Int J Environ Res Public Health. 11(6): 5684–5697.

Achanta S, Chintagari NR, Brackmann M, Balakrishna S, Jordt SE. 2018. TRPA1 and CGRP antagonists counteract vesicant-induced skin injury and inflammation. Toxicol Lett. 293:140–148.

Achanta S, Jordt SE. 2017. TRPA1: acrolein meets its target. Toxicol Appl Pharmacol. 324:45–50.

Ahmad S, Ahmad A, Hendry-Hofer TB, Loader JE, Claycomb WC, Mozziconacci O, Schoneich C, Reisdorph N, Powell RL, Chandler JD. 2015. Sarcoendoplasmic reticulum Ca(2+) ATPase. A critical target in chlorine inhalation-induced cardiotoxicity. Am J Respir Cell Mol Biol. 52(4):492–502.

Ahmad S, Ahmad A, Neeves KB, Hendry-Hofer T, Loader JE, White CW, Veress L. 2014. In vitro cell culture model for toxic inhaled chemical testing. J Vis Exp. 87:e51539.

Andres D, Keyser B, Benton B, Melber A, Olivera D, Holmes W, Paradiso D, Anderson D, Ray R. 2016. Transient receptor potential (TRP) channels as a therapeutic target for intervention of respiratory effects and lethality from phosgene. Toxicol Lett. 244:21–27.

Aratani Y. 2018. Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 640:47–52.

Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E. 2014. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. Am J Physiol Lung Cell Mol Physiol. 307(2):L158–L172.

Ballard-Croft C, Wang D, Sumpter LR, Zhou X, Zwischenberger JB. 2012. Large-animal models of acute respiratory distress syndrome. Ann Thorac Surg. 93(4):1331–1339.

Balte PP, Clark KA, Mohr LC, Karmaus WJ, Van Sickle D, Svendsen ER. 2013. The immediate pulmonary disease pattern following exposure to high concentrations of chlorine gas. Pulm Med. 2013:325869.

Barrett AM. 2009. Mathematical modeling and decision analysis for terrorism defense: assessing chlorine truck attack consequence and countermeasure cost effectiveness. Pittsburgh: Pennsylvania Carnegie Mellon University.

Batchinsky AI, Martini DK, Jordan BS, Dick EJ, Fudge J, Baird CA, Hardin DE, Cancio LC. 2006. Acute respiratory distress syndrome secondary to inhalation of chlorine gas in sheep. J Trauma. 60(5):944–956, discussion 956–947.

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell. 124(6): 1269–1282.

Bessac BF, Jordt SE. 2008. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. Physiology (Bethesda). 23:360–370.

Bessac BF, Jordt SE. 2010. Sensory detection and responses to toxic gases: mechanisms, health effects, and countermeasures. Proc Am Thorac Soc. 7(4):269–277.

Bessac BF, Sivula M, von Hehn CA, Caceres AI, Escalera J, Jordt SE. 2009. Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. FASEB J. 23(4): 1102–1114.

Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, Jordt SE. 2008. TRPA1 is a major oxidant sensor in murine airway sensory neurons. J Clin Invest. 118(5):1899–1910.

Branscomb L, Fagan M, Auerswald PE, Ellis R, Barcham R. 2010. Rail transportation of toxic inhalation hazards: policy responses to the safety and security externality. RPP-2010-01. https://ssrn.com/abstract=2397482

Brone B, Peeters PJ, Marrannes R, Mercken M, Nuydens R, Meert T, Gijsen HJ. 2008. Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor. Toxicol Appl Pharmacol. 231(2):150–156.

Buch T, Schafer E, Steinritz D, Dietrich A, Gudermann T. 2013. Chemosensory TRP channels in the respiratory tract: role in toxic lung injury and potential as "sweet spots" for targeted therapies. Rev Physiol Biochem Pharmacol. 165:31–65.

Carlisle M, Lam A, Svendsen ER, Aggarwal S, Matalon S. 2016. Chlorine-induced cardiopulmonary injury. Ann N Y Acad Sci. 1374(1):159–167.

CDC. 2005. Public health consequences from hazardous substances acutely released during rail transit—South Carolina, 2005; selected states, 1999–2004. Morb Mortal Weekly Rep (MMWR). 54(3):64–67.

Chang W, Chen J, Schlueter CF, Rando RJ, Pathak YV, Hoyle GW. 2012. Inhibition of chlorine-induced lung injury by the type 4 phosphodiesterase inhibitor rolipram. Toxicol Appl Pharmacol. 263(2):251–258.

