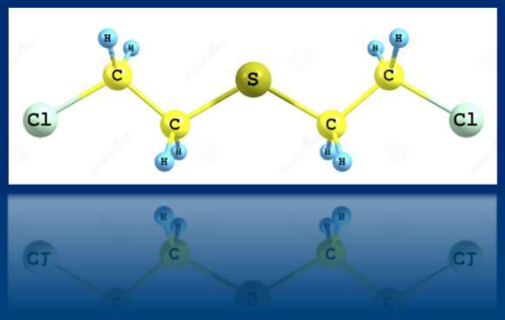
NIH Council 2024

Bethesda, MD

June 4th, 2024

Adverse Pulmonary Effects of Highly Toxic Chemicals - A Comparative Analysis of Chemical Inhalation Burns



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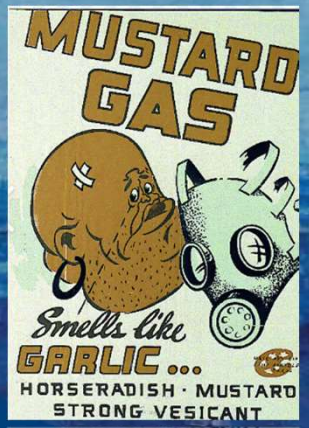

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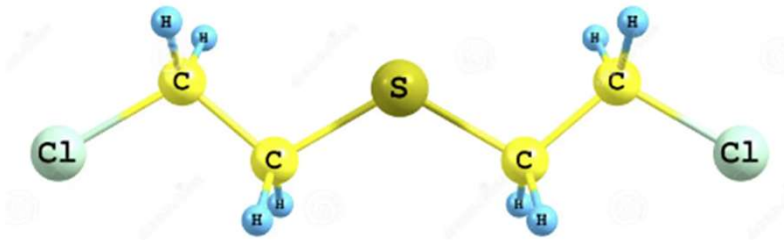
OBJECTIVES

- Review **Sulfur Mustard** Inhalation Injury – airway burn, alveolar fibrosis
- Review **Methyl-Isocyanate** Inhalation Injury – airway burn, alveolar fibrosis
- Review **Chlorine Gas** Inhalation Injury – airway burn, mild alveolar flooding

Historical context of the use of Sulfur Mustard

- Sulfur Mustard (SM) was first developed in 1822 by Cesar-Mansuete Despretz, by reacting Sulfur Dioxide with Ethylene
- In 1850-1860's, SM's harmful blistering properties were simultaneously reported by French (Riche), British (Guthrie), and German (Niemann) scientists
- SM was the first vesicant agent used as a chemical weapon
- Other chemical reactions were developed to produce a more potent SM



Ghanei 2016. Mustard Lung. *Elsevier*

Some Examples of the Use of SM as a Chemical Weapon

- 1917 – WWI, the German Army against the Allies (100,000 deaths)
- 1919 – UK against the Red Army
- 1921-1927 – Spain and France against Moroccan rebels
- 1930 – Italy against Libya
- 1934 – Soviet Union against China
- 1935 - 1940 – Italy against Ethiopia
- 1937 – 1945 – Japan against China
- 1963 – 1967 – Egypt against North Yemen
- 1983 – 1988 – Iraq against Iran (>1000 deaths)
- 1995 -1997 – Sudan against insurgents in Civil War
  - *Not Much Use in WWII*

Ghanei 2016. Mustard Lung. *Elsevier*

Modern use and potential threats of Sulfur Mustard

- After WWII, most of Germany's SM was dumped into the Baltic Sea
- In 1966-2002, >700 SM weapons were found near Bornholm island; location of others are unknown.
- All dumped SM weapons are still potent and lethal
- Causes **accidental exposures**, ex: 2010 (NY fishermen)
- Other SM was disposed of by undersea explosions, or in factories (or stored in Pueblo, Colorado)

Ghanei 2016. Mustard Lung. *Elsevier*

Regulations of Warfare Agents

- 1972 - Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction (BTWC)
- 1997 – Chemical Weapons Convention (CWC)

Chemical weapons watchdog concludes Islamic State used mustard gas in 2015 attack in Syria

BY JULIA ROBINSON | 28 FEBRUARY 2024

Ghanei 2016. Mustard Lung. *Elsevier*

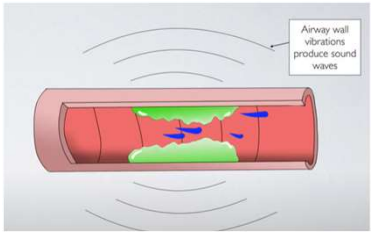
Clinical case presentation – Patient (S. Mustard) - Exposure

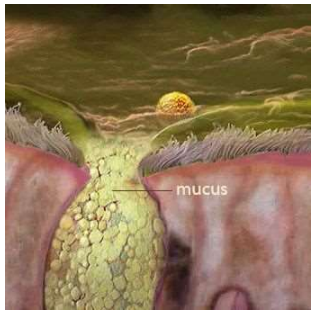
- 21 yr old soldier in WWI
- Premature burst of mustard gas munition during firing (primary exposure)
- No immediate symptoms
- He slept in the proximity of the artillery gun, acutely wakening after 4 hours with acute symptoms of:
  - **Nausea**
  - **Burning in the eyes**
  - **Vomiting**
  - **Dizziness**
  - **Headache**
  - **Tickle in the throat**

Heitzmann, Otto. 1920. *On Battefield Gas Poisoning*, Chapter VIII

Patient (S. Mustard) – cont. – 1st day symptoms

- Within 24hrs, the symptoms worsened:
  - **Conjunctivitis with photophobia**
  - **Erythematous oropharynx**
  - **Dyspnea**
  - **Chest rattling (rhonchi) and wheezing**
  - **Slimy foamy sputum**
  - **Fever of 40.1°C**
  - **Weakening**





Heitzmann, Otto. 1920. *On Battefield Gas Poisoning*, Chapter VIII

Patient (S.Mustard) – cont. – subsequent days

- On the 2nd day: temperature 39 to 40°, **cyanosis,** pulse 120, restlessness.
- On the 3rd day: **cyanosis,** somnolence; restlessness; 38.5°.
- On the 4th day: **death** after increasing dyspnea and tachycardia

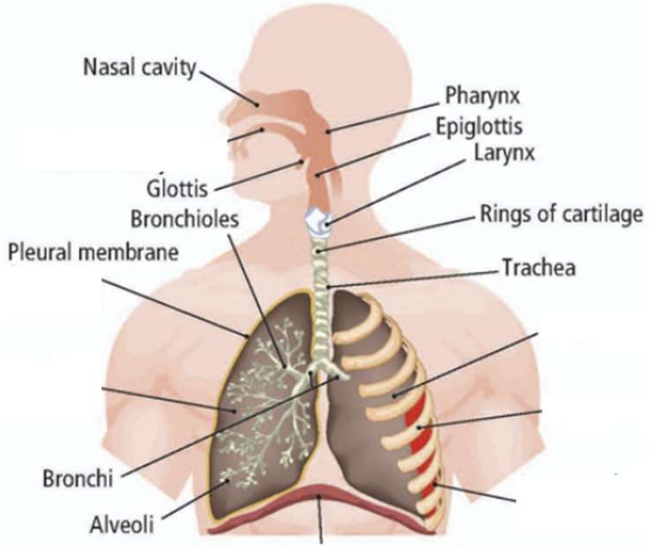
- Diagnosis:
  - croupous inflammation of the pharynx, larynx, trachea and larger bronchi;
  - purulent bronchitis and bronchiolitis
  - hemorrhagic bronchopneumonia
  - bullous emphysema of the anterior lung segments
  - hemorrhages in the brain (*purpura cerebri*), in the trachea, beneath the pleura, epicardium and endocardium, salivary gland, and renal tubules, cornea

Heitzmann, Otto. 1920. *On Battefield Gas Poisoning*, Chapter VIII

Patient (S. Mustard) – Autopsy Results

Patient Autopsy – Respiratory Organs

- **Nasal cavity** -- purulent mucus
- **Pharynx** --clogged with flaky purulent material
- **Posterior pharyngeal wall** -- fine, yellow, firmly adhering coatings
- **Larynx** --filled with purulent material; the inlet fold ulcerated
- **Epiglottis** edge – raw, erythematous
- **Soft palate** -- nearly entirely closed with a spanned pseudo/membrane
- **Trachea** -- lined with firmly or loosely adhering pseudo/membranes; the mucosa beneath is dark red with bright red spots (hemorrhages);
- **Mainstem Bronchi** -- adhering pseudo/membrane is formed into a tubular outlet;
- **Lobar bronchial branches** -- contiguous outlets alternate with containing thick liquid pus;
- **Bronchioles** - expanded; the mucosa overall is dark bluish-red.
- **Anterior lung** -- have large bubbles or blisters;
- **Pleural membrane** -- slightly opaque with isolated small hemorrhages.
- *Microscopic findings: hemorrhagic purulent bronchitis, fibrinous leukocytic exudate, edema.*



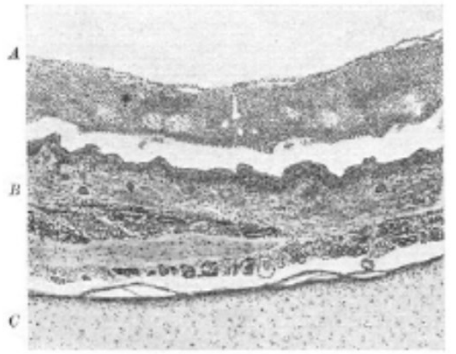


Fig. 1. Loosely adhering pseudomembrane in the trachea; A = pseudomembrane, B = mucosa, submucosa, C = cartilage

Heitzmann, Otto. 1920. *On Battefield Gas Poisoning*, Chapter VIII

Sulfur Mustard (SM) Inhalation-Exposed Humans - Airway Casts: The Willems report

Willems report: Report on the SM-exposed Iranians evacuated to European hospitals during 1984-87 war

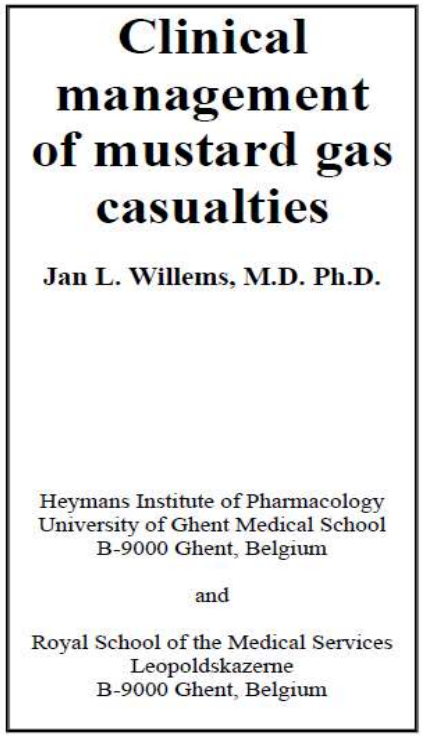
CASTS: 23% of SM-exposed patients had airway casts (15/65)

Mortality

- Of patients with casts, 50% died

Serious Morbidity

- Of patients with casts, 20% surviving patients had emergent tracheostomy due to sudden airway occlusion casts



Clinical management of mustard gas casualties

Jan L. Willems, M.D. Ph.D.

Heymans Institute of Pharmacology
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and

Royal School of the Medical Services
Leopoldskazerne
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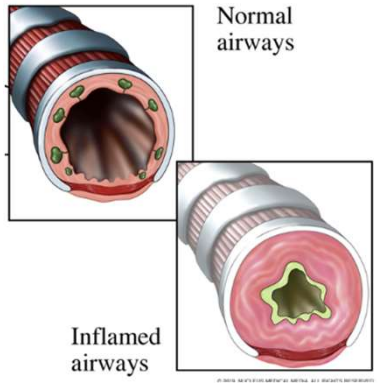
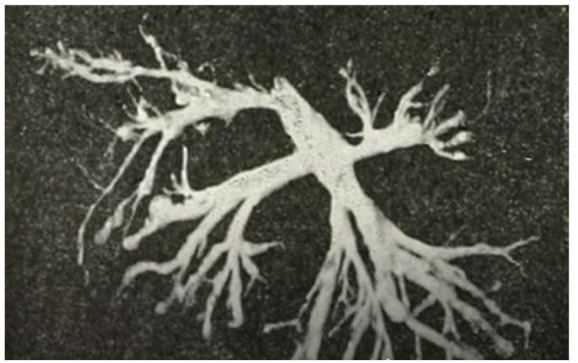
Acute Phase health effects of Sulfur Mustard inhalation exposure

Severity depends on SM dose, individual characteristic, temperature, humidity

Main feature is a **completely asymptomatic incubation period (hours – months)**



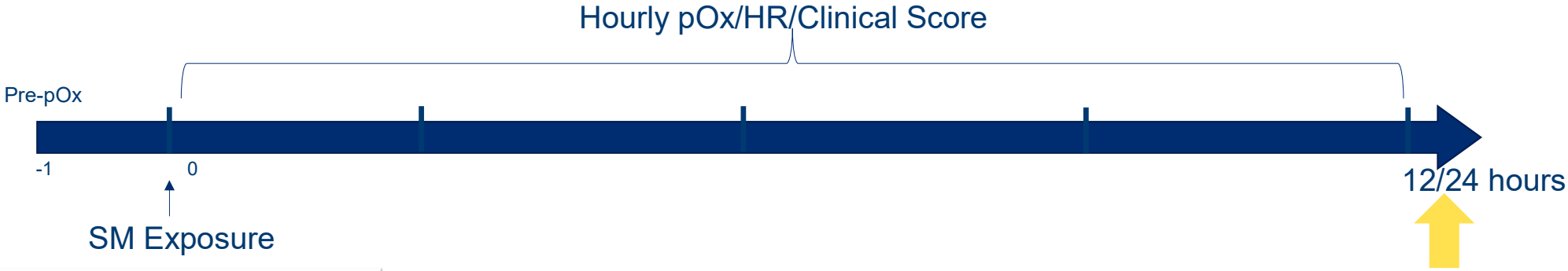


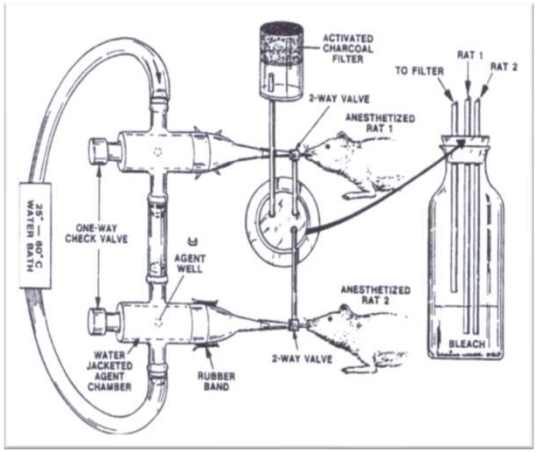
Acute Health Effects

- Acute airways edema
- Inflammation
- Destruction of Airway Epithelial Cells
- 'Pseudomembrane formation which may block airways and cause death'
- Multiple Organ Dysfunction Syndrome (MODS) – bone marrow, gastrointestinal and central nervous system injury

Ghanei 2016. Mustard Lung. *Elsevier*

Acute SM Inhalation Rat Model Development





Anderson et al, USAMRICD

<u>Species</u>
Sprague-Dawley rats, Charles River, Male
Weight – 250-275 g on exposure day

<u>Endpoints</u>
Clinically Translational (Vital Signs)
Blood Analysis (CBC, CMP, ABG)
BALF analysis
Lung histology/IHC, cast scoring
Coagulation assays
Cardiac Function (cath/echo)
Lung Function
SM metabolite analysis

