Countermeasures against pulmonary threat agents

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### Abbreviation Listing

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AHR</td>
<td>airway hyperresponsiveness</td>
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<tr>
<td>AKG</td>
<td>alpha ketoglutarate</td>
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<td>ALI</td>
<td>acute lung injury</td>
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<tr>
<td>ARDS</td>
<td>acute respiratory distress syndrome</td>
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<tr>
<td>ATI</td>
<td>alveolar type I cells</td>
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<td>ATII</td>
<td>alveolar type II cells</td>
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<td>BAR</td>
<td>beta-adrenergic receptors</td>
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<td>BO</td>
<td>bronchiolitis obliterans</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>CCl₃NO₂</td>
<td>chloropicrin</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>C₂H₄Cl₂S</td>
<td>sulfur mustard (SM)</td>
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<tr>
<td>C₃H₄O</td>
<td>acrolein</td>
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<td>Cl₂</td>
<td>chlorine gas</td>
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<td>CLDN5</td>
<td>claudin 5</td>
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<tr>
<td>CoC</td>
<td>chemicals of concern</td>
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<tr>
<td>COCl₂</td>
<td>phosgene (CG)</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<td>COx</td>
<td>carbon oxides</td>
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<td>CWA</td>
<td>chemical warfare agent</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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ERK1/2 extracellular signal-regulated protein kinase 1 and 2
Fas1 fasciclin 1
FiCO₂ fraction of inspired oxygen
Fv-HSP72 single-chain variable fragment of heat shock protein 72 (fusion protein)
GSH glutathione
HBECs human bronchial epithelial cells
HCl hydrogen chloride
HOCl hypochlorous acid
IL interleukins (IL1β, IL22, IL17)
ILD interstitial lung disease
iNOS inducible nitric oxide synthase
IPF idiopathic pulmonary fibrosis
LC₅₀ lethal concentration 50
LRT lower respiratory tract
L-TC L-thiocitrulline
MCMs medical countermeasures
miRNAs microRNAs
MSC mesenchymal stem cells
NAC N-acetylcysteine
NE neutrophil elastase
NET neutrophil extracellular trap
NH₃ anhydrous ammonia
NIH CCRP National Institutes of Health Chemical Countermeasures Research Program
NO nitric oxide
NOS-2 nitric oxide synthase 2
NOx nitrogen oxides
PaCO₂ partial pressure of carbon dioxide
PAI-1 plasminogen activator inhibitor 1
P-ALI  phosgene-induced acute lung injury
RADS  reactive airways disease
ROS/RNS  reactive oxygen species/reactive nitrogen species
SP-D  surfactant protein D
TNF-α  tumor necrosis factor alpha
TRP  transient receptor potential
TRPA1  transient receptor potential A1
URT  upper respiratory tract
Wnt/β-catenin  wingless-type MMTV integration site family/beta-catenin
WWI  world war I
I. Abstract: Inhaled toxicants are used for diverse purposes, ranging from industrial applications such as agriculture, sanitation, and fumigation, to crowd control and chemical warfare, and acute exposure can induce lasting respiratory complications. The intentional release of chemical warfare agents (CWAs) during World War I caused life-long damage for survivors, and CWA use is outlawed by international treaties. However, in the past two decades chemical warfare use has surged in the Middle East and Eastern Europe with a shift toward lung toxicants. The potential use of industrial and agricultural chemicals in rogue activities are a major concern, as they are often stored and transported near populated areas, where intentional or accidental release can cause severe injuries and fatalities. Despite laws and regulatory agencies that regulate use, storage, transport, emissions, and disposal, inhalational exposures continue to cause lasting lung injury.

Industrial irritants (e.g., ammonia) aggravate the upper respiratory tract, causing pneumonitis, bronchoconstriction, and dyspnea. Irritant gases (e.g., acrolein, chloropicrin) affect epithelial barrier integrity and cause tissue damage, through reactive intermediates or by direct adduction of cysteine-rich proteins. Symptoms from CWAs (e.g., chlorine gas, phosgene, sulfur mustard) progress from airway obstruction and pulmonary edema to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) that results in respiratory depression days later. Emergency treatment is limited to supportive care using bronchodilators to control airway constriction, and rescue with mechanical ventilation to improve gas-exchange. Complications from acute exposure can promote obstructive lung disease and/or pulmonary fibrosis which require long-term clinical care.