Chen J, Mo Y, Schlueter CF, Hoyle GW. 2013. Inhibition of chlorine-induced pulmonary inflammation and edema by mometasone and budesonide. Toxicol Appl Pharmacol. 272(2):408–413.

Chierakul N, Rittayamai N, Passaranon P, Chamchod C, Suntiwuth B. 2013. Respiratory health effect of persons accidentally expose to high concentration of chlorine gas. J Med Assoc Thai. 96(Suppl. 2):S17–S21.

Clark KA, Karmaus WJ, Mohr LC, Cai B, Balte P, Gibson JJ, Ownby D, Lawson AB, Vena JE, Svendsen ER. 2016. Lung function before and after a large chlorine gas release in Graniteville, South Carolina. Ann Am Thorac Soc. 13(3):356–363.

Das R, Blanc PD. 1993. Chlorine gas exposure and the lung: a review. Toxicol Ind Health. 9(3):439–455.

de Genaro IS, de Almeida FM, Hizume-Kunzler DC, Moriya HT, Silva RA, Cruz JCG, Lopes RB, Righetti RF, de Paula Vieira R, Saiki M, et al. 2018. Low dose of chlorine exposure exacerbates nasal and pulmonary allergic inflammation in mice. Sci Rep. 8(1):12636.

Demnati R, Fraser R, Ghezzo H, Martin JG, Plaa G, Malo JL. 1998. Time-course of functional and pathological changes after a single high acute inhalation of chlorine in rats. Eur Respir J. 11(4):922–928.

Demnati R, Fraser R, Martin JG, Plaa G, Malo JL. 1998. Effects of dexamethasone on functional and pathological changes in rat bronchi caused by high acute exposure to chlorine. Toxicol Sci. 45(2):242–246.

Evans RB. 2005. Chlorine: state of the art. Lung. 183(3):151–167.

Fanucchi MV, Bracher A, Doran SF, Squadrito GL, Fernandez S, Postlethwait EM, Bowen L, Matalon S. 2012. Post-exposure antioxidant treatment in rats decreases airway hyperplasia and hyperreactivity due to chlorine inhalation. Am J Respir Cell Mol Biol. 46(5):599–606.

Ford DA, Honavar J, Albert CJ, Duerr MA, Oh JY, Doran S, Matalon S, Patel RP. 2016. Formation of chlorinated lipids post-chlorine gas exposure. J Lipid Res. 57(8):1529–1540.

Fukuda S, Ihara K, Lopez E, Cox R, Southan G, Salzman A, Salsbury JR, Prough DS, Enkhbaatar P. 2016. R-107 attenuates severity of acute respiratory distress syndrome induced by chlorine gas inhalation in ovine model. Am J Respir Crit Care Med. 193:A6299.

Gessner MA, Doran SF, Yu Z, Dunaway CW, Matalon S, Steele C. 2013. Chlorine gas exposure increases susceptibility to invasive lung fungal infection. Am J Physiol Lung Cell Mol Physiol. 304(11):L765–L773.

Govier P, Coulson JM. 2018. Civilian exposure to chlorine gas: a systematic review. Toxicol Lett. 293:249–252.

Grace MS, Baxter M, Dubuis E, Birrell MA, Belvisi MG. 2014. Transient receptor potential (TRP) channels in the airway: role in airway disease. Br J Pharmacol. 171(10):2593–2607.

Güloğlu C, Kara IH, Erten PG. 2002. Acute accidental exposure to chlorine gas in the Southeast of Turkey: a study of 106 cases. Environ Res. 88(2):89–93.

Gunnarsson M, Walther SM, Seidal T, Bloom GD, Lennquist S. 1998. Exposure to chlorine gas: effects on pulmonary function and morphology in anaesthetised and mechanically ventilated pigs. J Appl Toxicol. 18(4):249–255.

Hamamoto Y, Ano S, Allard B, O'Sullivan M, McGovern TK, Martin JG. 2017. Montelukast reduces inhaled chlorine triggered airway hyperresponsiveness and airway inflammation in the mouse. Br J Pharmacol. 174(19):3346–3358.

Hemstrom P, Larsson A, Elfsmark L, Astot C. 2016. L-alpha-phosphatidylglycerol chlorohydrins as potential biomarkers for chlorine gas exposure. Anal Chem. 88:9972–9979.

Hickey MJ. 2011. MPO and neutrophils: a magnetic attraction. Blood. 117(4):1103–1104.