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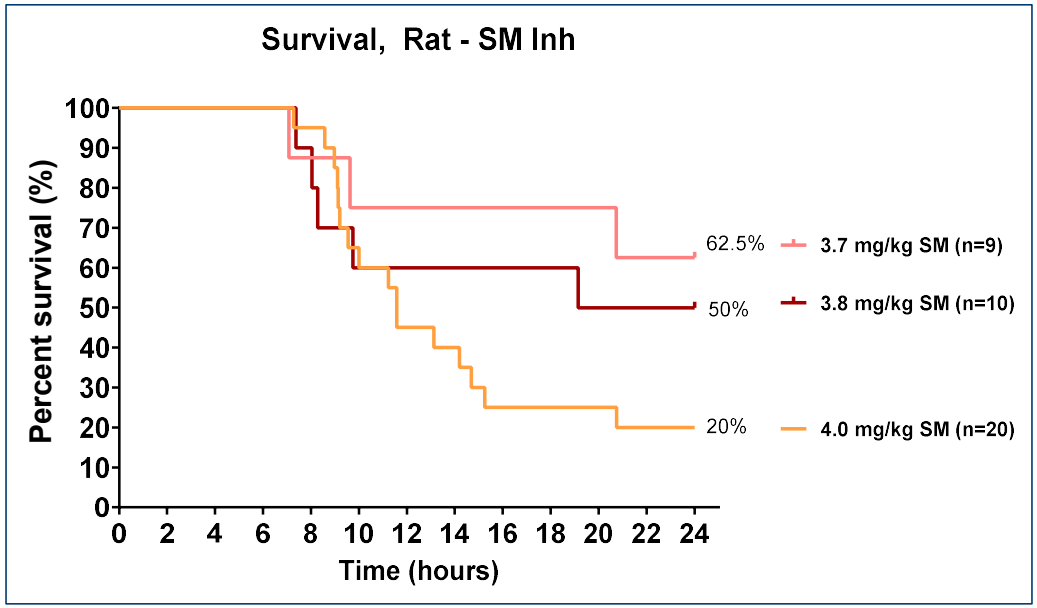
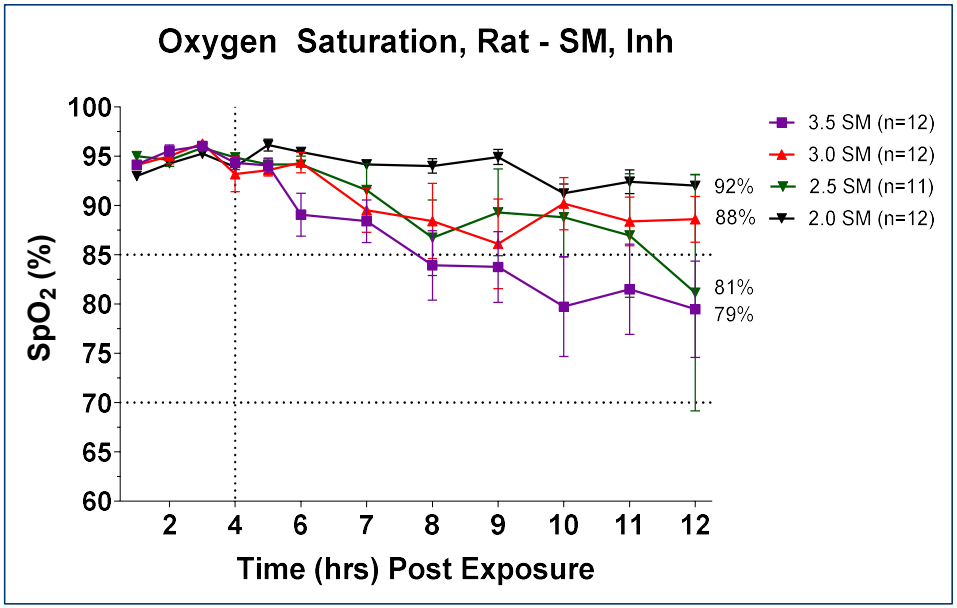
Acute SM Inhalation exposure causes dose-dependent survival and oxygen desaturation





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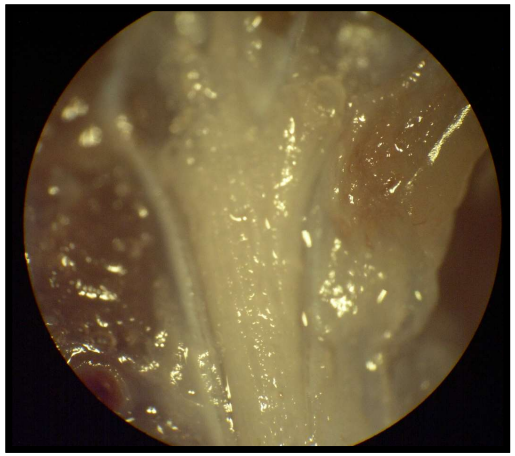
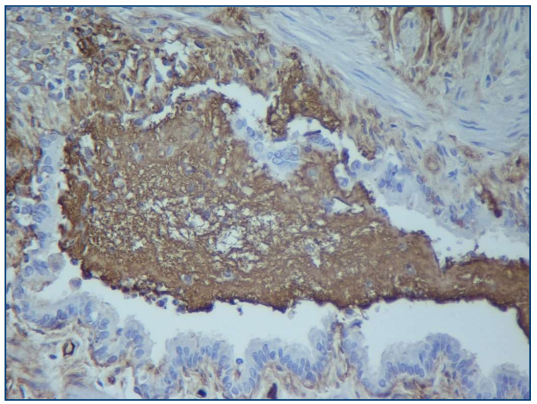
Acute SM Inhalation morbidity and mortality is due to airway thrombosis (fibrin rich casts) in rat model

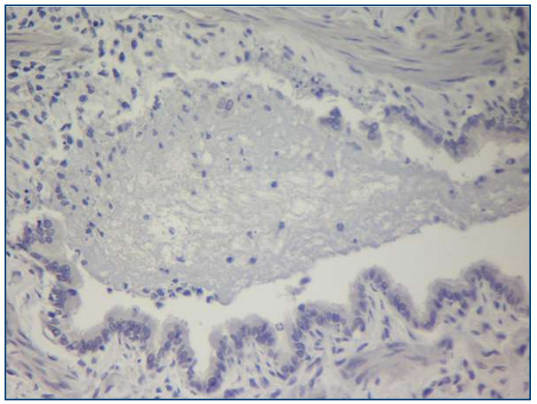


Fibrin-rich casts (aka pseudomembranes) form in the airways after SM Inhalation

Fibrinogen IHC



IgG Control IHC



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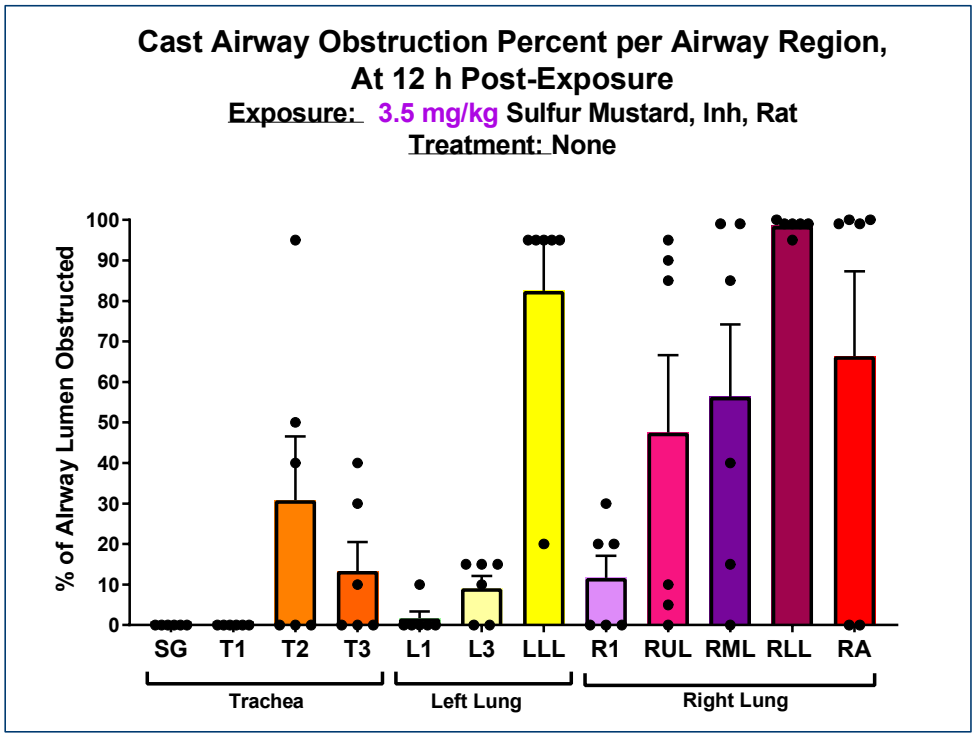
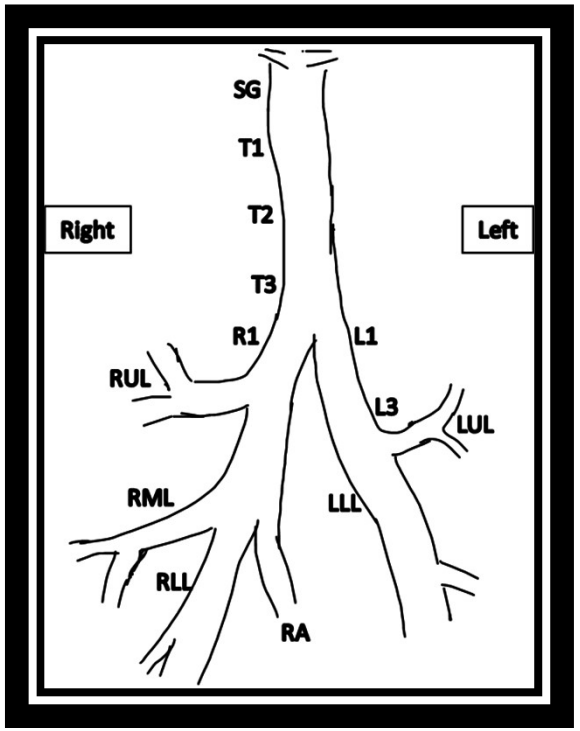
Fibrinogen IHC

Airway Casts in Right Lower Lobes Predominantly





SG – subglottis
T1 – proximal trachea
T2 – mid-trachea
T3 – distal trachea
R1 – right mainstem at carina
L1 – left mainstem at carina
L3 – distal left mainstem
LLL – left lower lobe (most proximal)
RUL – right upper lobe
RML – right middle lobe
RLL – right lower lobe
RA – right accessory

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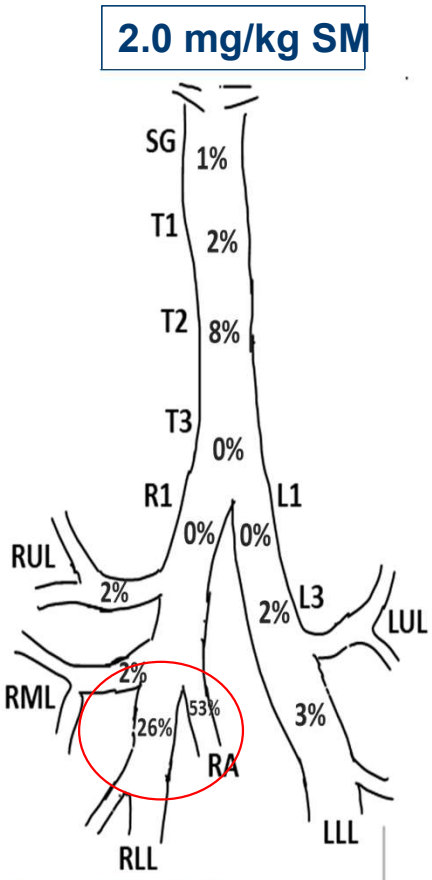
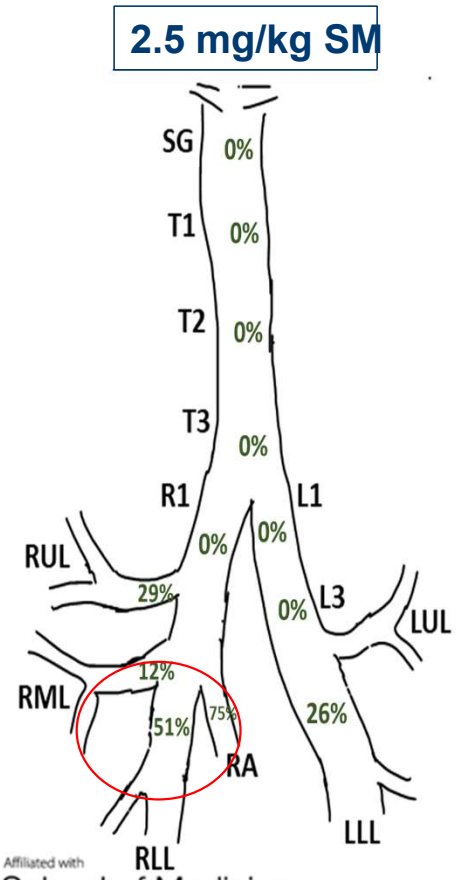
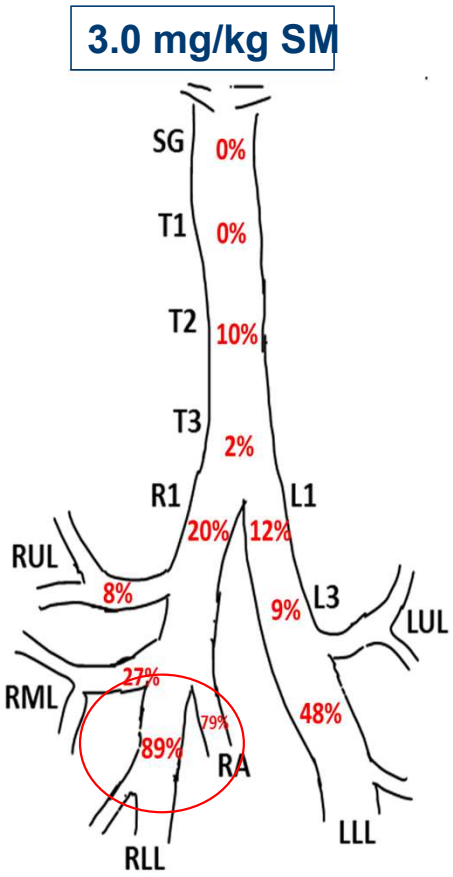
Cast Obstruction (%) per Airway Region at 12 hours Post-Exposure to SM (Inhaled) in Rats



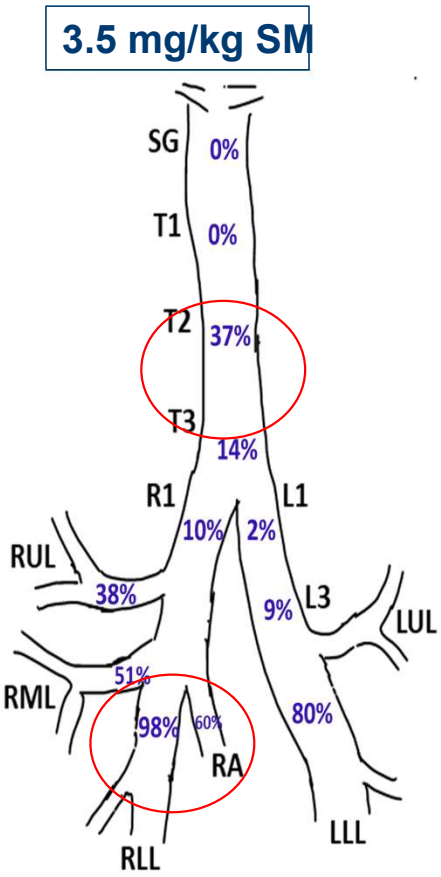
Mean, n=6



Mean, n=5



Mean, n=5



Mean, n=5

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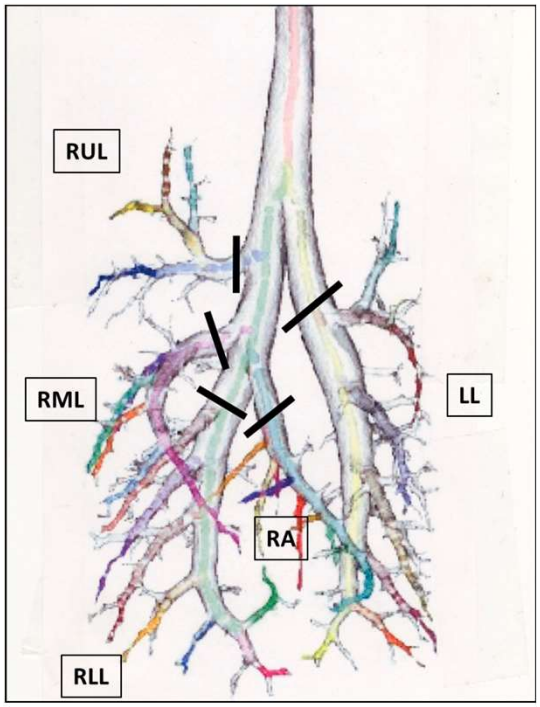
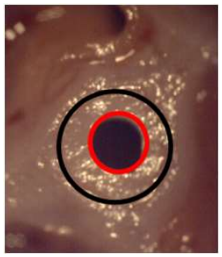
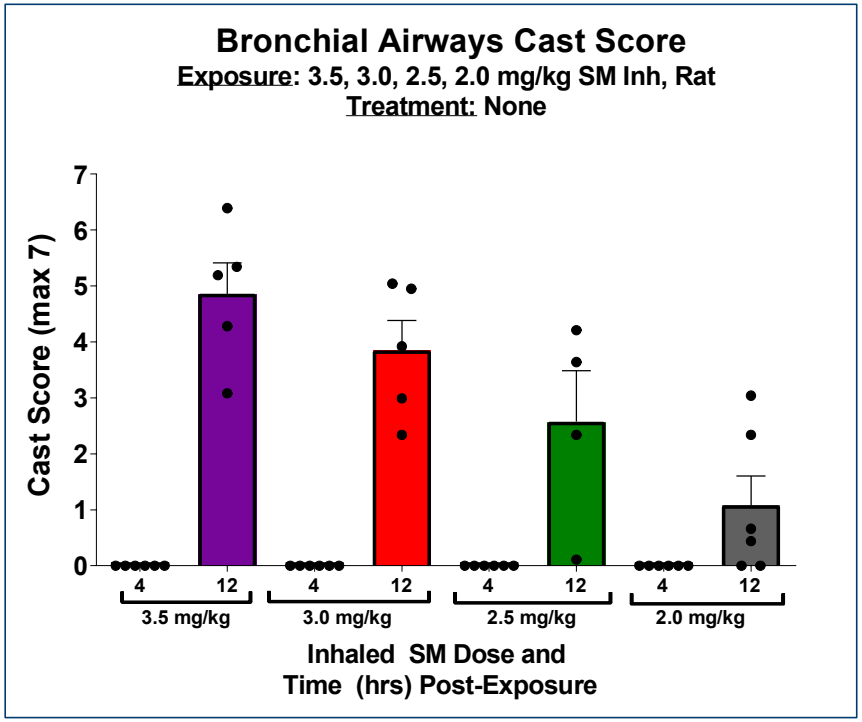
Airway Bronchial Cast Formation is Dose-Dependent - Composite Bronchial Cast Score