Significance Statement: Inhaled chemical threats are of growing concern in both civilian and military settings, and there is increased need to reduce acute lung injury and delayed clinical
complications from exposures. This minireview highlights our current understanding of acute toxicity and pathophysiology of a select number of chemicals of concern. It discusses potential early-stage therapeutic development, as well as challenges in developing countermeasures applicable for administration in mass casualty situations.

**Keywords:** Chemical threat, lung toxicant, nitrosative stress, inflammation, ALI, ARDS, RADS, COPD, BO, ILD, IPF, bronchitis, pulmonary edema, respiratory failure
II. Introduction

Over two hundred chemicals of concern (CoC) have been identified by the U.S. Department of Homeland Security as high consequence public health threats and a quarter of these toxicants affect the lungs. CWAs are high priority health concerns with short exposure windows that cause immediate casualties and lung injury and can induce progressive lung disease. Industrial and agricultural chemicals (irritants) are of significant concern, as accidental or intentional exposures affect civilian populations. Lung irritants cause reversible non-immunologic reactions after direct contact with the nose, throat, and respiratory tract.

Lung tissue in the lower respiratory tract is composed of numerous alveoli that mediate gas-exchange and is highly sensitive to inhaled toxicants due its extensive surface area. Toxicants are inhaled through absorption of chemical vapors, particulates, and incomplete combustion byproducts into the lungs. Clinical symptoms and severity depend on dose and proximity, water solubility, and toxicant size. Highly water soluble (hydrophilic, e.g., ammonia) and larger compounds (> 10 um) mainly affect the upper airways, stimulating the trigeminal nerves of the nasal passages, with rapid symptom onset that presents as burning, irritation, and cough. Those of intermediate size and solubility (5-10 um, e.g., chlorine gas) cause greater parenchymal injury, and small, poorly water soluble (hydrophobic) toxicants < 5um (e.g., phosgene gas) penetrate to the respiratory bronchioles and alveoli of the lower respiratory tract to cause detrimental lung injury (WR 1994, Muskat 2008, Nelson LS 2014). Oxidant gases (e.g., chlorine, phosgene, ammonia) react initially with antioxidants in the epithelial lining fluid, and during acute, high-dose toxicant exposures, this first line of defense can be overwhelmed (Addis, Aggarwal et al. 2021). Understudied chemicals and some of the acute toxicities that lead to lung complications are described in Table 1.
Mechanisms of early lung injury are shared among irritants and CWAs, while the development and progression of delayed lung complications are toxicant-specific. CWAs cause respiratory distress, rapid airway constriction, and pulmonary edema that can lead to respiratory failure. Similarly, CWAs can cause bronchoconstriction and airway smooth muscle activation that accelerates cardiopulmonary injury. Exposure induces two phase effects, which initially present as pneumonitis, bronchitis, with latent symptoms of pulmonary edema, bronchiolitis, and ALI/ARDS days to weeks later (Muskat 2008). High-dose irritants initially cause persistent rhinosinusitis, airway hyperresponsiveness (AHR), and accelerated lung function decline. Complications from exposure may proceed from airway obstruction to persistent reactive airways disease (RADS), and at high doses from pulmonary edema to ALI/ARDS (Leduc, Gris et al. 1992, Lu, Mundy et al. 2017, Pesonen and Vähäkangas 2020).

ARDS develops as a cascading consequence of ALI, and often progresses to respiratory failure. Current research is aimed at studying ALI mechanisms, and intervention strategies that can be applied before ARDS develops. Symptoms of ALI and ARDS overlap, with clinical distinction based on the degree of hypoxemia present \([\text{PaO}_2/\text{FiO}_2\text{ ratio, or oxygenation index, is the ratio of partial pressure of oxygen (PaO}_2\text{) to fraction of inspired oxygen (FiO}_2\text{): ALI } \leq 300 \text{ mm Hg; ARDS } \leq 200 \text{ mm Hg}].\) The term ALI/ARDS is now widely used (Butt, Kurdowska et al. 2016), and diagnosis pairs deteriorating oxygenation levels with evidence of bilateral lung infiltrates on chest X-ray. ALI/ARDS represents a continuum of physiological changes that disrupt integrity of the endothelial and epithelial barriers of the lung, causing diffuse alveolar damage and increased lung permeability that impairs gas exchange. It is characterized by three phases: exudative (days 1-6), proliferative (days 7-14) and fibrotic (>14 days), and diverse
chemicals included on the CoC list may induce phenotypically diverse effects along the trajectory of lung injury (Radbel, Laskin et al. 2020).