Honavar J, Doran S, Oh JY, Steele C, Matalon S, Patel RP. 2014. Nitrite therapy improves survival postexposure to chlorine gas. Am J Physiol Lung Cell Mol Physiol. 307(11):L888–L894.

Honavar J, Doran S, Ricart K, Matalon S, Patel RP. 2017. Nitrite therapy prevents chlorine gas toxicity in rabbits. Toxicol Lett. 271:20–25.

Honavar J, Samal AA, Bradley KM, Brandon A, Balanay J, Squadrito GL, MohanKumar K, Maheshwari A, Postlethwait EM, Matalon S, et al. 2011. Chlorine gas exposure causes systemic endothelial dysfunction by inhibiting endothelial nitric oxide synthase-dependent signaling. Am J Respir Cell Mol Biol. 45(2):419–425.

Hox V, Vanoirbeek JA, Callebaut I, Bobic S, De Vooght V, Ceuppens J, Hoet P, Nemery B, Hellings PW. 2011. Airway exposure to hypochlorite prior to ovalbumin induces airway hyperreactivity without evidence for allergic sensitization. Toxicol Lett. 204(2–3):101–107.

Hoyle GW, Chen J, Schlueter CF, Mo Y, Humphrey DM Jr, Rawson G, Nino JA, Carson KH. 2016. Development and assessment of countermeasure formulations for treatment of lung injury induced by chlorine inhalation. Toxicol Appl Pharmacol. 298:9–18.

Hoyle GW, Svendsen ER. 2016. Persistent effects of chlorine inhalation on respiratory health. Ann N Y Acad Sci. 1378(1):33–40.

Hoyle GW. 2010. Mitigation of chlorine lung injury by increasing cyclic AMP levels. Proc Am Thorac Soc. 7(4):284–289.

Jani DD, Reed D, Feigley CE, Svendsen ER. 2016. Modeling an irritant gas plume for epidemiologic study. Int J Environ Health Res. 26(1):58–74.

Jian MY, King JA, Al-Mehdi AB, Liedtke W, Townsley MI. 2008. High vascular pressure-induced lung injury requires P450 epoxygenase-dependent activation of TRPV4. Am J Respir Cell Mol Biol. 38(4):386–392.

Johansson M, Gustafsson A, Johanson G, Oberg M. 2017. Comparison of airway response in naïve and ovalbumin-sensitized mice during short-term inhalation exposure to chlorine. Inhal Toxicol. 29(2):82–91.

Jonasson S, Koch B, Bucht A. 2013. Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury. Toxicology. 303:34–42.

Jonasson S, Wigenstam E, Koch B, Bucht A. 2013. Early treatment of chlorine-induced airway hyperresponsiveness and inflammation with corticosteroids. Toxicol Appl Pharmacol. 271(2):168–174.

Jones R, Wills B, Kang C. 2010. Chlorine gas: an evolving hazardous material threat and unconventional weapon. West J Emerg Med. 11(2):151–156.

Jurkuvenaite A, Benavides GA, Komarova S, Doran SF, Johnson M, Aggarwal S, Zhang J, Darley-Usmar VM, Matalon S. 2015. Upregulation of autophagy decreases chlorine-induced mitochondrial injury and lung inflammation. Free Radic Biol Med. 85:83–94.

Kales SN, Polyhronopoulos GN, Castro MJ, Goldman RH, Christiani DC. 1997. Mechanisms of and facility types involved in hazardous materials incidents. Environ Health Perspect. 105(9):998–1000.

Kilburn KH. 2000. Chlorine-induced damage documented by neurophysiological, neuropsychological, and pulmonary testing. Arch Environ Health. 55(1):31–37.

Klonne DR, Ulrich CE, Riley MG, Hamm TE Jr, Morgan KT, Barrow CS. 1987. One-year inhalation toxicity study of chlorine in rhesus monkeys (*Macaca mulatta*). Fundam Appl Toxicol. 9(3):557–572.

Kose A, Kose B, Acikalin A, Gunay N, Yildirim C. 2009. Myocardial infarction, acute ischemic stroke, and hyperglycemia triggered by acute chlorine gas inhalation. Am J Emerg Med. 27(8):1022.e1–1024.

Lee J, Giordano S, Zhang J. 2012. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J. 441(2):523–540.

Leikauf GD, Pope-Varsalona H, Concel VJ, Liu P, Bein K, Berndt A, Martin TM, Ganguly K, Jang AS, Brant KA, et al. 2012. Integrative assessment of chlorine-induced acute lung injury in mice. Am J Respir Cell Mol Biol. 47(2):234–244.