Scoring System:

0	0 - 4%
1	5 -14%
2	15 - 29%
3	30 - 49%
4	50 - 64%
5	65 – 79%
6	80 – 99%
7	100%



Cast Scoring system via dissection of fixed lungs

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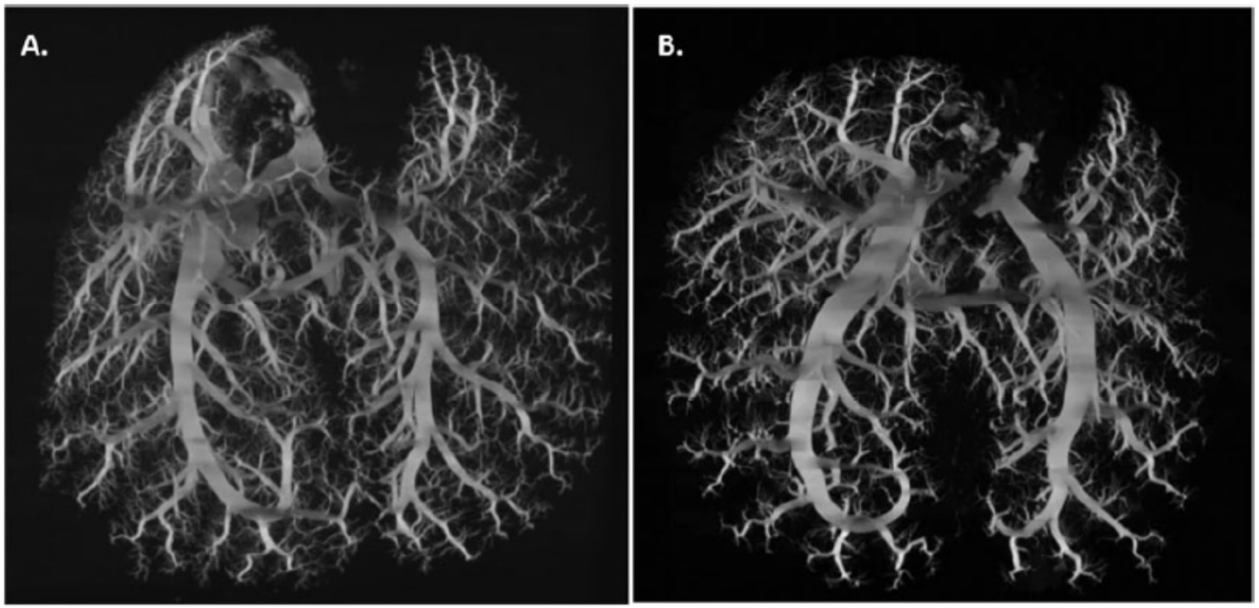
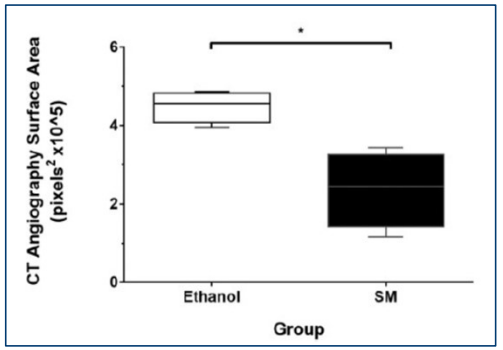
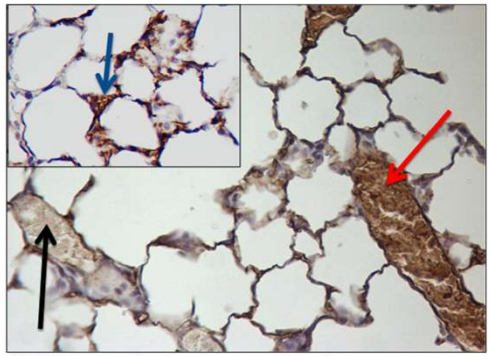
Acute high dose SM Inhalation causes vascular pruning (rats) due to acute vascular pathology (thrombosis)



CT quantitation





Red arrow - small pulmonary artery thrombus
Black arrow – small pulmonary artery without thrombus
Blue arrow – microvascular (capillary) pulmonary thrombus

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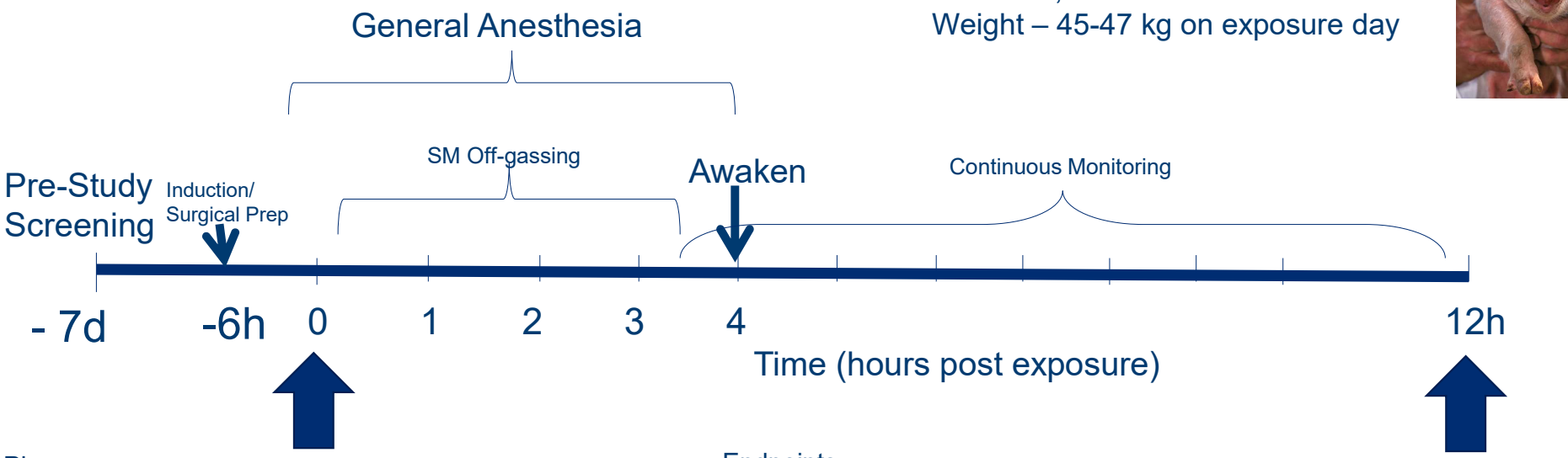
McGraw et al, Tox Sci, 2017

Acute SM Inhalation Pig Model Development

Species
Yorkshire, Female
Weight – 45-47 kg on exposure day



Pig:
Intubated
General anesthesia (4 drug - IV)
Spontaneously ventilating
(5-7 ml/kg Tidal Volume, RR 10-15 bpm)

Endpoints
Clinically Translational (Vital Signs)
Blood Analysis (CBC, CMP, ABG)
BALF analysis
Lung histology/IHC, cast scoring
Coagulation assays
Lung Function
SM metabolite analysis

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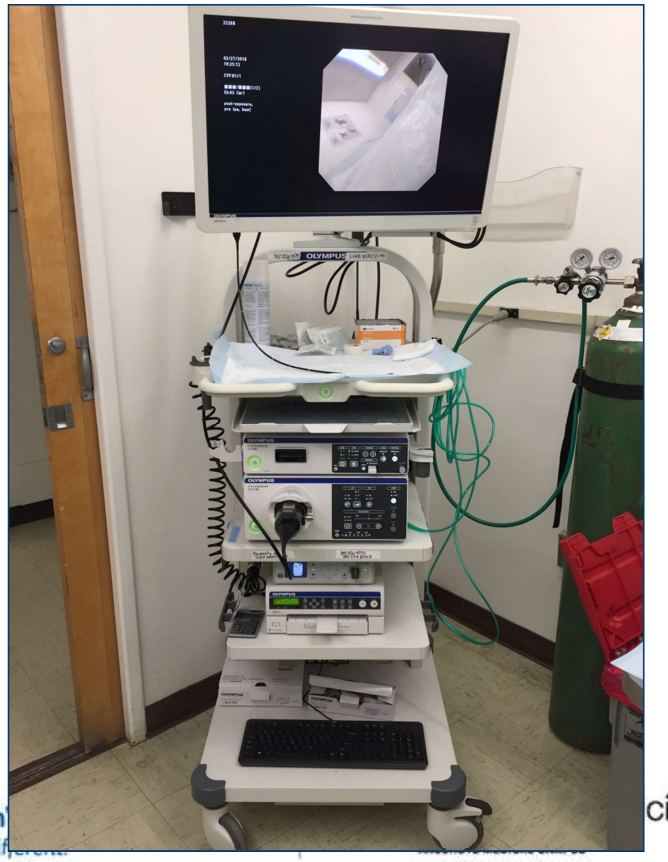
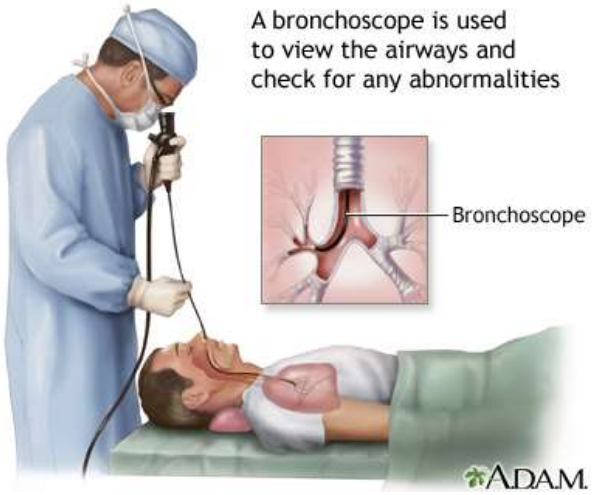
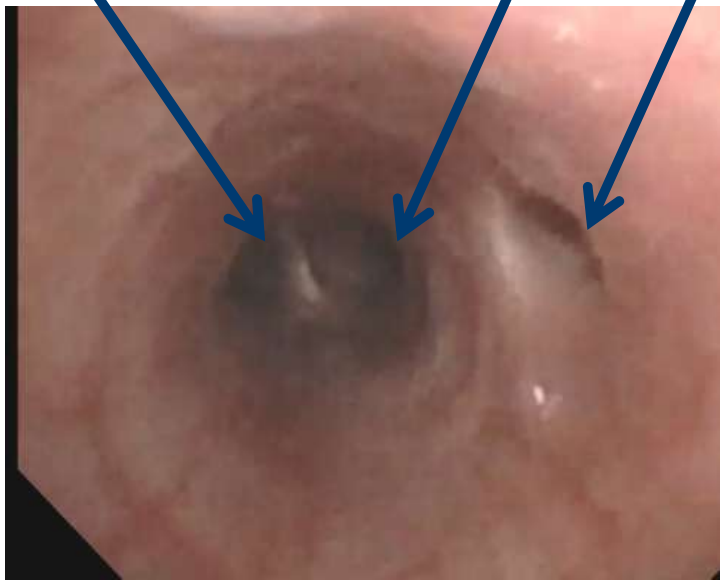
Bronchoscopy (Human and Pig)

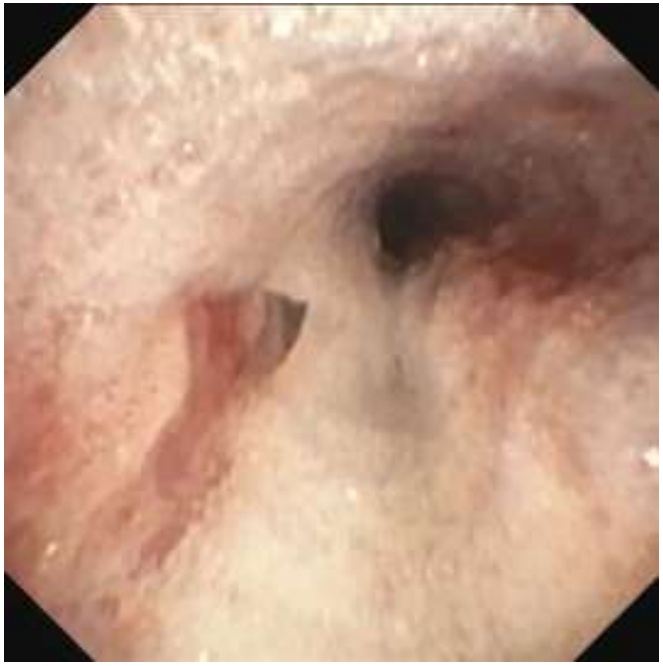




Bronchoscopy in Pigs After SM Inhalation

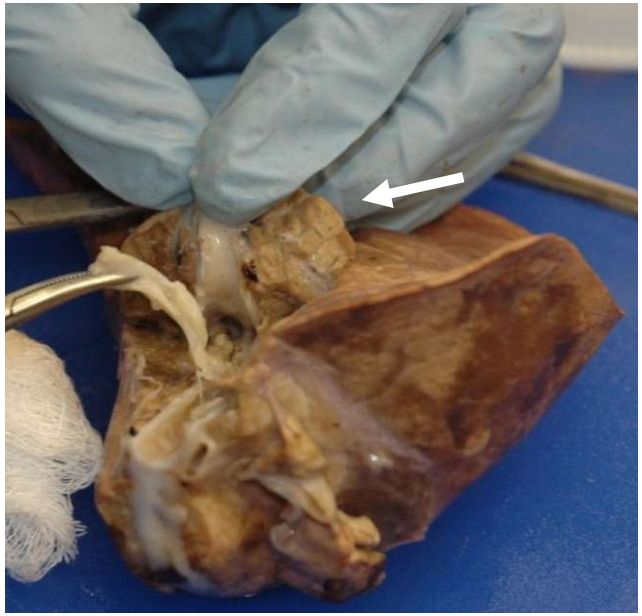


Normal Pig Trachea

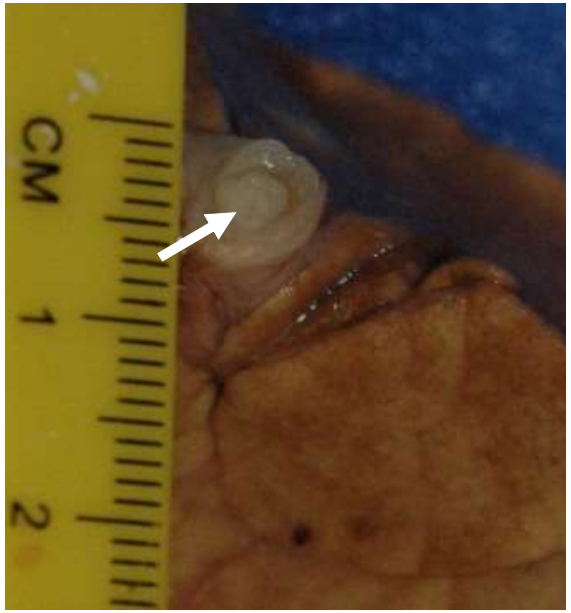


Pig Trachea 10.5h
Post-SM Inhalation

Pig Model of SM Inhalation Causes Airway Thrombosis (Fibrin Casts)



Cast pulled out via hemostat

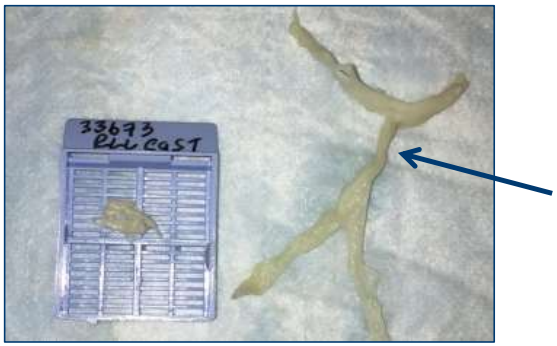


Cast completely obstructing opening to Right Upper Lobe (RUL)

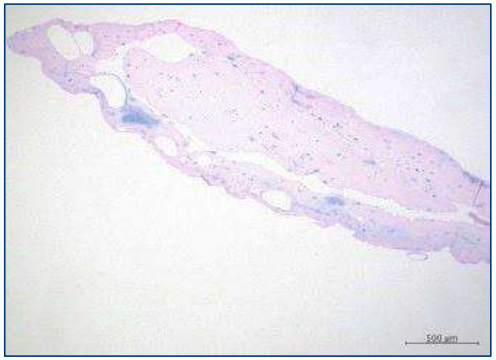
Entire cast pulled out of RUL

Airway Casts Are Present in Pig Lobes After SM Inhalation

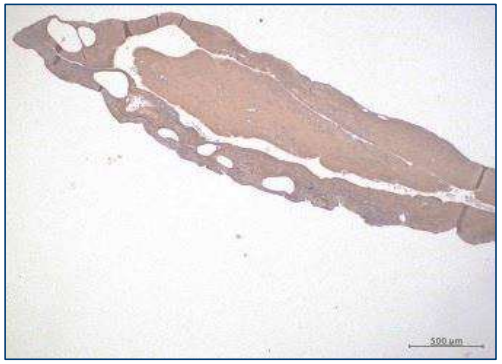
Right Middle Lobe Cast In-situ *(arrow)*



Right Lower Lobe Cast Ex-vivo *(arrow)*



Tracheal Cast Histology: Mucus (4X AB/PAS Stain for Mucus)



Tracheal Cast Histology: Fibrin (10X Fibrinogen IHC)

