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VISUAL AND HISTOPATHOLOGICAL CHANGES OF RAT LUNGS FOLLOWING INHALATION OF A LETHAL DOSE OF PHOSGENE

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ABSTRACT

Background: Phosgene, CAS 75 – 44 – 5, is a highly toxic gas at ambient temperature and pressure. It is widely used in manufacture of plastics, dyes, pharmaceuticals and pesticides. It can also be generated in advertently during fire involving plastic and other chemicals and solvents containing chlorine. Inhalation is the main route of exposure. Besides occupational exposure, phosgene is also of concern to emergency teams including early responders. Lethal dose of phosgene in humans is approximately 500 ppm / min of exposure or exposure to 3 ppm for 170 minis equally as fatal as exposure at 30 ppm for 17min.Exposure through inhalation to 3 – 4 ppm can produce an immediate irritant reaction that typically lasts 3 – 30 minutes and includes lacrimation, conjunctiva irritation, burning sensation in mouth and throat. Inhalation of high concentrations of phosgene may cause initially symptoms of respiratory tract irritation, patients may feel temporarily fine but a day later may manifests difficulty or shortness of breath, nausea and vomiting, and chocking may develop within 2 – 6. Skin contact can result in lesions similar to those of burns and frost. Methodology: Liquid **phosgene** (69.6 mg) was injected into a 20 liter static exposure chamber (Fig.1) to obtain phosgene concentration of 3.48 mg / liter. Based on conversion factor and calculation method reported in literature (CHSR, 2006) this was found to account to 868 ppm. This was checked by Gas Chromatography according to the method reported by (OSHA,1980). Animals: Male albino rats (Ratus ratus) weighing 170 – 250 g, whole body exposed to a single lethal concentration of phosgene gas at 868 ppm for 2 min. The exposure chamber was then immediately recondition for 30 min, the animals were then individually re-caged and kept under pre- exposure conditions. The animals were then sacrificed at different time intervals within the observation period (**2 – 8 h**). The animals within the control group were treated similar way but without exposure to phosgene gas. The lungs of all exposed and non - exposed animals were subjected to visual and histopathological examinations. Lung section for histopathological studies were prepared following the method described by Baker and Silverton (1976). The statistical analysis was carried out using one – way classification analysis of unequal variance test (Duncan, 1955).

KEYWORDS: Rats, whole body, phosgene exposure, visual and histopathological changes lungs.

INTRODUCTION

Carbonyl chloride **(**$COCl_2$**)** – most commonly known as phosgene is a colorless toxic gas at ambient temperature and pressure. It boils at 7.5 ^{o} C, odor threshold,0.4 – 1.5 ppm, and irritation threshold of 3 ppm. Phosgene – is widely used in the manufacture of plastics, dyes, pharmaceuticals and pesticides (Gutch et al., 2012). It is synthetized through reaction between carbon monoxide and chlorine in the presence of activated charcoal. It can also be released during decomposition of chlorinated hydrocarbons or plastics pyrolysis (Mehlman, 1987). When liquid phosgene is released, it quickly turns into a gas, and at low concentrations it has a pleasant odor of newly mown hay or green corn, but its odor may not be noticed by all. Therefore odor provides in sufficient warning of phosgene gas. One of the most life threatening properties of phosgene is that; at an early phase of exposure; it exerts only a mild irritant to the eyes and upper respiratory airways; therefore, it may be inhaled deeply into the lungs with little discomfort (Jacobs, 1967, Currie et al, 1987, Lipsett et al, 1994). Lethal dose of phosgene in humans is approximately 500 ppm for one min of exposure or 3 ppm for 170 min is equally as fatal as exposure at 30 ppm for 17 min (Diller and Zante,1982,Currie et al.,1987, IPCS, 1997, Vaish et al., 2013), and LCT_{50} of phosgene gas in rats was reported to be between 500 – 800 ppm (Currie et al., 1987, IPCS, 1997) and, the 10 - min LD_{50} value was 80 ppm, and the 30 – and 60 min LC_{50} values were 20 and 12 ppm (Zwart et al., 1990), and LD50 value of about 500 ppm for one min(Diller and Zante, 1982).