Leikauf GD, Pope-Varsalona H, Concel VJ, Liu P, Bein K, Brant KA, Dopico RA, Di YP, Jang AS, Dietsch M, et al. 2010. Functional genomics of chlorine-induced acute lung injury in mice. Proc Am Thorac Soc. 7(4):294–296.

Leube G, Kreiter H. 1971. Acute chlorine poisoning. Case reports of 90 patients with acute poisoning. Med Klin. 66(10):354–357.

Leustik M, Doran S, Bracher A, Williams S, Squadrito GL, Schoeb TR, Postlethwait E, Matalon S. 2008. Mitigation of chlorine-induced lung injury by low-molecular-weight antioxidants. Am J Physiol Lung Cell Mol Physiol. 295(5):L733–L743.

Luo S, Trubel H, Wang C, Pauluhn J. 2014. Phosgene- and chlorine-induced acute lung injury in rats: comparison of cardiopulmonary function and biomarkers in exhaled breath. Toxicology. 326:109–118.

Mackie E, Svendsen E, Grant S, Michels JE, Richardson WH. 2014. Management of chlorine gas-related injuries from the Graniteville, South Carolina, train derailment. Disaster Med Public Health Prep. 8(5):411–416.

Massa CB, Scott P, Abramova E, Gardner C, Laskin DL, Gow AJ. 2014. Acute chlorine gas exposure produces transient inflammation and a progressive alteration in surfactant composition with accompanying mechanical dysfunction. Toxicol Appl Pharmacol. 278(1):53–64.

Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM, Acute Lung Injury in Animals Study Group. 2011. An Official American Thoracic Society Workshop Report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol. 44(5):725–738.

Matute-Bello G, Frevert CW, Martin TR. 2008. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 295(3):L379–L399.

McGovern T, Day BJ, White CW, Powell WS, Martin JG. 2011. AEOL10150: a novel therapeutic for rescue treatment after toxic gas lung injury. Free Radic Biol Med. 50(5):602–608.

McGovern TK, Goldberger M, Allard B, Farahnak S, Hamamoto Y, O'Sullivan M, Hirota N, Martel G, Rousseau S, Martin JG. 2015. Neutrophils mediate airway hyperresponsiveness after chlorine-induced airway injury in the mouse. Am J Respir Cell Mol Biol. 52(4):513–522.

McGovern TK, Powell WS, Day BJ, White CW, Govindaraju K, Karmouty-Quintana H, Lavoie N, Tan JJ, Martin JG. 2010. Dimethylthiourea protects against chlorine induced changes in airway function in a murine model of irritant induced asthma. Respir Res. 11(1):138.

Mo Y, Chen J, Humphrey DM Jr, Fodah RA, Warawa JM, Hoyle GW. 2015. Abnormal epithelial structure and chronic lung inflammation after repair of chlorine-induced airway injury. Am J Physiol Lung Cell Mol Physiol. 308(2):L168–L178.

Mo Y, Chen J, Schlueter CF, Hoyle GW. 2013. Differential susceptibility of inbred mouse strains to chlorine-induced airway fibrosis. Am J Physiol Lung Cell Mol Physiol. 304(2):L92–L102.

Morris JB, Wilkie WS, Shusterman DJ. 2005. Acute respiratory responses of the mouse to chlorine. Toxicol Sci. 83(2):380–387.

Morty RE, Kuebler WM. 2014. TRPV4: an exciting new target to promote alveolocapillary barrier function. Am J Physiol Lung Cell Mol Physiol. 307(11):L817–L821.

Murphy MP. 2009. How mitochondria produce reactive oxygen species. Biochem J. 417(1):1–13.

Musah S, Chen J, Hoyle GW. 2012. Repair of tracheal epithelium by basal cells after chlorine-induced injury. Respir Res. 13:107.