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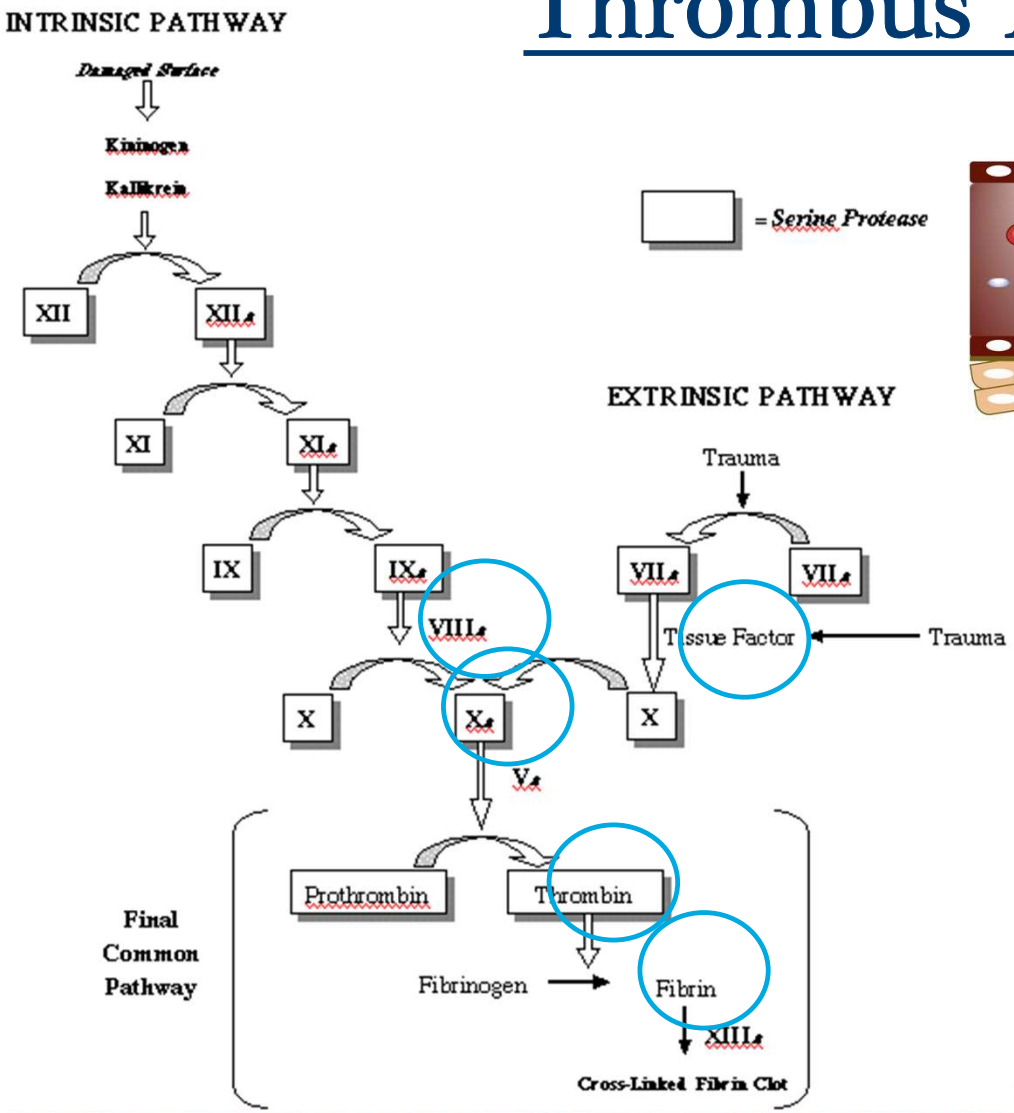
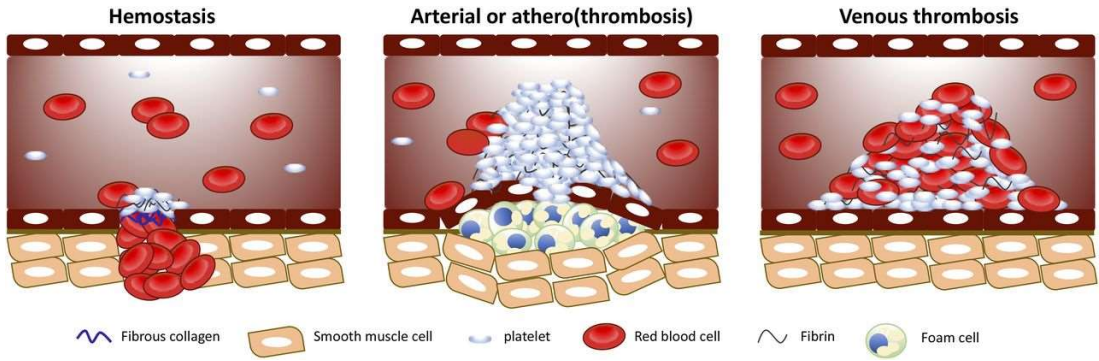
Thrombus Formation





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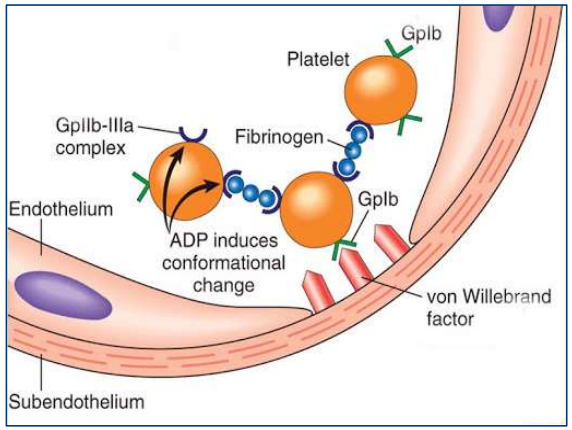


Platelet binding to vWF on EC

Endo cell injury
↓
vWF expression
↓
Platelet activation
↓
Platelet recruitment
↓
Platelet aggregation
↓
Thrombin activation
↓
Fibrin binding

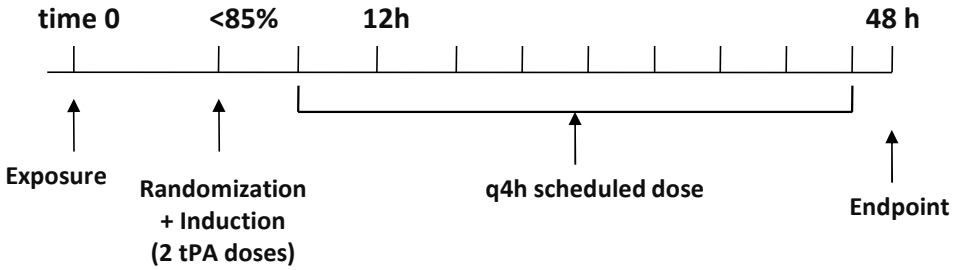
Cast Lysis with Alteplase (tissue plasminogen activator, tPA)

Alteplase

- Fibrinolytic drug, converts (inactive) plasminogen to (active) plasmin
- FDA approved for use in Myocardial infarction, Strokes, Pulmonary embolism, Clearance of clots in central venous devices and chest tubes

Groups:

1. **HD alone (NT)**
2. **HD + tPA q4**
3. **HD + PBS q4**

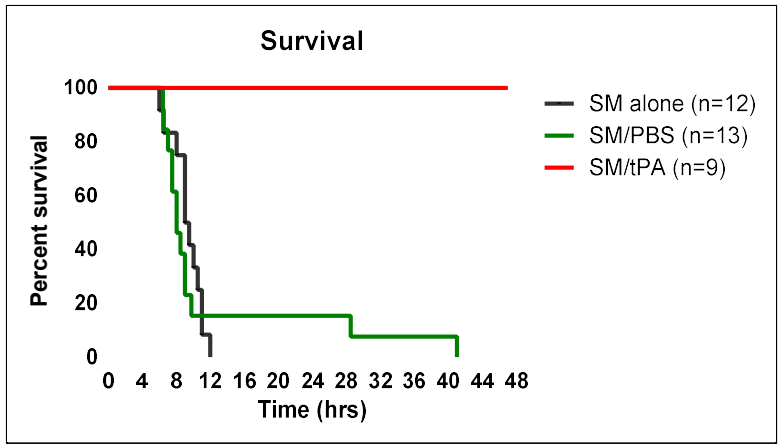


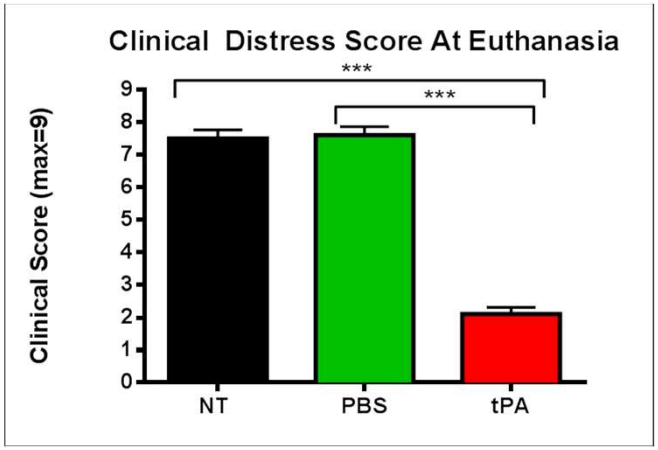
tPA dosing: 0.7mg/kg (induction is 2 doses 1h apart), then q4h maintenance dose (1 dose)
-administered under isoflurane anesthesia via Penn-Century microsprayer

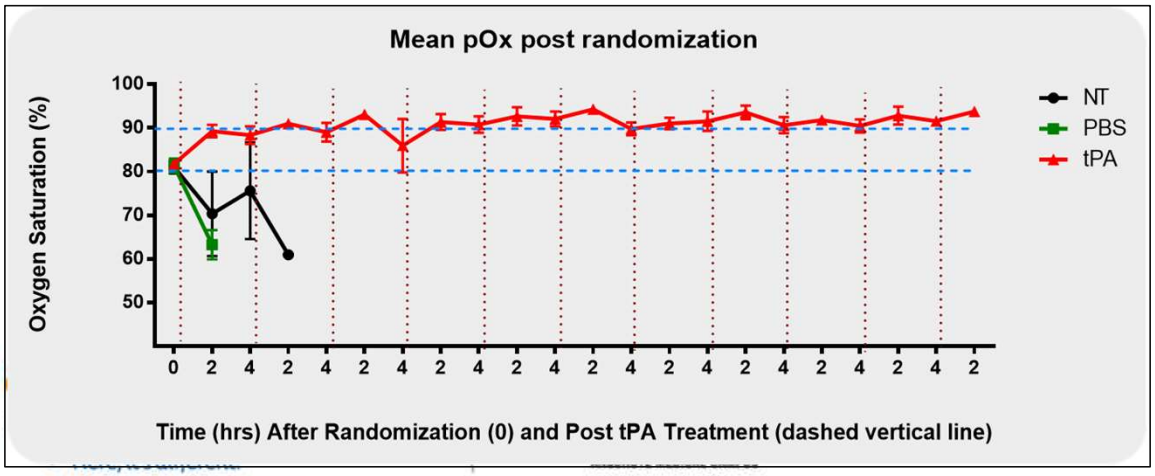
Euthanasia criteria (must meet both):

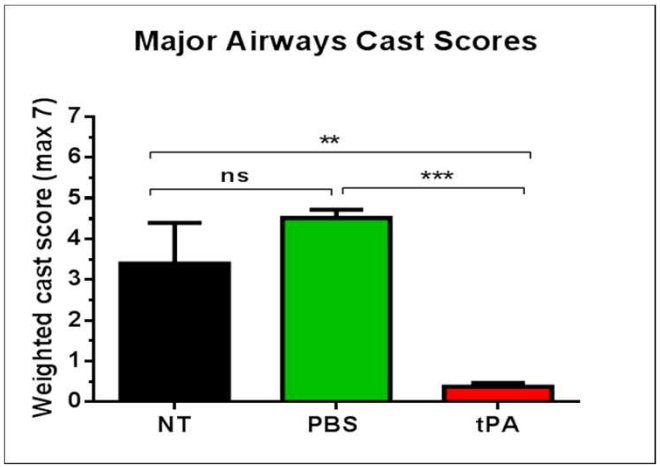
1. pOx <70%
2. Clinical score > or = 7 (new scoring system)