The exudative phase is indicated by inflammation, surfactant deficiency, and tissue factor dysfunction, which contribute to intravascular and intra-alveolar coagulation, and hypoxemia. During this phase lymphatic drainage from the lung cannot remove fluid that filters from the lung vasculature, and potentially fatal pulmonary edema develops (Lindsay 2011). The proliferative phase is largely mediated by pro-resolution macrophages, which recruit specialized pro-resolving mediators to initiate resolution. In this phase alveolar type II (ATII) cells proliferate due to Wnt/β-catenin signaling and differentiate into ATI cells to repair the damaged epithelial barrier (Radbel, Laskin et al. 2020). Impaired resolution prompts the debilitating fibrotic phase which is characterized by collagen deposition and persistent thickening of the alveolar interstitium that impedes gas exchange. This promotes obstructive lung diseases [e.g., bronchiolitis obliterans (BO), chronic obstructive pulmonary disease (COPD)] that require long-term clinical management. For comprehensive reviews of ALI/ARDS, see Radbel et al (Radbel, Laskin et al. 2020) and Butt et al (Butt, Kurdowska et al. 2016).

In CWA-induced lung injury, the additional extravascular coagulation and fibrin formation in the alveolar compartment may promote development of pulmonary fibrosis. Early symptoms include unproductive cough, weight loss, and fatigue, largely due to hypoxia (Savin, Zenkova et al. 2022). Diagnosis is based on lung function measures, and blood and imaging tests are used to rule out other lung-related illnesses. Pulmonary fibrosis is a heterogeneous disease with distinct tissue pathology, encompassing multiple chronic lung outcomes including interstitial lung disease (ILD) and idiopathic pulmonary fibrosis (IPF). Left unresolved, pulmonary fibrosis progressively destroys the alveolar structure of the lung and can lead to
respiratory failure. For a comprehensive review of fibrosis, see Savin et al (Savin, Zenkova et al. 2022).

The NIH Chemical Countermeasures Research Program (CCRP) is focused on toxicant-induced ALI/ARDS and delayed effects of lung injury. Its mission is to understand molecular and cellular perturbations and identify common mechanisms of lung injury in order to develop safe and effective medical countermeasures (MCMs) that reduce acute mortality, and complications of chronic lung disease.

III. Recent Advances in Inhalational Research

Irritants – ammonia

Anhydrous ammonia (NH₃) is a compressed liquid used as an agricultural fertilizer and refrigerant and is widely transported through highly populated areas. Upon release, ammonia reacts with water to form ammonium hydroxide which irritates the mucosa and at high concentrations causes severe upper airways irritation and respiratory failure within two to five minutes of exposure (Saeed, Boyer et al. 2018). Tissue damage occurs through exothermic reactions with body tissues, causing alkali skin burns and liquefactive necrosis (Pangeni, Timilsina et al. 2022). In some cases, survivors may develop complications such as bronchiectasis, AHR, BO, COPD, ILD, and end-stage disease that requires lung transplantation. Diagnosis is based on exposure, by physical exam and body-system focused lab tests. Alpha ketoglutarate (AKG) and fluticasone propionate (Flonase) have been shown in animal and cell culture models to reduce injury (Ali, Mittal et al. 2012, Ali, Mittal et al. 2013), however, there are currently no antidotes or tests for extent of toxicity.
Recent animal studies have established an LD₅₀ value and mechanisms by which ammonia induces ALI/ARDS and systemic changes (Perkins, Wong et al. 2017, Elfsmark, Ågren et al. 2019). Intratracheal ammonia instillation causes severe lung injury and increased vascular resistance in mice, with persistent respiratory acidosis and alveolar damage at one day, and interstitial hemorrhage and mortality within seven days of exposure (Elfsmark, Ågren et al. 2022). Early activation of surfactant protein D (SP-D), which regulates pulmonary innate immunity and defense against xenobiotics (Madsen, Kliem et al. 2000), and sustained activation of coagulation factors such as plasminogen activator inhibitor 1 (PAI-1) and fibrinogen are observed. Ammonia induces biphasic injury, with an acute inflammatory phase that deteriorates to obstructive lung disease within days. Comparable symptoms of dyspnea, acute neutrophilic airway inflammation, and pulmonary edema are seen in ammonia-induced ALI/ARDS. Case reports show some patients develop delayed lung injuries without initial upper respiratory tract obstruction (Close, Catlin et al. 1980) and persistent RADS after a single high-dose exposure (Brooks, Weiss et al. 1985, Brooks 2008).