Musah S, Schlueter CF, Humphrey DM Jr, Powell KS, Roberts AM, Hoyle GW. 2017. Acute lung injury and persistent small airway disease in a

rabbit model of chlorine inhalation. Toxicol Appl Pharmacol. 315: 1–11.
Nodelman V, Ultman JS. 1999. Longitudinal distribution of chlorine absorption in human airways: a comparison to ozone absorption. J Appl Physiol. 87(6):2073–2080.
O'Koren EG, Hogan BL, Gunn MD. 2013. Loss of basal cells precedes bronchiolitis obliterans-like pathological changes in a murine model of chlorine gas inhalation. Am J Respir Cell Mol Biol. 49(5):788–797.
Okponyia OC, McGraw MD, Dysart MM, Garlick RB, Rioux JS, Murphy AL, Roe GB, White CW, Veress LA. 2018. Oxygen administration improves survival but worsens cardiopulmonary functions in chlorine-exposed rats. Am J Respir Cell Mol Biol. 58(1):107–116.
Park GD, Mitchel JT. 2016. Working with the U.S. Food and Drug Administration to obtain approval of products under the Animal Rule. Ann N Y Acad Sci. 1374(1):10–16.
Rastrick J, Birrell M. 2014. The role of the inflammasome in fibrotic respiratory diseases. Minerva Med. 105(1):9–23.
Robaszkiewicz A, Bartosz G, Soszynski M. 2011. Detection of 3-chlorinated tyrosine residues in human cells by flow cytometry. J Immunol Methods. 369(1–2):141–145.
Rothenberg C, Achanta S, Svendsen ER, Jordt SE. 2016. Tear gas: an epidemiological and mechanistic reassessment. Ann N Y Acad Sci. 1378(1):96–107.
Runkle JR, Zhang H, Karmaus W, Brock-Martin A, Svendsen ER. 2013. Long-term impact of environmental public health disaster on health system performance: experiences from the Graniteville, South Carolina chlorine spill. South Med J. 106(1):74–81.
Samal A, Honovar J, White CR, Patel RP. 2010. Potential for chlorine gas-induced injury in the extrapulmonary vasculature. Proc Am Thorac Soc. 7(4):290–293.
Schneider T, Lütkefend T. 2019. Nowhere to hide: the logic of chemical weapons use in Syria. Berlin, Germany: Global Public Policy Institute.
Soffritti M, Belpoggi F, Lenzi A, Maltoni C. 1997. Results of long-term carcinogenicity studies of chlorine in rats. Ann N Y Acad Sci. 837: 189–208.
Song W, Wei S, Liu G, Yu Z, Estell K, Yadav AK, Schwiebert LM, Matalon S. 2011. Postexposure administration of a {beta}2-agonist decreases chlorine-induced airway hyperreactivity in mice. Am J Respir Cell Mol Biol. 45(1):88–94.
Song W, Yu Z, Doran SF, Ambalavanan N, Steele C, Garantziotis S, Matalon S. 2015. Respiratory syncytial virus infection increases chlorine-induced airway hyperresponsiveness. Am J Physiol Lung Cell Mol Physiol. 309(3):L205–L210.
Spickett CM. 2007. Chlorinated lipids and fatty acids: an emerging role in pathology. Pharmacol Ther. 115(3):400–409.
Summerhill EM, Hoyle GW, Jordt S-E, Jugg BJ, Martin JG, Matalon S, Patterson SE, Prezant DJ, Sciuto AM, Svendsen ER, et al. 2017. An Official American Thoracic Society Workshop Report: chemical inhalational disasters. Biology of lung injury, development of novel therapeutics, and medical preparedness. Ann Am Thorac Soc. 14(6): 1060–1072.
Sutiakova I, Sutiak V, Rimkova S, Poracova J. 2004. Chromosome damage in peripheral lymphocytes of sheep induced by chlorine in drinking water. Int J Environ Health Res. 14(5):381–390.
Thorneloe KS, Cheung M, Bao W, Alsaid H, Lenhard S, Jian MY, Costell M, Maniscalco-Hauk K, Krawiec JA, Olzinski A, et al. 2012. An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure. Sci Transl Med. 4(159):159ra148.
Tian X, Tao H, Brisolara J, Chen J, Rando RJ, Hoyle GW. 2008. Acute lung injury induced by chlorine inhalation in C57BL/6 and FVB/N mice. Inhal Toxicol. 20(9):783–793.
Tiruppathi C, Ahmmed GU, Vogel SM, Malik AB. 2006. Ca^{2+} signaling, TRP channels, and endothelial permeability. Microcirculation. 13(8):693–708.
Tuck SA, Ramos-Barbon D, Campbell H, McGovern T, Karmouty-Quintana H, Martin JG. 2008. Time course of airway remodelling after an acute chlorine gas exposure in mice. Respir Res. 9(1):61.