Alteplase treats acute airway injury after SM inhalation in Rats

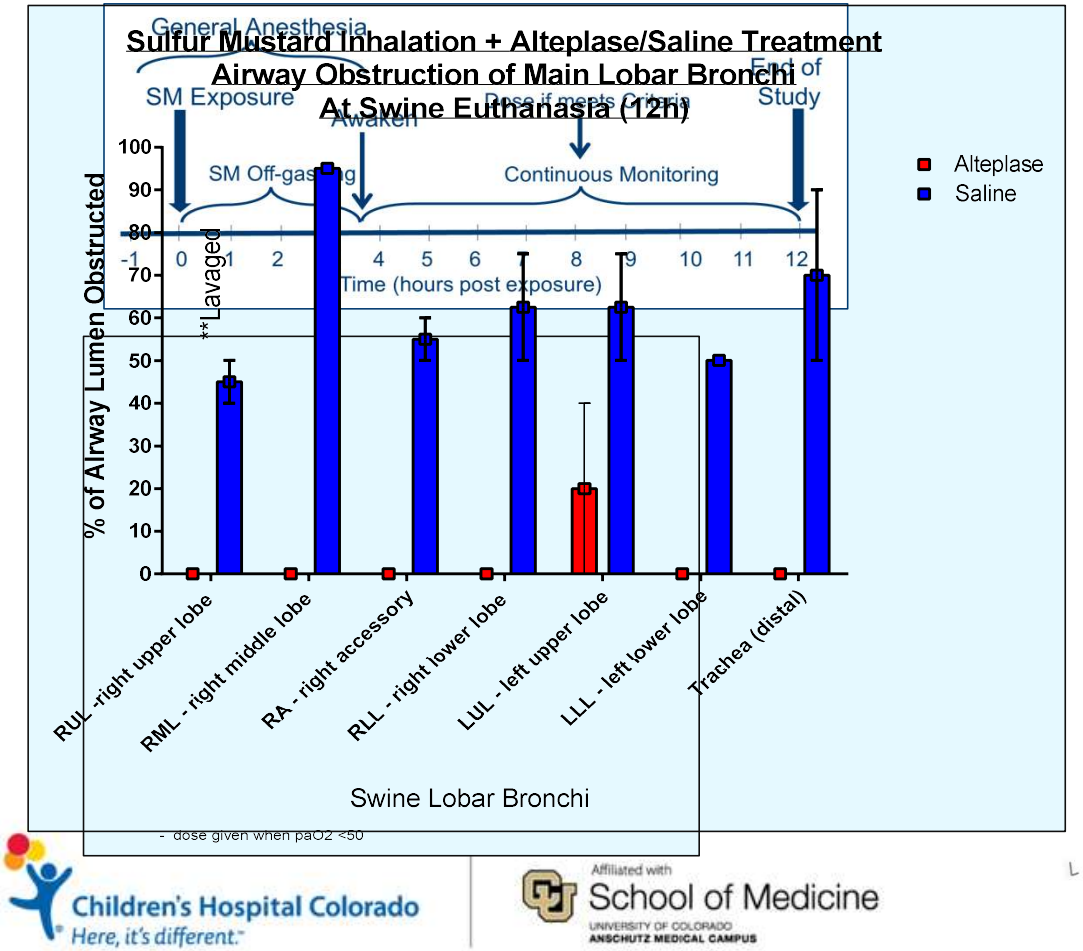


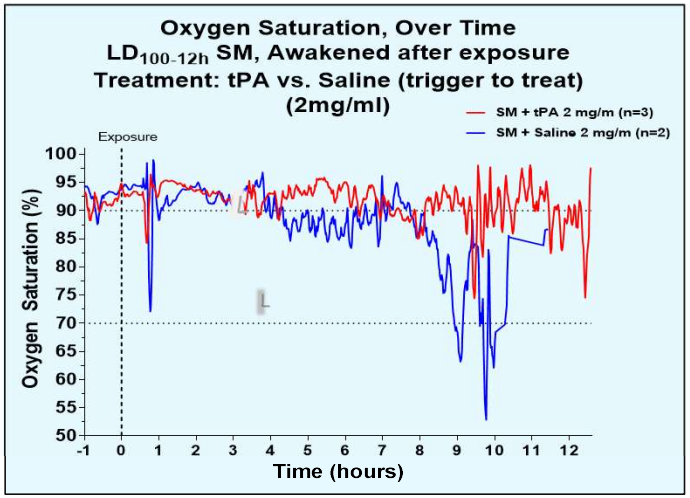






Alteplase treats acute airway injury after SM inhalation in Swine





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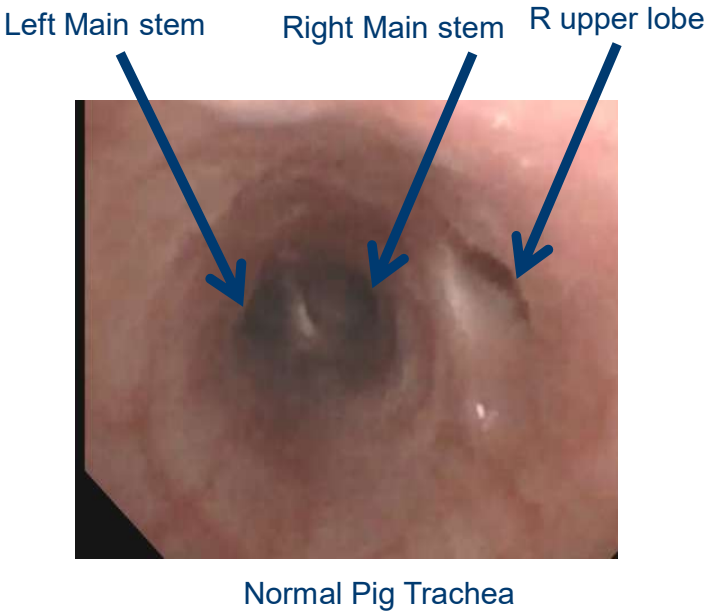
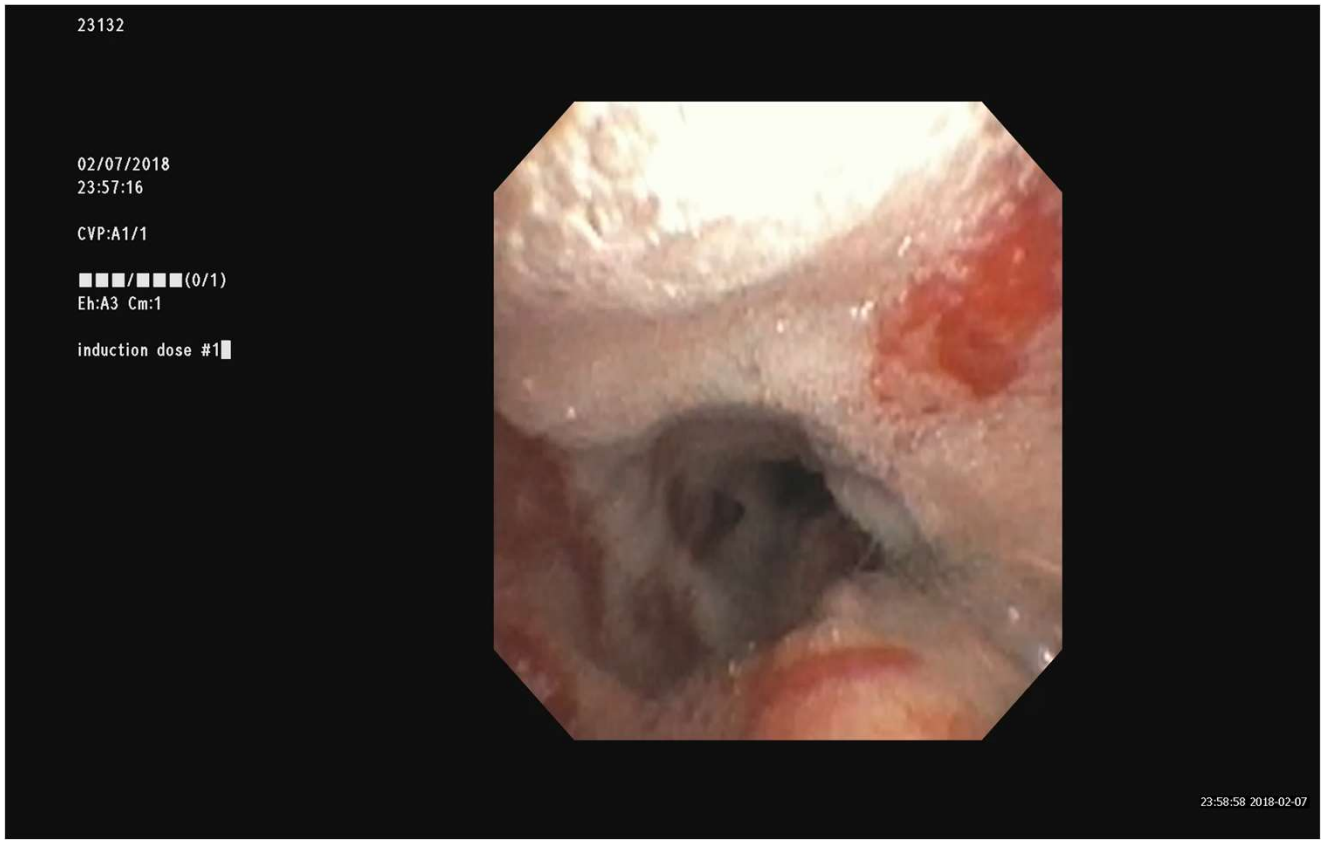
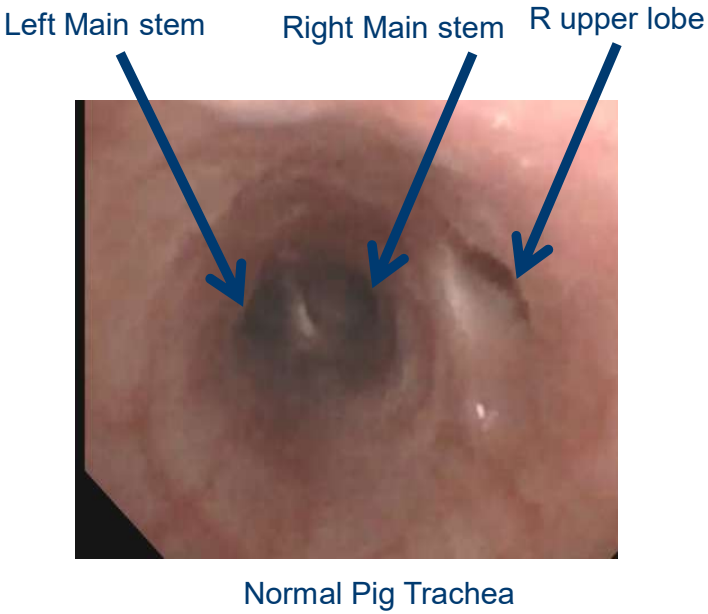
Bronchoscopy Video – SM inh, 10.5hr



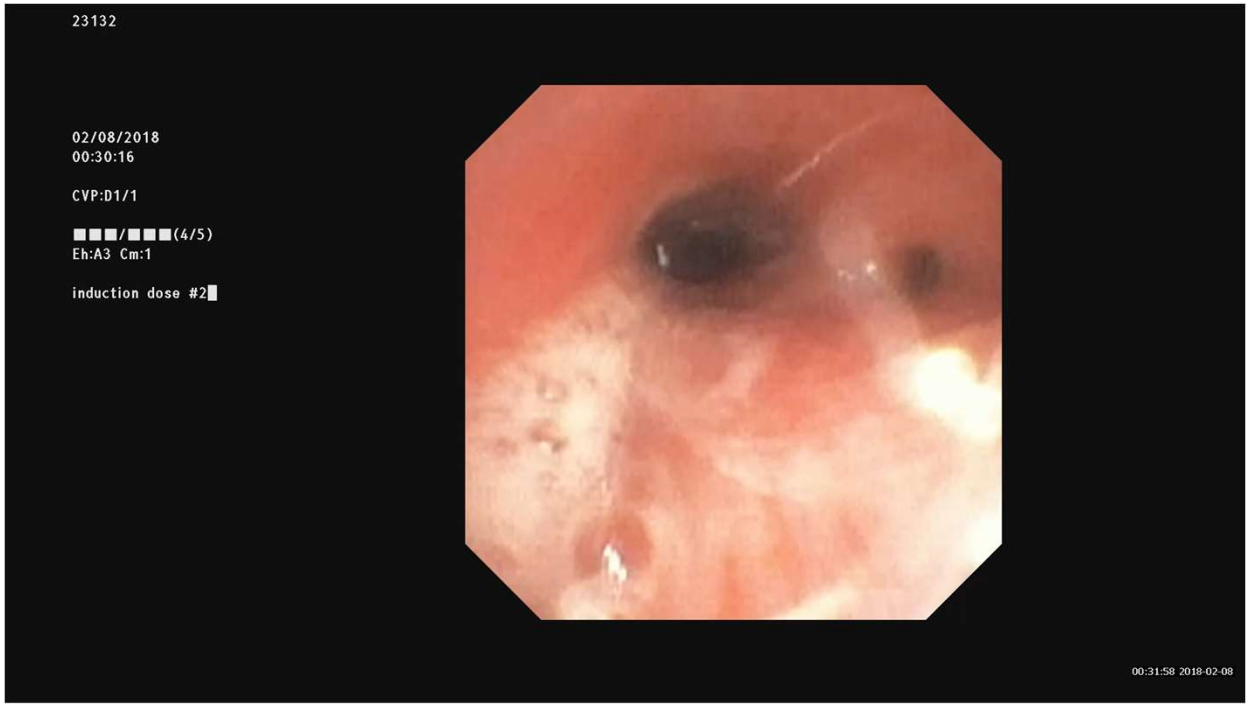
Normal Pig Trachea



Bronchoscopy Video – SM inh, 11 hr 30 min after Alteplase

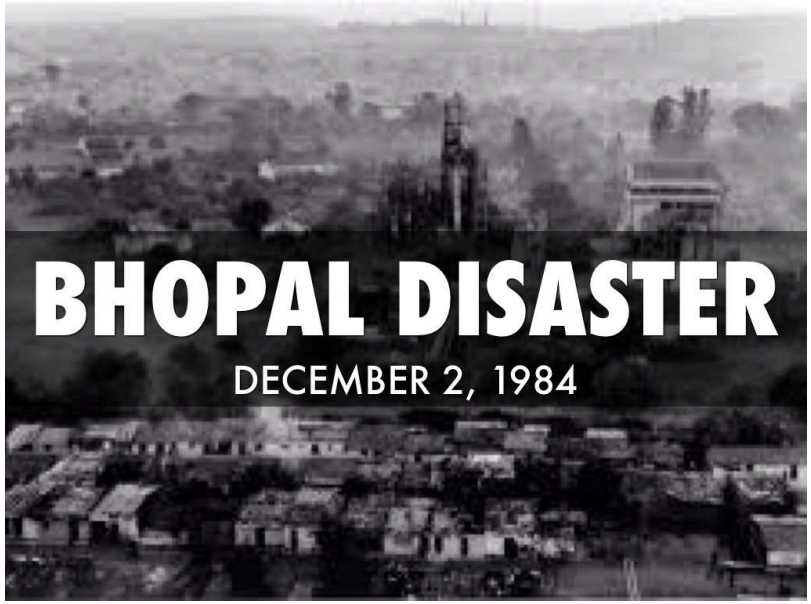


Normal Pig Trachea



Methyl Isocyanate - Introduction

1. Bhopal Agent = Methyl Isocyanate (MIC)
2. Accident of 1984 Bhobal, India --30-40 tons of MIC leaked from Union Carbide pesticide plant.
    1. ~4000 people died overnight, another 10,000 over next days/weeks
    2. Necropsy findings of obstructed airways, inflammation, hemorrhage, acute bronchiolitis --- *fibrinous exudate in the entire respiratory tract*



The Many Uses of Methyl-Isocyanate in Industrial Manufacturing

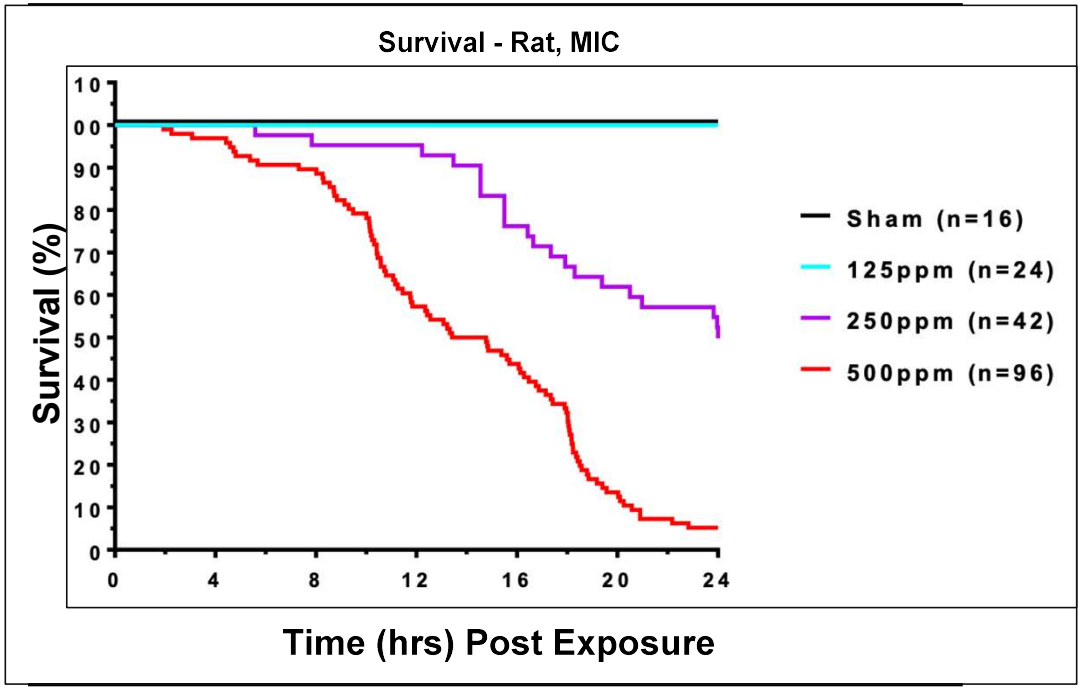
1. Carbamate pesticides and herbicides
2. Polyurethane/plastics
3. Paints/adhesives
4. Rubber
5. Used in production of, and often stored in proximity to, other toxic chemicals, some of which also can be required MIC for synthesis. Many of these are flammable and/or explosive.
6. Many other isocyanates and diisocyanates are used in industry, but MIC is the most toxic/lethal

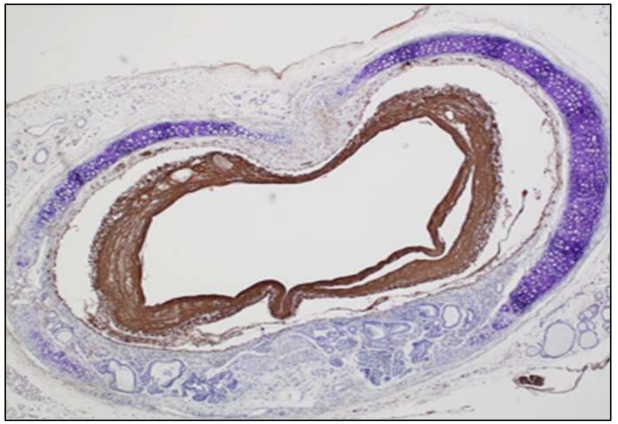
Patient (MIC)

1. Female age 26, cloud of gas from nearby chemical plant
2. Immediate eye burning, and watering, rhinorrhea
3. Within 4 hours: Coughing– ***strongly associated with mortality***
4. Within 8-12 hours: By Diarrhea, shortness of breath, vomiting, wheezing, respiratory distress, rales
5. Second day: Worsening Cyanosis, coughing up copious amounts of thick and frothy sputum, severe respiratory distress, ongoing eye watering, rhinorrhea
6. Death within 3 days: **cyanosis, seizures, frothing at mouth → death**

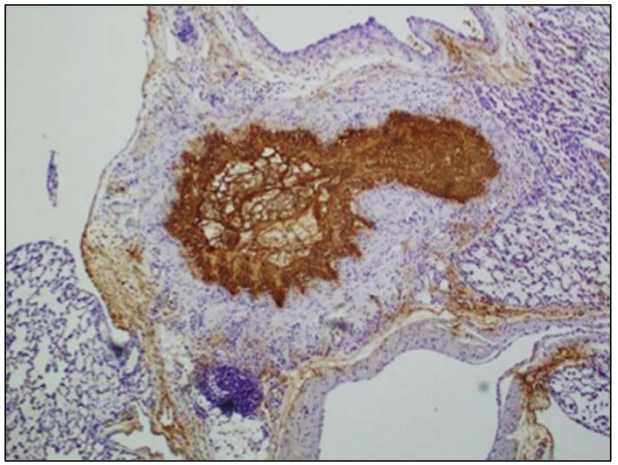
7. **AUTOPSY: severe epithelial fibrinous necrosis, sloughing, pseudomembranes, pulmonary edema, airway edema, inflammation, ARDS**
8. **Cause of Death: Asphyxia due to respiratory failure**