**Irritant gases—acrolein and chloropicrin**

Acrolein (C₃H₄O) and chloropicrin (CCl₃NO₂) were deployed as CWAs in WWI and are now used for industrial and agricultural applications. Acrolein is a volatile aldehyde formed during incomplete combustion of fossil fuels, and large quantities are synthesized for industrial use as a biocide (Conklin 2016). It reacts with the respiratory lining fluid and cellular macromolecules, altering gene regulation, mucociliary transport, and compromising endothelial barrier integrity. Acrolein induces AHR and mucus hypersecretion, which obstructs the lumen, and leads to airspace enlargement with loss of lung elasticity. At high concentrations it promotes epithelial cell hyperplasia with lower respiratory tract necrosis that induces pulmonary edema.
and ALI/ARDS if not resolved (Xiong, Wu et al. 2018). Acrolein-exposed lungs have histological features of squamous cell differentiation and dysfunctional tissue remodeling (Beauchamp, Andjelkovich et al. 1985).

Mechanistic studies show acrolein affects energy balance, with profound effects on lipid and mitochondrial metabolism (Fabisiak, Medvedovic et al. 2011, Agarwal, Yin et al. 2013, Snow, McGee et al. 2017). RNASeq analyses indicate sex-dependent differences in injury severity and increased neutrophil extracellular trap (NET) formation (Bein, Birru et al. 2021). *In vitro* studies using acrolein vapor suggest interleukins (IL)1β, IL22 and IL17 pathway genes play a central role in toxicity (Johanson, Dwivedi et al. 2020). Potential therapies include chemosensory transient receptor potential A1 (TRPA1) antagonists (Conklin 2016), antioxidants (Hochman, Collaco et al. 2014), and targeting claudin 5 (CLDN5) to preserve endothelial barrier integrity (Jang, Concel et al. 2011). Investigators show increased perivascular edema and reduced CLDN5 expression in acrolein-sensitive mice (Jang, Concel et al. 2011), and CLDN5 is being studied in other models of chemically induced ALI/ARDS (Geng, Ma et al. 2018, Geng, Yu et al. 2020).

Chloropicrin is a halogenated fumigant and disinfectant, that causes respiratory complications when inhaled. Decomposition releases toxic gases such as phosgene, chlorine, and nitrogen oxides, and it can dehalogenate in aqueous systems to form nitromethane. While initial exposure irritates the lungs, at higher concentrations chloropicrin induces vomiting and breathing difficulties, and airway damage that triggers bronchitis and pulmonary edema. This can progress to respiratory failure or in some instances life-long complications such as COPD. Histological lesions including ulceration and necrosis are seen in animal models, and chloropicrin is toxic to other organs (Pesonen, Rysä et al. 2017).
Mechanisms of chloropicrin-induced lung damage are not well understood, and animal studies have mainly focused on ocular injury. Chloropicrin exposed primary bronchial epithelial cells (HBECs) show elevated levels of ERK1/2 that induce ER stress (Pesonen, Storvik et al. 2015) and increase cytoplasmic vacuolization (Pesonen, Rysä et al. 2017). HBECs show deformed cytoskeletal ultrastructure, with weakened cell attachments, and mitochondrial dysfunction. This suggests chloropicrin targets β-tubulin, and interferes with the tubulin network, inducing apoptosis. Chloropicrin is mutagenetic, and modifies sulfhydryl groups on cysteines and enzymes important for energy metabolism (Sparks, Quistad et al. 2000). There are no specific biomarkers of exposure, and therapy is limited to supportive care and the use of antioxidants. N-acetylcysteine (NAC) acts as thiol-reducing agent to scavenge reactive oxygen species/reactive nitrogen species (ROS/RNS) and stimulate glutathione (GSH) synthesis and prevents chloropicrin-induced cytotoxicity and vacuolization in cell culture models (Pesonen, Häkkinen et al. 2014).