Vajner JE 3rd, Lung D. 2013. Case files of the University of California San Francisco Medical Toxicology Fellowship: acute chlorine gas inhalation and the utility of nebulized sodium bicarbonate. J Med Toxicol. 9(3): 259–265.
Van Sickle D, Wenck MA, Belflower A, Drociuk D, Ferdinands J, Holguin F, Svendsen E, Bretous L, Jankelevich S, Gibson JJ, et al. 2009. Acute health effects after exposure to chlorine gas released after a train derailment. Am J Emerg Med. 27(1):1–7.
Wang HM, Bodenstein M, Markstaller K. 2008. Overview of the pathology of three widely used animal models of acute lung injury. Eur Surg Res. 40(4):305–316.
Wang J, Abu-Zidan FM, Walther SM. 2002. Effects of prone and supine posture on cardiopulmonary function after experimental chlorine gas lung injury. Acta Anaesthesiol Scand. 46(9):1094–1102.
Wang J, Oldner A, Winskog C, Edston E, Walther SM. 2006. Effects of endothelin receptor antagonism on acute lung injury induced by chlorine gas. Crit Care Med. 34(6):1731–1737.
Wang J, Winskog C, Edston E, Walther SM. 2005. Inhaled and intravenous corticosteroids both attenuate chlorine gas-induced lung injury in pigs. Acta Anaesthesiol Scand. 49(2):183–190.
Wang J, Zhang L, Walther SM. 2002. Inhaled budesonide in experimental chlorine gas lung injury: influence of time interval between injury and treatment. Intensive Care Med. 28(3):352–357.
Wang J, Zhang L, Walther SM. 2004. Administration of aerosolized terbutaline and budesonide reduces chlorine gas-induced acute lung injury. J Trauma. 56(4):850–862.
Wenck MA, Van Sickle D, Drociuk D, Belflower A, Youngblood C, Whisnant MD, Taylor R, Rudnick V, Gibson JJ. 2007. Rapid assessment of exposure to chlorine released from a train derailment and resulting health impact. Public Health Rep. 122(6):784–792.
White CW, Martin JG. 2010. Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models. Proc Am Thorac Soc. 7(4):257–263.
Wigenstam E, Elfsmark L, Koch B, Bucht A, Jonasson S. 2016. Acute respiratory changes and pulmonary inflammation involving a pathway of TGF-beta1 induction in a rat model of chlorine-induced lung injury. Toxicol Appl Pharmacol. 309:44–54.
Wigenstam E, Koch B, Bucht A, Jonasson S. 2015. N-acetyl cysteine improves the effects of corticosteroids in a mouse model of chlorine-induced acute lung injury. Toxicology. 328:40–47.
Winder C. 2001. The toxicology of chlorine. Environ Res. 85(2):105–114.
Wolf DC, Morgan KT, Gross EA, Barrow C, Moss OR, James RA, Popp JA. 1995. Two-year inhalation exposure of female and male B6C3F1 mice and F344 rats to chlorine gas induces lesions confined to the nose. Fundam Appl Toxicol. 24(1):111–131.
Yadav AK, Bracher A, Doran SF, Leustik M, Squadrito GL, Postlethwait EM, Matalon S. 2010. Mechanisms and modification of chlorine-induced lung injury in animals. Proc Am Thorac Soc. 7(4):278–283.
Yadav AK, Doran SF, Samal AA, Sharma R, Vedagiri K, Postlethwait EM, Squadrito GL, Fanucchi MV, Roberts LJ 2nd, Patel RP, et al. 2011. Mitigation of chlorine gas lung injury in rats by postexposure administration of sodium nitrite. Am J Physiol Lung Cell Mol Physiol. 300(3): L362–L369.
Zaky A, Bradley WE, Lazrak A, Zafar I, Doran S, Ahmad A, White CW, Dell'Italia LJ, Matalon S, Ahmad S. 2015. Chlorine inhalation-induced myocardial depression and failure. Physiol Rep. 3(6):e12439.
Zarogiannis SG, Jurkuvenaite A, Fernandez S, Doran SF, Yadav AK, Squadrito GL, Postlethwait EM, Bowen L, Matalon S. 2011. Ascorbate and deferoxamine administration after chlorine exposure decrease mortality and lung injury in mice. Am J Respir Cell Mol Biol. 45(2): 386–392.
Zarogiannis SG, Wagener BM, Basappa S, Doran S, Rodriguez CA, Jurkuvenaite A, Pittet JF, Matalon S. 2014. Postexposure aerosolized heparin reduces lung injury in chlorine-exposed mice. Am J Physiol Lung Cell Mol Physiol. 307(5):L347–L354.