Methyl Isocyanate- Rat Model





Trachea



Accessory lobe main bronchus

Fibrinogen IHC

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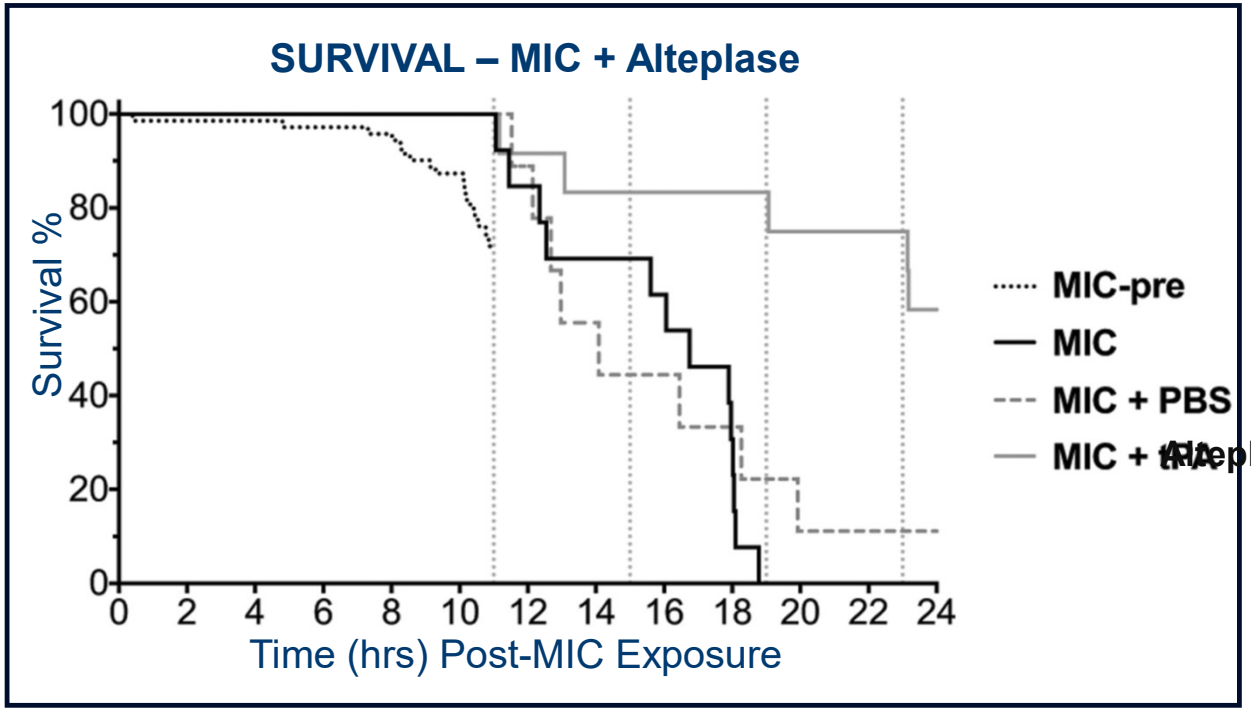
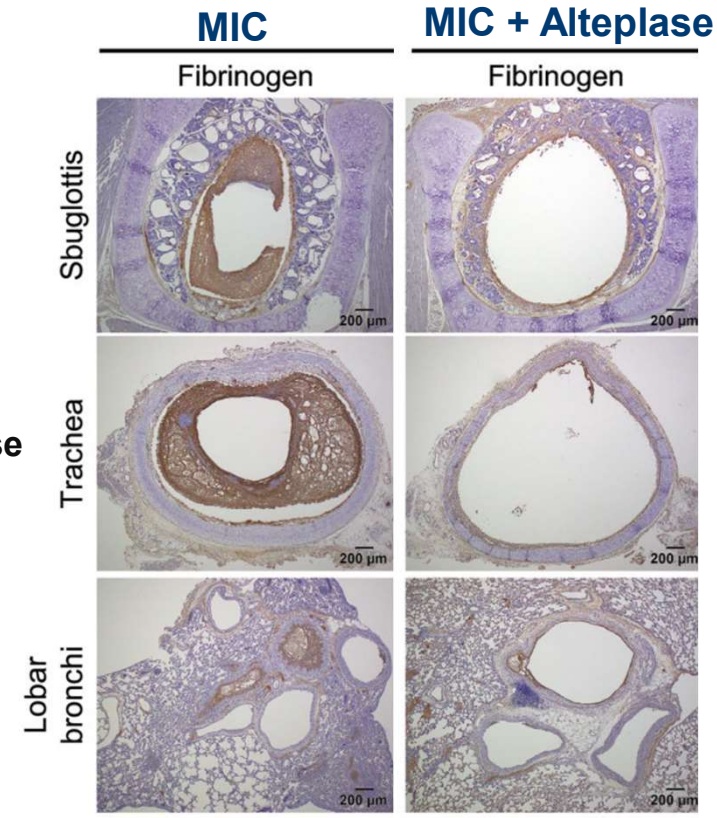
Methyl Isocyanate Treatment with Alteplase - Rat





Hb

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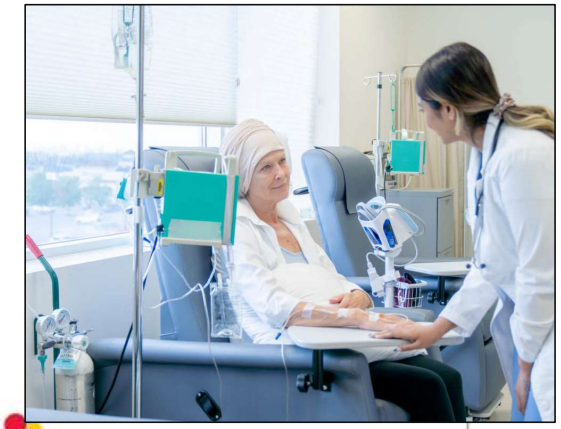
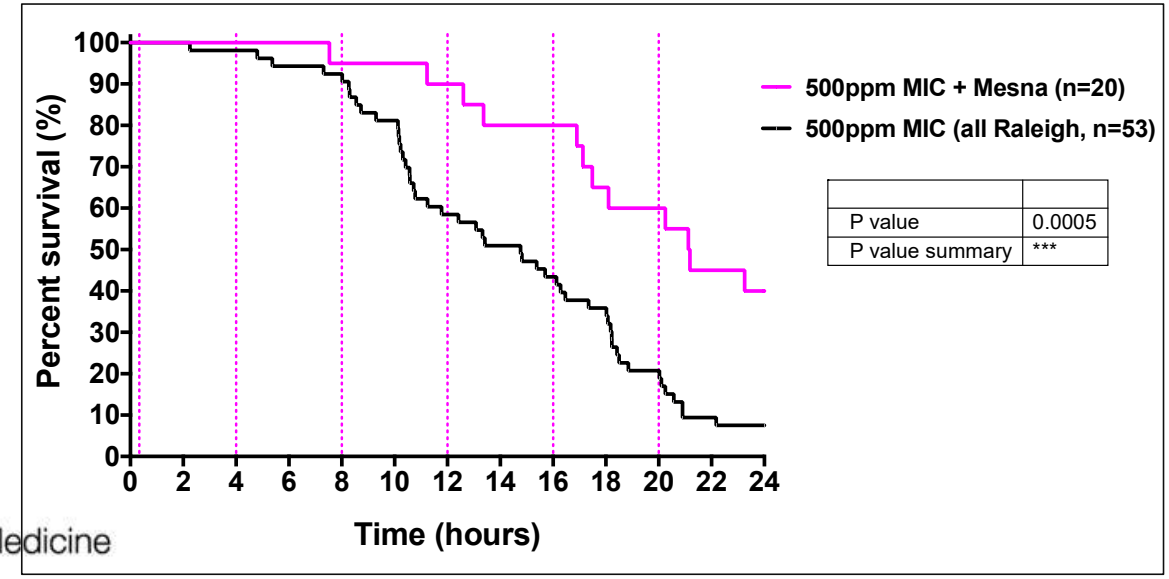
Nick et al, *Ann NY Acad Sci,* 2020

Methyl Isocyanate and MESNA Treatment

Mesna = sodium 2-mercaptoethane sulfonate

- Thiol
- Chemoprotective agent, used to detoxify metabolites of ifosfamida and cyclophosphamide during chemotherapy for cancer treatment
- Antioxidant, as thiol can scavange reactive oxygen species





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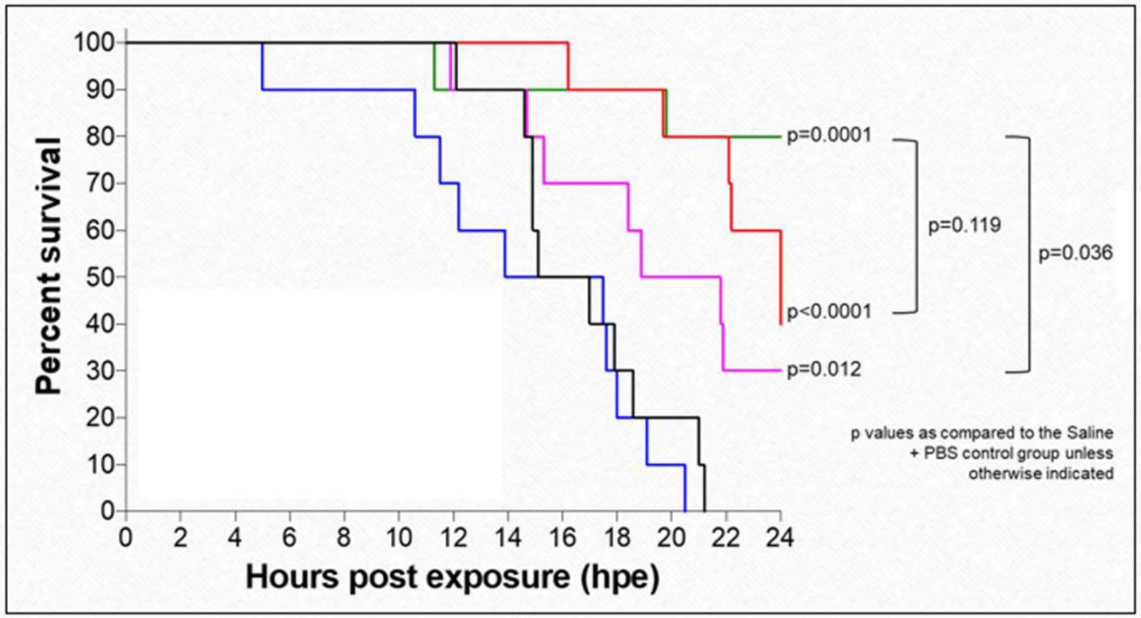
MESNA Synergizes with tPA to Further Improve Survival after MIC Inhalation (80% survival at 24 h)



- MIC (n=10)
- MIC + Saline + PBS (n=10)
- MIC + Mesna (n=10)
- MIC + tPA (n=10)
- MIC + Mesna + tPA (n=10)

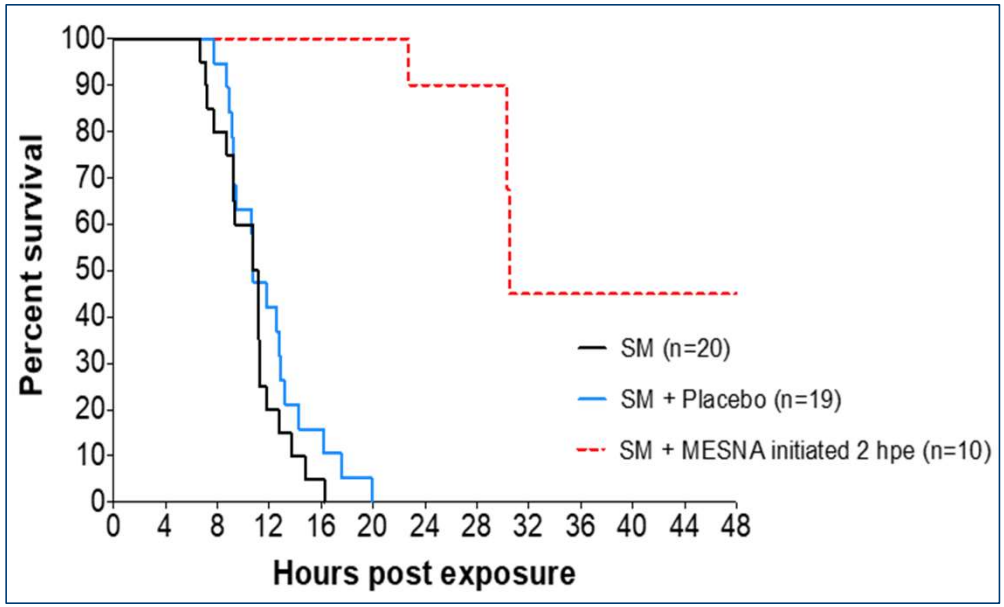
Methyl isocyanate (MIC):
500 ppm for 30 min, nose-only

MESNA:
300 mg/kg, IP, at 0.33, 4 and 8 h

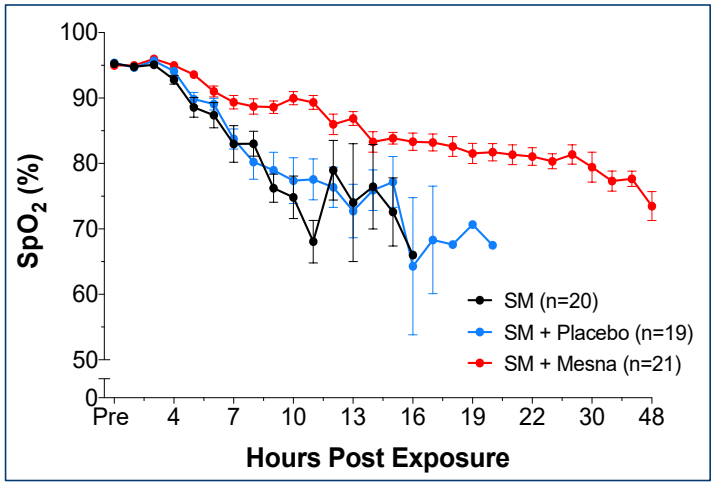
tPA:
0.7 mg/kg, IT, at 6, 11, 16 and 21 h

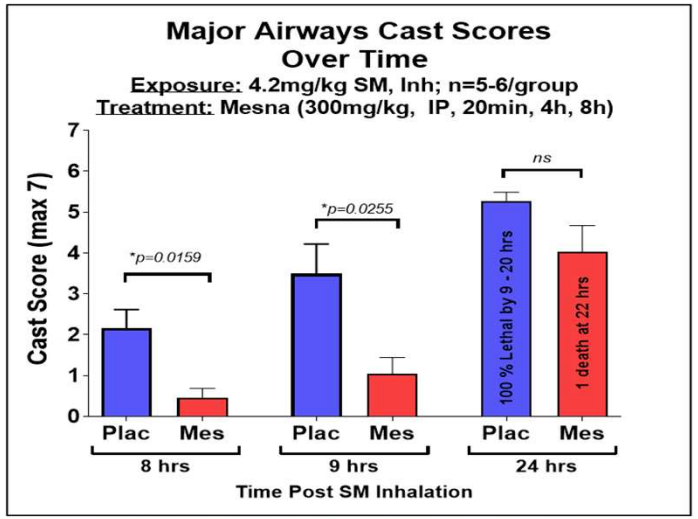
Sulfur Mustard and Mesna Treatment

First Mesna Treatment Delayed to 2 hours



Alternative Tx Protocol:
Mesna: 300mg/kg, IP, at 2, 4, and 8h post-exposure





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Chronic Phase health effects of Sulfur Mustard inhalation exposure

Prevalence– 78%

***Bronchiolitis Obliterans *** (53-55%)**
- 55% at 6wks to 1yr
- 53% at >4 yrs

Pulmonary fibrosis (5 – 24%)
- 24% at 6wk to 1 yr
- 5- 12% at >4 yrs

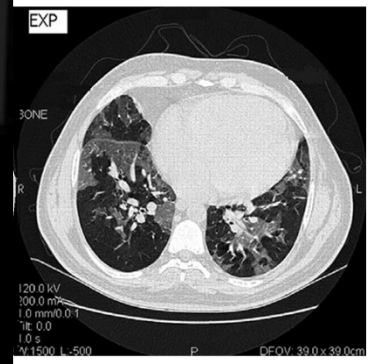
Chronic Bronchitis - (22 – 80%)
- Recurrent Pneumonia, COPD

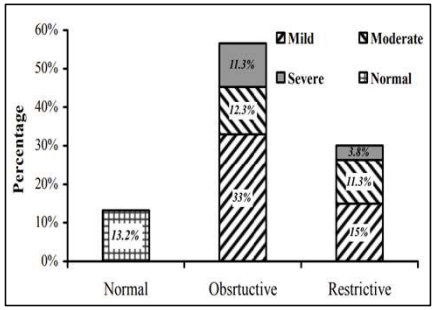
Tracheobronchomalacia - (14%)

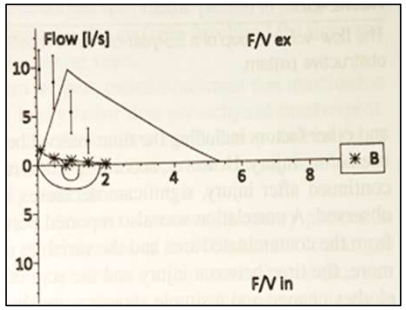
RADS– (11 - 30%)
- Emphysema

Large Airway Stenosis – (10%)

Bronchiectasis - (8.8%)