**Chemical warfare agents – chlorine, phosgene, and sulfur mustard**

Chlorine gas (Cl\textsubscript{2}) is used industrially for sanitation and water purification and is also used as a CWA. It reacts with the mucosal lining to form hydrochloric and hypochlorous acids, and the oxidative byproduct hypochlorite, which mediate cytotoxic effects in the lungs (Achanta and Jordt 2021). Chlorine initially causes symptoms of bronchospasm and chest pain, and at high concentrations destroys lung structure, inducing hemorrhage, pulmonary edema, and respiratory collapse within one hour of exposure. Survivors of acute exposure may develop symptoms of pulmonary fibrosis and RADS, with cardiovascular complications that last for years (Zaky, Bradley et al. 2015, Carlisle, Lam et al. 2016).
Animal models of chlorine injury show histological features of epithelial desquamation, leukocyte infiltration, atelectasis, and necrosis (Balakrishna, Song et al. 2014). Biologic changes observed include protease activation, coagulopathies, compromised barrier integrity, and fibrin deposition (Mo, Chen et al. 2015, Musah, Schlueter et al. 2017), as well as increased susceptibility to infection (Gessner, Doran et al. 2013, Song, Yu et al. 2015). Potential therapeutics to counteract chlorine toxicity include antioxidants, corticosteroids, and combined therapies. TRP inhibitors, nitrite administration, heparin, and autophagy activators also show limited benefits (Achanta and Jordt 2021). The recent identification of chlorinated lipids as biomarkers of exposure provides a potential diagnostic measure of chlorine toxicity (Spickett 2007, Ford, Honavar et al. 2016).

Phosgene (COCl₂; CG) is a toxic colorless gas used for chemical synthesis and as a CWA. CG is hydrophobic and slowly reacts with water to form carbon dioxide and hydrochloric acid, which directly damages the respiratory tract (Pauluhn 2021, Cao, Zhang et al. 2022). CG bypasses chemosensory perception through limited retention in the upper airways and reflex bradypnea is largely absent (Pauluhn 2021). CG collects in the lower respiratory tract, and noncardiogenic pulmonary edema often goes unnoticed, with development of secondary hypoxemia and deterioration of the blood-gas barrier (Pauluhn, Carson et al. 2007). Phosgene-induced ALI (P-ALI) is preceded by persistent respiratory depression and cardiogenic pulmonary edema within 15-20 hours of exposure (Pauluhn 2021). This occult or asymptomatic period is not evident on clinical exam. Fluid shifts from the pulmonary to systemic circulation induce hypovolemia and hypotension that parallel pulmonary edema onset (Pauluhn 2021). In late-stage P-ALI, survivors often develop fibrosis and chronic obstructive lung disease (Cao, Zhang et al. 2022).
A consistent LC$_{50}$ in animal models has been difficult to determine (Hobson, Richieri et al. 2021), and toxic load depends linearly on dose x exposure duration, such that acute high dose is no more toxic than chronic low levels of exposure. CG is electrophilic and acylates nucleophilic biomolecules (Holmes, Keyser et al. 2016), inducing pulmonary edema as the blood-gas barrier is permeabilized. CG undergoes hemolytic cleavage to form highly reactive carbamoyl chloride derivatives (Arroyo, Feliciano et al. 1993, Holmes, Keyser et al. 2016) that further alter surfactant levels. Increased nitrosative stress affects neuronal cells that innervate the lungs, as well as epithelial, endothelial, and blood cells. Ongoing debate on the role of inflammation in vasopermeability and pulmonary edema (Russell, Blain et al. 2006, Chen, Bai et al. 2013, Holmes, Keyser et al. 2016) has impacted agreement on which biomarkers and therapeutics should be explored.

Anti-inflammatories show little benefit for CG-induced injury, and TRP antagonists are limited to reducing neurogenic inflammation (Pauluhn 2021). Administration of antioxidants such as NAC increase GSH availability (Ji, Liu et al. 2010) and melatonin attenuates CG-induced lung injury through Wnt/b-catenin signaling (Zhang, Zhang et al. 2017). Strategies using ulinastatin and NOS-2 inhibitors show promise in small animal models (Shen, Gan et al. 2014, Filipczak, Senft et al. 2015, Zhang, Zhang et al. 2017) but early treatment during the asymptomatic phase is critical to minimize the risk of cardiogenic edema (Lu, Huang et al. 2021). Mesenchymal stem cells (MSC) that target miRNAs (Xu, Shao et al. 2019, Qu, Zhang et al. 2020, Jiang, Shao et al. 2021) or overexpress angiogenic (Shao, Shen et al. 2018) or heat shock proteins (Jin, Zhou et al. 2020) are shown to counteract toxicity in vitro, and administration of cardioprotective Fv-HSP72 may ameliorate injury (Hobson, Richieri et al. 2021).
Sulfur mustard (C4H8Cl2S; SM) is a toxic vesicant primarily used as a CWA, with short and long-term lung injury effects. It causes blistering and erythema of the respiratory tract (Schmidt, Steinritz et al. 2018) that destroys bronchial tissue and obstructs the airway minutes to hours after exposure. At high doses, this is followed by hemorrhagic pulmonary edema, secondary pneumonia, and respiratory failure twenty-four hours to one week later (Ghanei, Khalili et al. 2005). Acute exposures can induce pathogenic lesions and fibrosis that promotes BO and/or ILD decades later (Ghanei, Tazelaar et al. 2008). In addition, acute SM exposure causes systemic injury and increases cancer risk (Razavi, Abdollahi et al. 2016).