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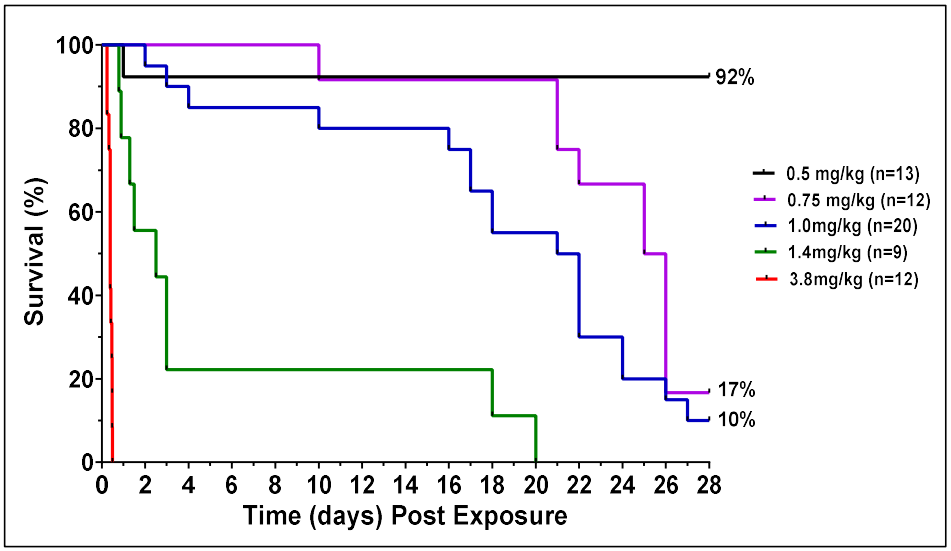
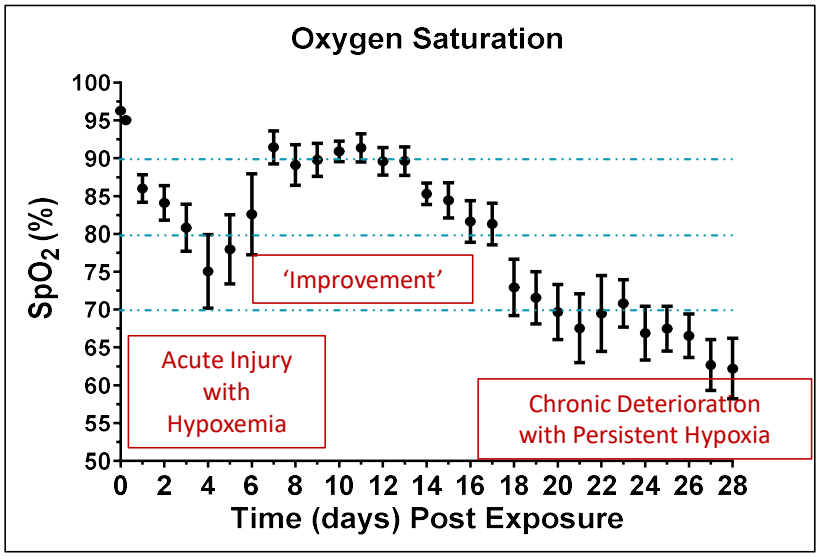
Idani et al 2012. *Acta Medica Iranica*

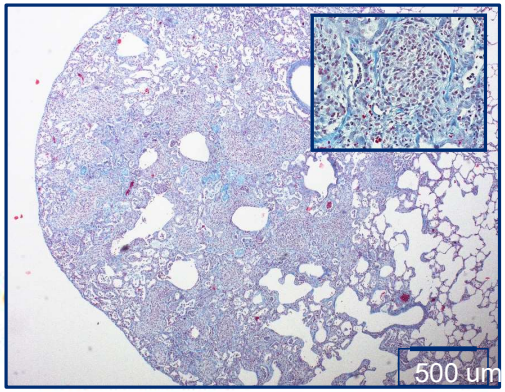
Ghanei 2016. Mustard Lung. *Elsevier*

Ghabili et al 2010. *J. of Appl Tox*

Late effects (Chronic Lung Disease) – Sulfur Mustard - rat







- Predominance for sub-pleura
- Greater in dependent lobes
- Patchy, heterogeneous

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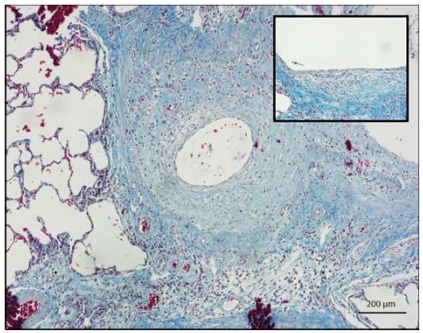
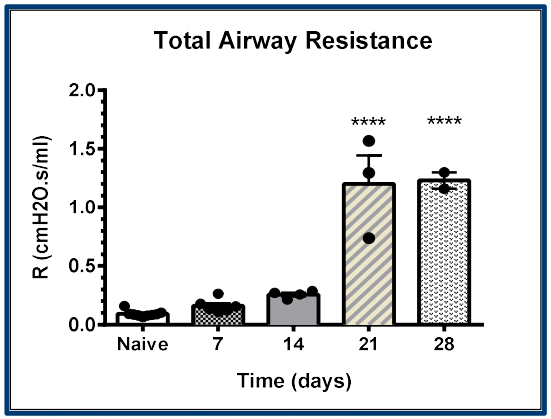
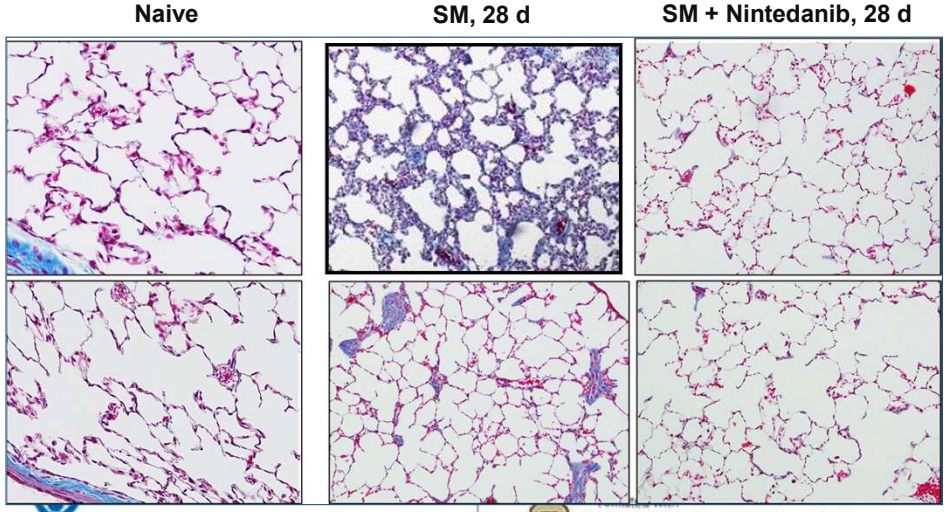
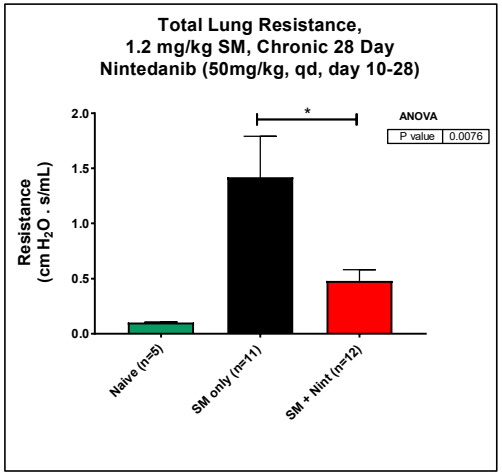


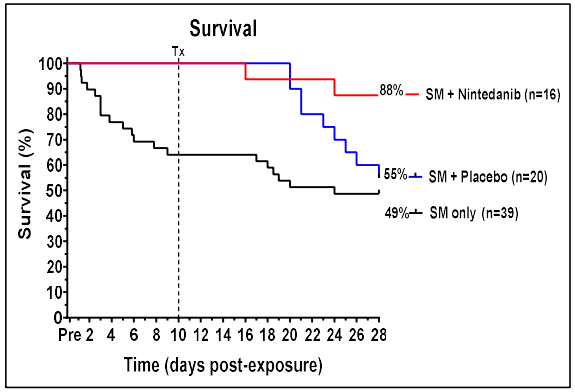


Nintedanib (antifibrotic) for Pulmonary Fibrosis due to SM

- Nintedanib is a “triple kinase inhibitor” and acts on tyrosine kinase receptors for:
  - PDGF (platelet derived growth factor)
  - VEGF (vascular endothelium growth factor)
  - FGF (fibroblast growth factor)
- Inhibits fibroblast migration, proliferation, and myofibroblast transformation.
- FDA approved for treatment of idiopathic pulmonary fibrosis







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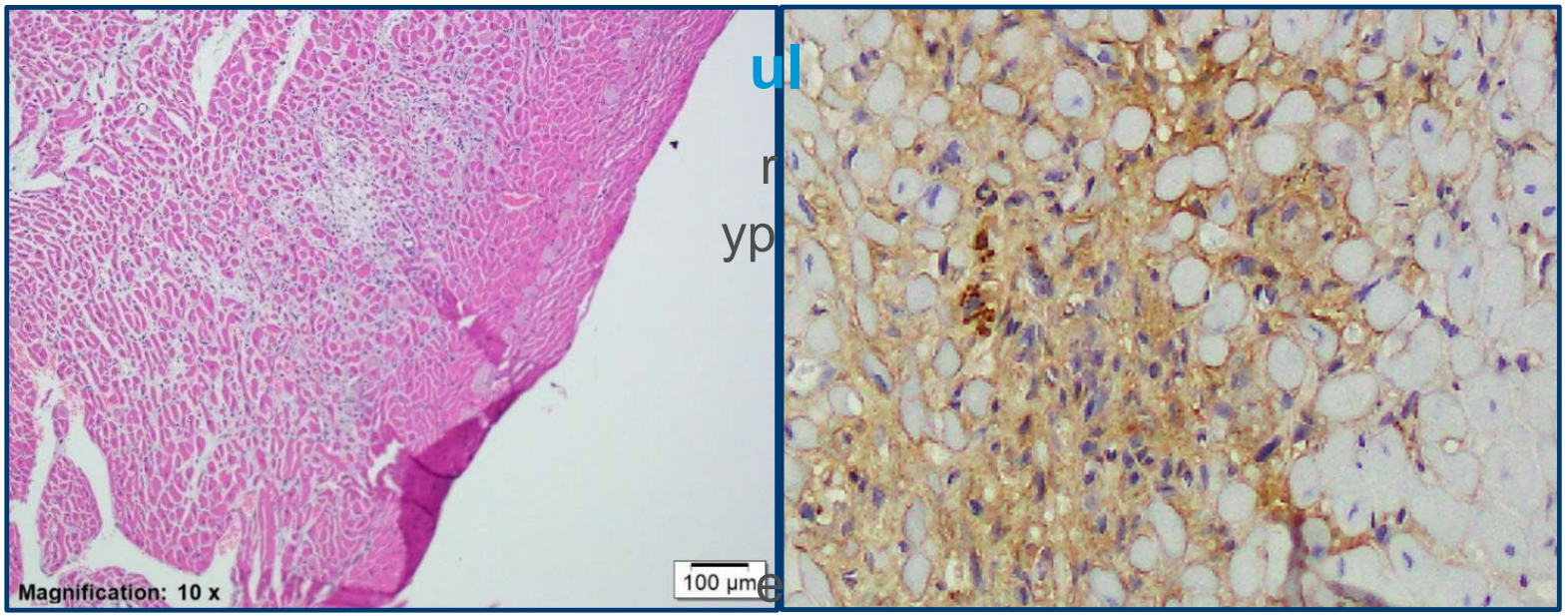
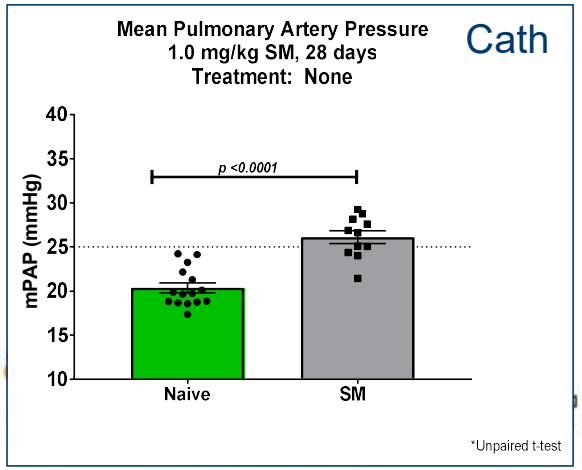


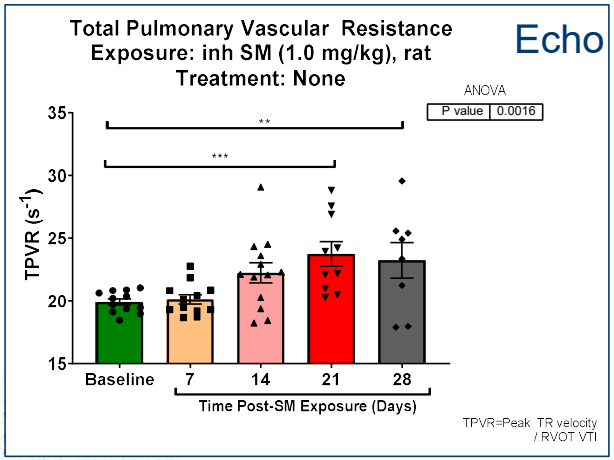
Shabestari, et al *CV Tox,* 2019

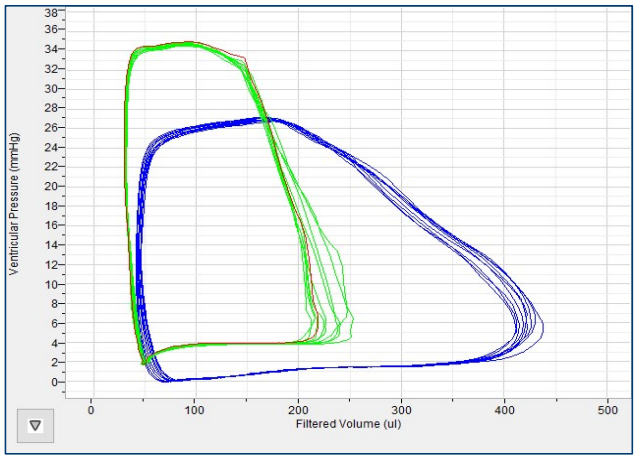
Khosravi et al *J. Clin U/S,*2018

Cath



Echo





Chlorine Gas

1. Yellow-green color
2. Highly reactive oxidant
3. Slightly water soluble
4. Density 2x that of air (settles near ground)
5. Intermediate reactivity with water (both upper and lower airways injured)
    1. forming a strong acid, HCl, and a powerful oxidant, HOCl
6. Reacts directly with organic materials in tissues
    1. Forming oxidized and chlorinated derivatives (chlorotyrosine, other chlorinated amines)

$Cl_2 + H_2O \rightarrow HOCl^- + tyrosine \text{ ----} > Chlorotyrosine$

H_2O_2 — MPO

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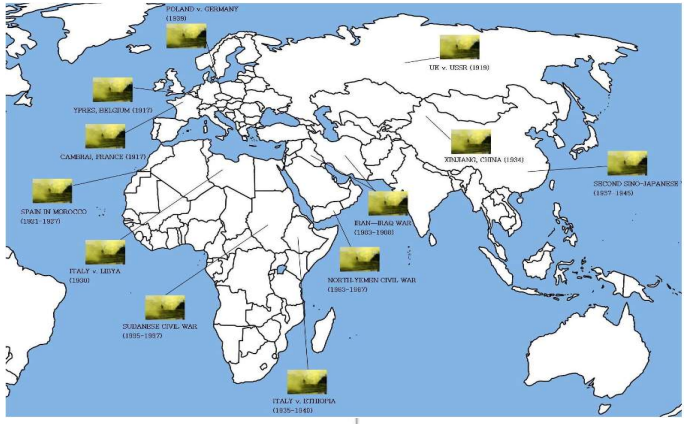
Chlorine – situations for exposure

1. Industrial Uses – plastics, PVC, solvents, water purification, pesticides
2. Chlorine - As a Chemical Weapon
   - First used in Ypres, France, WWI
   - Syria – more than 30 attacks since 2013 (most Chlorine)
     - Kafr Zita, Harasta, Damascus, Ghouta, Aleppo,
     - Last Use: Douma, April 7th, 2018

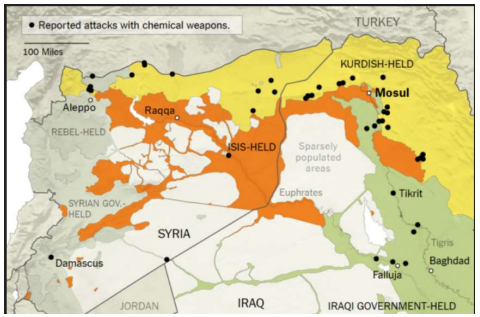
Global Attacks 1917 - Present



WWI Estimated Gas Casualties

Nation	Fatal	Total (Fatal & Non-fatal)
Russia	56,000	419,340
Germany	9,000	200,000
France	8,000	190,000
British Empire (includes Canada)	8,109	188,706
Austria-Hungary	3,000	100,000
United States	1,462	72,807
Italy	4,627	60,000
Total	90,198	1,230,853

Reported Attacks in the Middle East 2014 - 2017



Syrian Child After Suspected Gas Attack

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Acute effects of chlorine

TIME COURSE:

- Respiratory symptoms may be immediate or delayed for several hours or days after exposure to chlorine.
- Symptoms generally resolve within 6 hours after mild exposures, but may continue for several days after severe exposures.
- Deterioration may continue for several hours.

DEATH:

1. Deaths (1.5%) – most within 4 hours of exposure
2. Autopsy findings:
   - cardiomegaly (89%)
   - pulmonary congestion
   - pulmonary edema
   - frothy fluid in airways
   - tracheal and bronchial mucosal erythema,
   - purplish red and firm lung parenchyma
   - lactic acidosis (ICU patient)
   - cause of death: asphyxiation or acute respiratory failure

Table 1. Dose response relationship of chlorine (Reprinted with permission from Winder [65]).