While the lungs are a major target of exposure, SM is highly lipophilic, and enters the body through multiple routes. It eliminates chloride ions through intramolecular substitution to form cyclic sulfonium ions. These reactive intermediates alkylate DNA and other nuclear components inducing genotoxicity and cytotoxic damage that suppresses the immune system and inducible nitric oxide synthase (iNOS) signaling (Tahmasbpour, Ghanei et al. 2019). Inhaled SM activates phagocytic leukocytes and inflammatory mediators that alter serum cytokines, C reactive protein (CRP), and soluble pro-apoptotic fasciclin 1 (Fas1) expression. Vascular leakage and endothelial permeability are reported in animal models (Calvet, Jarreau et al. 1994, Rancourt, Veress et al. 2013, Malaviya, Abramova et al. 2020), as well as fibrin cast formation that occludes the airways (White, Rancourt et al. 2016). Increased fibrinogen suppresses surfactant, and can prolong macrophage activation and inflammation. (Wygrecka, Jablonska et al. 2008).

There are currently no approved therapies for SM inhalation, however, combined administration of vitamin E and L-thiocitrulline (L-TC) helps replenish NO and manage respiratory symptoms. Therapies being explored include anti-inflammatory, antioxidants, and...
protease inhibitors, and some benefits from natural products are shown (Boskabady and Farhadi 2008, Hossein, Nasim et al. 2008). Studies show restoration of surfactant may protect against SM-induced lung injury (van Helden, Kuijpers et al. 2004), and that SP-D, a non-specific alveolar injury marker, may serve as an indicator of early respiratory disease (Starosta and Griese 2006, Sorensen 2018, Malaviya, Abramova et al. 2020).

**IV. Early mechanisms of toxicant-induced lung injury**

While ALI/ARDS progression and delayed respiratory complications are toxicant-specific, toxicant-induced inflammation and injury share overlapping protective mechanisms to limit lung damage (Fig 1). Acute exposure induces nitrosative stress and endothelial damage that occurs concurrently with inflammation (Radbél, Laskin et al. 2020, Elfsmark, Ågren et al. 2022). Reactive intermediates (e.g., ROS/RNS) induce inflammatory cytokines/chemokines that activate iNOS which generates excess nitric oxide (NO) that can magnify oxidative stress (Sun, Druhan et al. 2010). Peripheral lung inflammation stimulates histamine release and endothelial NO production, which amplifies the inflammatory cascade (Branco, Yoshikawa et al. 2018, Radbel, Laskin et al. 2020). In CWA models, the overwhelming magnitude of nitrosative stress depletes NO stores from alveolar macrophages (Malaviya, Gardner et al. 2023) and aggravates histamine intolerance (Branco, Yoshikawa et al. 2018). It also reduces glutathione levels and alters phospholipid production, exhausting surfactant reserves in multiple models of toxicity (Tahmasbpour, Ghanei et al. 2019, Pesonen and Vähäkangas 2020, Elfsmark, Ågren et al. 2022).

Chemosensory TRP channels respond to a variety of inhaled toxicants and induce changes in intracellular calcium that alter alveolar and vascular permeability. Activation of TRP channels in the respiratory epithelium prompts membrane depolarization and inflammatory neuropeptide release (Bessac and Jordt 2010, Achanta and Jordt 2020, Pesonen and Vähäkangas
Irritation from chloropicrin and acrolein is mediated by TRPA1 (Conklin 2016, Pesonen and Vähäkangas 2020), stimulating afferent airway fibers that trigger reflex bradypnea and respiratory braking. Chlorine and sulfur mustard directly activate TRP channels (Stenger, Zehfuss et al. 2015, Zellner and Eyer 2020), and this complex interplay of inflammatory mediators and nitrosative stress prompts bronchoconstriction that restricts airflow.

For sulfur mustard and analogs, the heightened nitrosative burden desensitizes β-adrenergic receptors (βAR) in the distal airways, affecting cyclic adenosine monophosphate (cAMP) levels, and impairing endothelial barrier function (Ghanei, Shohrati et al. 2007, Kabir, Mukherjee et al. 2009, Rambacher and Moniri 2020). This allows blood plasma to enter the interstitium and exacerbates pulmonary edema and inflammation (Radbel, Laskin et al. 2020). Inhaled toxicants can induce lung hemorrhage and activation of the coagulation cascade that mediates fibrinolysis, exacerbating ALI/ARDS. Coagulation is elevated in ammonia and chlorine models (Zarogiannis, Wagener et al. 2014, Elfsmark, Ågren et al. 2022), and extravascular coagulation and impaired fibrinolysis is pronounced in SM injury (White, Rancourt et al. 2016). In phosgene models, hemolysis destroys red blood cells, which obstructs the pulmonary capillaries (Aggarwal, Jilling et al. 2019).

V. Challenges to Medical Countermeasures Development

The development of intervention strategies to counteract acute toxicant exposures presents innumerable challenges. There are ample opportunities to advance our fundamental mechanistic understanding of toxicant-induced ALI and delayed lung effects such as pulmonary fibrosis. As discussed earlier, the resultant lung injury involves endothelial and epithelial damage, alveolar permeability dysfunction, and dysregulated lung inflammation, which reduce
lung compliance and compromise respiration. An immediate challenge in mass casualty scenarios is to develop strategies to protect survivors without prior knowledge of the inhaled toxicant. In addition, animal studies using pregnant dams and neonates have shown vulnerable subgroups are highly susceptible to pulmonary toxicants and may require population specific care (Addis, Molyvdas et al. 2020, Addis, Aggarwal et al. 2021).

Immediate treatment for acute exposures involves personal protective equipment for first responders, removal of victims from the area, and decontamination. As there are few antidotes for inhaled toxicants, therapy largely depends on supportive care, with administration of warm, humidified oxygen, antitussives, and bronchodilators (Walker, Buehner et al. 2015). In some instances, survivors with compromised airways need endotracheal intubation and low tidal volume mechanical ventilation. Those with severe epithelial injury and sloughing may require bronchoscopic lavage to maintain airflow. Inhaled and systemic corticosteroids can be used to reduce inflammation; however, demonstrations of efficacy are limited to small, uncontrolled studies from other airway diseases. Follow-on clinical therapies are based on long-term outcomes (e.g., ALI/ARDS, BO, RADS, IPF).

To date, several laboratories have explored therapeutic approaches to reduce toxicant-induced ALI using antioxidants, angiotensin converting enzyme inhibitors, neutrophil elastase (NE) inhibitors, anti-TNF-α therapy, β-agonists, heparin, and phosphodiesterase inhibitors, and some combinations of these drugs have shown limited success. However, the high attrition rate of ARDS drugs may be due to a missed therapeutic window between ALI development and progression to full blown ARDS. The translation of findings from other lung disease models (e.g., lipopolysaccharide, bleomycin) to high-dose CWA exposures that involve rapid transition of ALI to ARDS is especially difficult.
Lung injury intensity is determined by multiple factors including proximity to the exposure epicenter, duration, and population demographics (e.g., age, sex, preexisting disease status). The NIH CCRP supports the development of therapeutic agents that can be administered in the field to treat ALI, and in trauma/critical care centers to reduce the potential for delayed onset lung complications, as well as uncontrolled pulmonary edema from low dose exposure. Current research efforts have focused on understanding the acute pathophysiological consequences of exposure to a limited number of CWAs and industrial toxicants. Given the number of potential CoCs, it is prohibitive to develop a countermeasure specific to each chemical.

Instead, research efforts toward medical countermeasures development should focus on identifying common molecular, cellular, and pathophysiological pathways involved in toxicant-induced ALI using available in vitro, in silico, and organoid systems, as well as animal models. Ideally genomic, epigenomic, and metabolomic approaches could be used to establish kinetic models that demonstrate the evolution and progression of disease, and fully characterize specific cell types involved throughout the process. Defining mechanisms of ALI after industrial and agricultural toxicant exposures would be beneficial, as we have limited understanding of their molecular pathophysiology. High throughput screens could accelerate the identification of potential therapeutic targets that counteract acute and delayed effects from diverse toxicant insults.