Chlorine exposure concentration	Effect on human health
1-3 ppm	Mild irritation of mucous membranes
> 5 ppm	Eye irritation
> 15 ppm	Throat irritation
15–30 ppm	Cough, choking, burning
>50 ppm	Chemical pneumonitis
430 ppm	Death after 30 minutes exposure
> 1000 ppm	Death within minutes

Chlorine Late effects - human

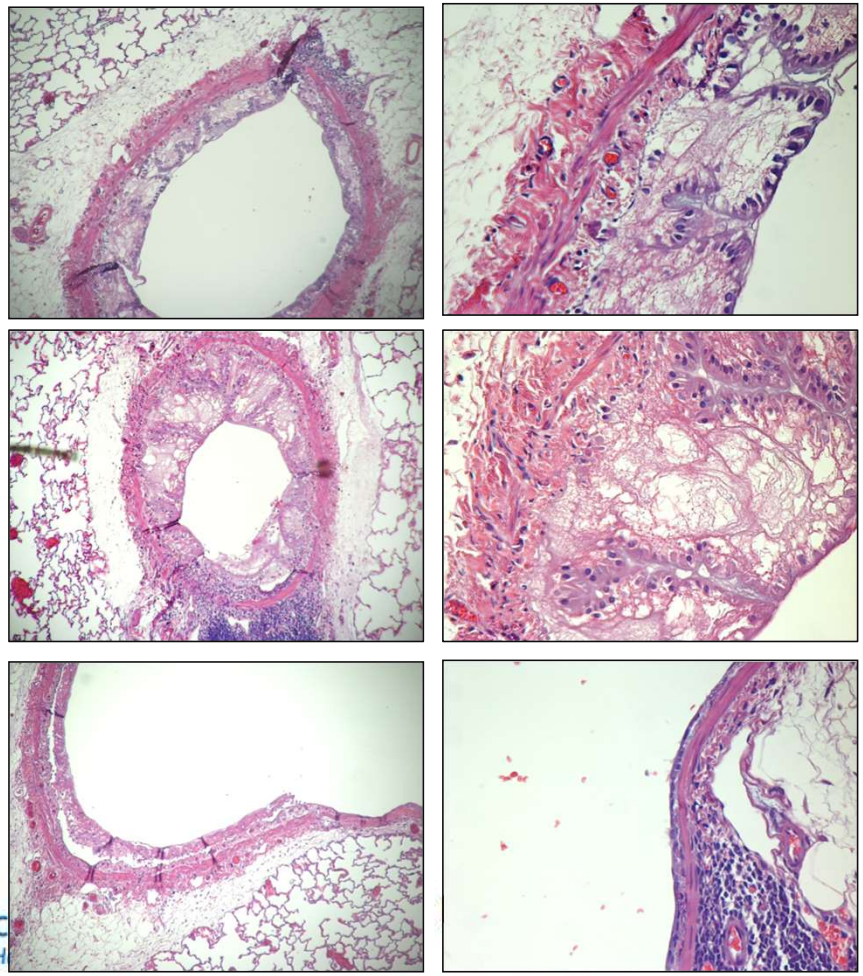
- Most individuals who suffer single chlorine gassings recover normal pulmonary function, even if the exposure is overwhelming.
- Ongoing low level exposures can cause obstructive airways disease, including asthma.
- Few sporadic cases of bronchiolitis obliterans have been reported
- Long-term neuropsychiatric, neurocognitive dysfunction is being reported at high rates

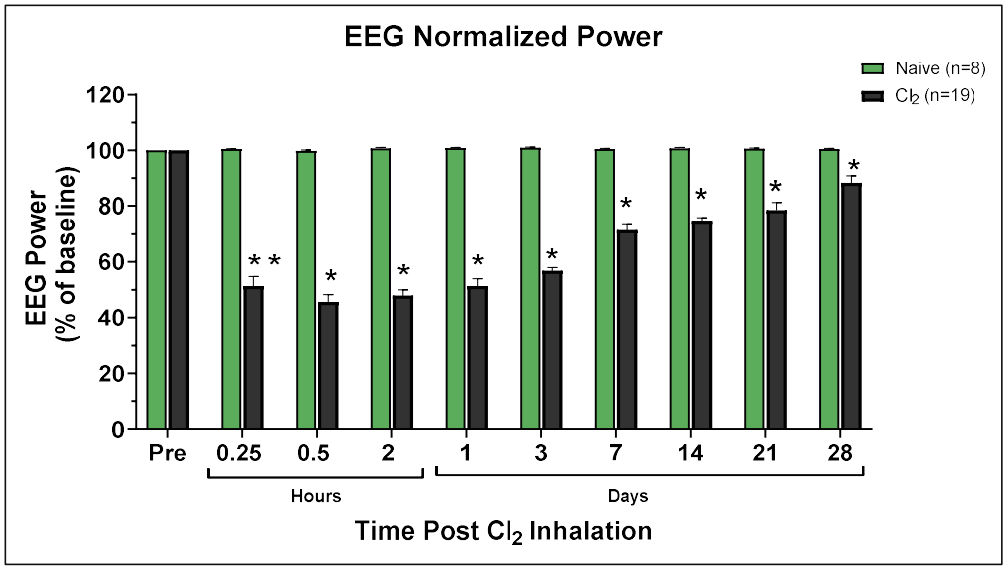
Chlorine Rat Model

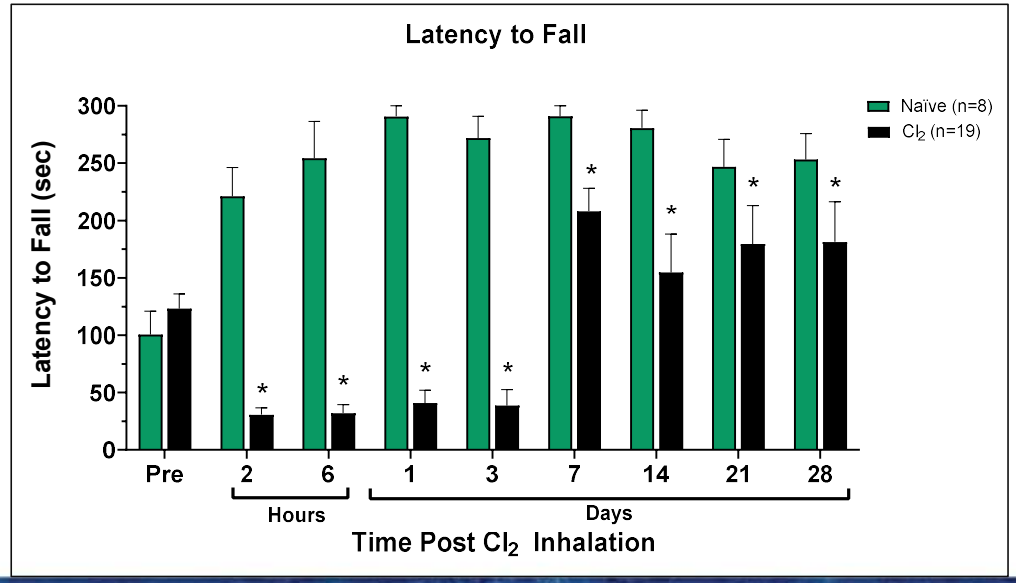
1 hr

6 hr

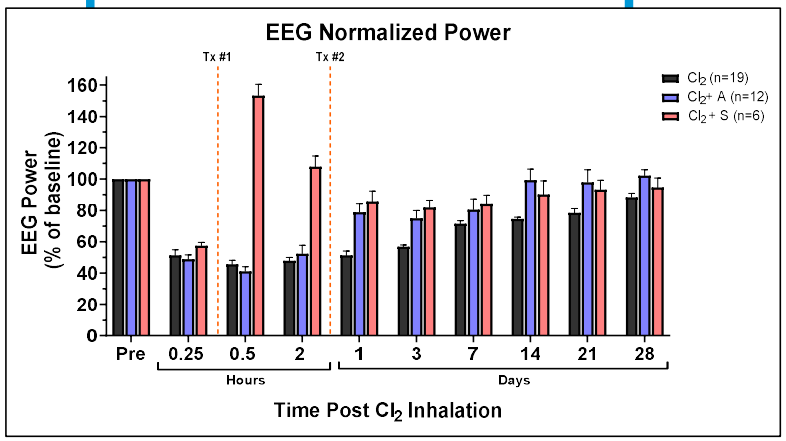
24 hr

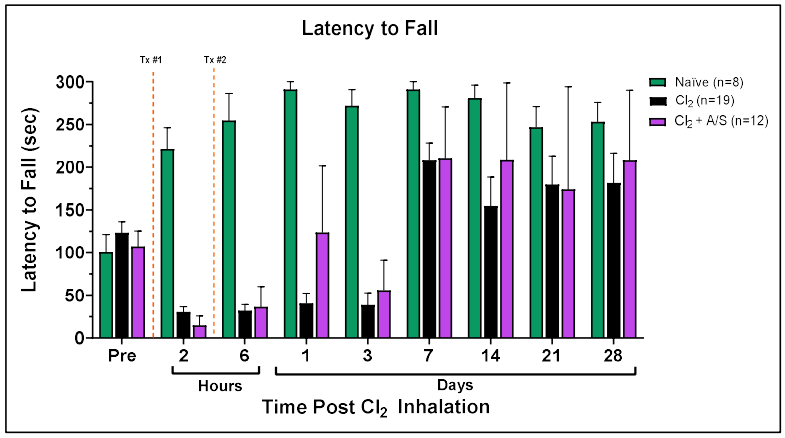


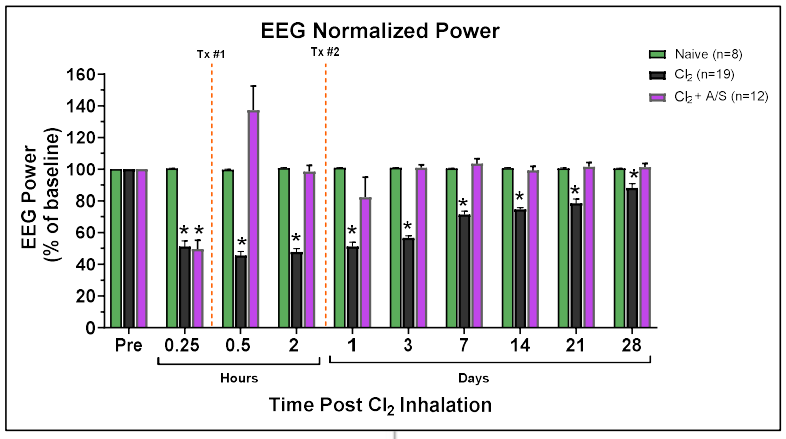


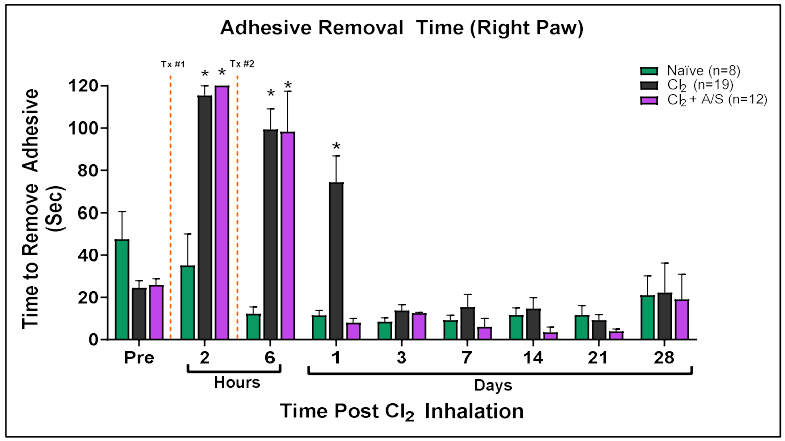


Scopolamine and Atropine in our rat chlorine









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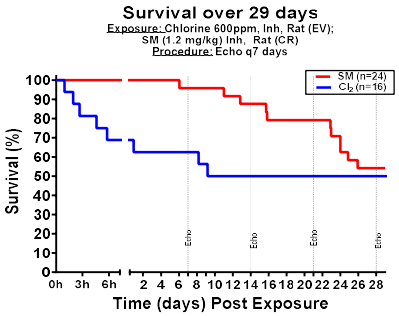
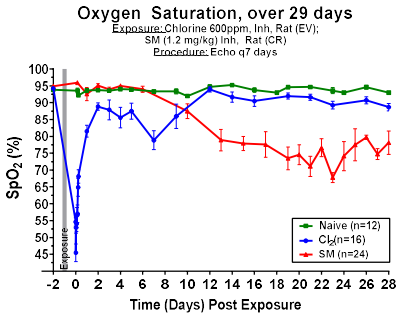
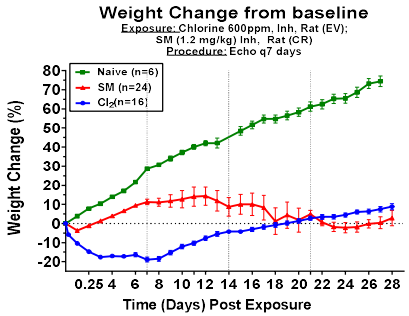
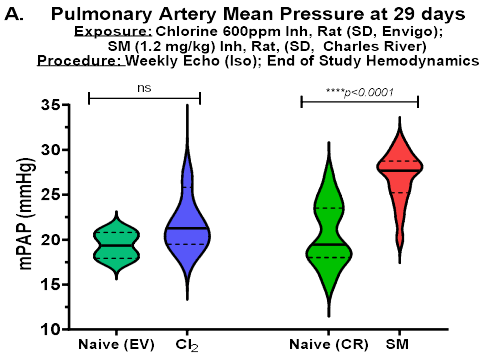
Scopolamine 10mg/kg at 15min, 2hr (IM)
Atropine 1 mg/kg at 15min, 2hr (IM)

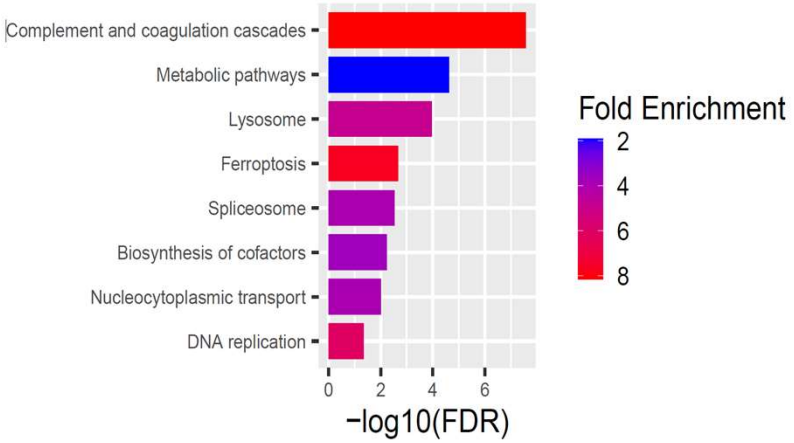
Current R01 Project at UC-CADD

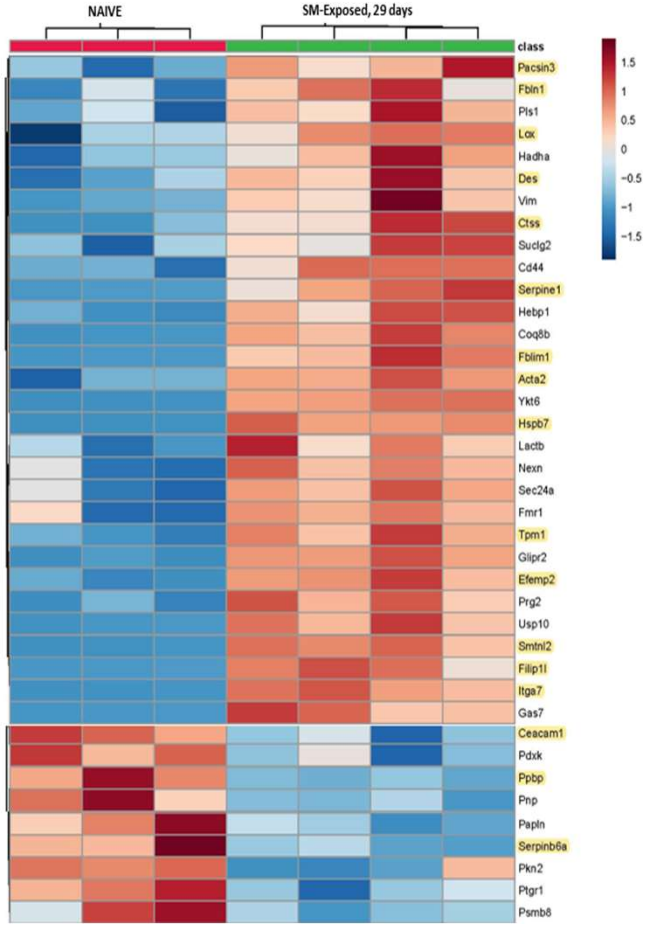












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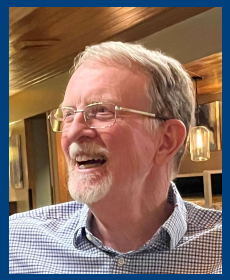
Summary

1. Sulfur Mustard, acute – airway casts - Alteplase, Mesna
2. Sulfur Mustard, delayed – fibrosis, PH, RV dysfxn – Nintedanib, (Sildenafil)
3. Methyl Isocyanate, acute – airway casts - Alteplase, Mesna
4. Methyl Isocyanate, delayed - ??fibrosis?? PH/RV? (*?Nintedanib, ?Sildenafil)*
5. Chlorine, acute – croup; neurotoxicity – N/A; Scopolamine, Atropine
6. Chlorine, delayed – encephalopathy – Scopolamine, Atropine

THANK YOU

Center for Advanced Drug Development - Team



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Q & A

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