There is compelling need for better mechanistic understanding of the temporal etiology of lung injury following exposure. Advances in the field are largely driven by findings in animal models and are complicated by variability due to species and strain-specific differences, and duration and routes of exposure. There is renewed need to develop and refine animal models of
exposure, and to increase efficacy and specificity of drug candidates. Significant challenges exist due to ethical constraints on human research and testing. Longitudinal population studies that follow large-scale chemical accidents could delineate long-term effects of toxicant exposures, and appropriate global infrastructure is needed to support these efforts. Ultimately, these comprehensive research efforts will further our understanding of toxicant-induced lung injury progression and support the development of countermeasures against pulmonary threats.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.
Figure Legend

**Figure 1.** Mechanisms by which inhaled toxicants affect the bronchioles and alveoli of the lung, leading to nitrosative stress and inflammation. Activation, elevation, and depletion of shared signaling pathways enhances injury. DNA damage, protein adduction, and lipid peroxidation, if left unresolved, can develop into bronchitis, pulmonary edema, and ultimately ALI/ARDS. Current therapies for symptoms include supportive care, with bronchodilator use and mechanical ventilation.
Table 1. Acute toxicity of pulmonary chemicals of concern (CoCs).

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<thead>
<tr>
<th>Chemical</th>
<th>Ammonia</th>
<th>Acrolein</th>
<th>Chloropicrin</th>
<th>Chlorine</th>
<th>Phosgene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>NH₃</td>
<td>C₃H₄O</td>
<td>CCl₃NO₂</td>
<td>Cl₂</td>
<td>COCl₂</td>
</tr>
<tr>
<td>Deposition</td>
<td>URT</td>
<td>URT</td>
<td>URT</td>
<td>URT/LRT</td>
<td>LRT</td>
</tr>
<tr>
<td>Industrial/agricultural use</td>
<td>Refrigerant Fertilizer</td>
<td>Biocide Livestock feed</td>
<td>Disinfectant Soil fumigant</td>
<td>Disinfectant Sanitation Plastics Paper</td>
<td>Chemicals Adhesives Plastics</td>
</tr>
<tr>
<td>Reactive by-products</td>
<td>NH₂OH</td>
<td>C₃H₄O₂, COx</td>
<td>COCl₂, Cl₂, NOx, (intermediates: HOCI, CH₃NO₂)</td>
<td>HCl, HOCl</td>
<td>HCl</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>Irritant, exothermically reacts with water, damages tissue</td>
<td>Irritant, strong electrophile, damages tissue</td>
<td>Irritant, induces ER stress, mutagen</td>
<td>Reacts with epithelial lining, lyses cells, causes fibrin deposition</td>
<td>Hydrolyses with mucus layer, acylates biomolecules, depletes surfactant</td>
</tr>
<tr>
<td>Acute toxicity</td>
<td>Bronchiectasis, BO, COPD, ILD, respiratory failure</td>
<td>Bronchial obstruction, LRT necrosis</td>
<td>Bronchitis, pulmonary edema, respiratory failure</td>
<td>ALI/ARDS, RADS, pulmonary fibrosis, respiratory failure</td>
<td>Pulmonary edema, hypoxemia, ALI/ARDS, respiratory failure</td>
</tr>
</tbody>
</table>

BO = bronchiolitis obliterans; COPD = chronic obstructive pulmonary disease; ILD = interstitial lung disease; AKG = alpha ketoglutarate; URT = upper respiratory tract; LRT = lower respiratory tract; ALI/ARDS = acute lung injury/acute respiratory distress syndrome; RADs = reactive airways disease.
Authorship Contributions

- Participated in research design: N/A
- Conducted experiments: N/A
- Contributed new reagents or analytic tools: N/A
- Performed data analysis: N/A
- Wrote or contributed to the writing of the manuscript: Marzec J, and Nadadur S
References:


**Nitrosative stress**

**Inflammation**
- DNA damage
- Protein adduction
- Lipid peroxidation

**Activation**
- TRP channels
- β-adrenergic receptors

**Elevation**
- iNOS
- Histamine
- Wnt/β-catenin signaling
- Coagulation

**Depletion**
- NOx
- Surfactant

**Supportive Care**
- Bronchodilator use
- Mechanical ventilation
- Pulmonary edema
- ALI/ARDS