Skin contact with liquid phosgene can result in lesions similar to those from severe burns or frost (Sullivan and Krieger, 1992). However, although persons exposed only to phosgene gas do not pose substantial risks of secondary contamination, but at an ambient temperature below 8.2^{o}C, can secondarily contaminate response personnel through direct contact or off - gassing vapor (ATSDR, 2014). An increased incidence of chronic pneumonitis incidence and acute fibrinous pneumonia have been observed in cases of exposure to extremely high concentrations of phosgene . Fibrinous pneumonia is an indication of sever lung injury, characterized by the presence of intra – alveolar fibrinin**,** the form of fibrin balls (Santos et al., 2019). Some studies in animal models, show that at extremely high concentrations, death may occur before the development of pulmonary edema (Tobias, 1949). The primary findings in such cases were attributed to the plugging of pulmonary capillaries by haemolized red blood cells. It has also been reported that emphysematous lesions which are seen in animals may occur as a result of exposure to high concentrations of phosgene gas (Clay and Rossing, 1964, Ghio & Hatch, 1996). The present investigation was part of expanded project aiming at the end (second part of the study) to find out how a therapeutic protocol can be linked to the concentration and expected histopathological effects of inhaled phosgene gas.

RESULTS AND DISCUSSION

In the past, phosgene gas was classified as a chemical warfare agent due to its high toxicity, but in modern era it is widely used in chemical industries. The toxic effect of phosgene depends on how much phosgene is taken in and for how long. Its warning properties are slight, and there is no specific therapy for exposure except supportive treatments. Therefore, occupational exposure of phosgene is of great health concern. Cases of exposure have been reported whereby acute lung damages have been documented (Polednak, 1980, Lim et al., 1996, Vaish et al., 2013, Harrison et al., 2014). The lower respiratory system, namely the lungs are the main and most affected organs. Thus, exposure to even low phosgene concentration of (2 ppm for 90 min) by inhalation, and concentration lower than this may produce produces chronic pneumonitis (Gross et al., 1965). Studies in animals have shown that in cases of chronic exposure to phosgene gas, chronic pneumonitis and acute fibrotic pneumonia have been observed (Sittig, 1985). Acute exposure to high concentrations induces severe pathophysiological and pathophysiological changes in the lower respiratory system. It has been reported that at least 5 ppm for 10 min exposure was necessary for the production of alveolar edema in rats (Diller et al 1985). The median lethal concentration (LD_{50}) for phosgene in humans was found to be in the range of 500 – 800 ppm (Currie et al., 1987). In controversy to most animal studies where facial exposure method was employed, this study was designed to use whole body exposure fo**r** only 2 min which is more realistic t o the possible l accidental exposure accidental exposure to high doses of phosgene. Moreover, facial exposure for long time creates discomfort for the animals, causing lacrimation, eyes (conjunctiva), and irritation of airways and throat. Besides, prolong exposure duration, especially in static whole body exposure, may affect the humidity resulting from animals breathing and sweating, and from released faces and urine. This study was designed to investigate acute visual and histopathology changes in lungs of male rats exposed to a single lethal phosgene concentration at 868 ppm level for 2 min using static exposure chamber (Figure – 1). Mortality occurred within 2 – 6 hours. As an early sign of exposure**,** acidosis was clear as indicated by difficulty of breathing, significant increase in breathing rate, accompanied by unique voice; wheezing as an indication begging of lung edema. Such observation has also been reported by (Diller et al., 1985). The visual examination of the lungs of the exposed animal showed severe congestion, an increase in the size, weight, and rigidity (Table – 1).Such increase in the weight and size of the lungs of exposed animals may be due to the accumulation of fluid in the lung cells of exposed animals. This may be due to direct contact of phosgene gas with pulmonary alveoli affecting blood – gas barrier of alveoli leading to alteration in their semi-permeability nature resulting in accumulation of liquid inside the alveoli leading to non – cardiogenic edema. Similar conclusion has been reported by other researchers (Coman et al., 1977, Frosolono and Pawlowski, 1977, Hobson et al., 2019)). The hardness of the lung (as we not intra – alveolar fibrin in the form of " fibrin balls" within the alveolar spaces, with a patchy distribution as an indication of acute lung injury. The results presented in (Table.1) show significant differences as percentage ratio and standard error between the dry and wet of lungs of control and exposed animals. Such differences may be due to accumulation of fluids in the lungs (cellular infiltration) as shown in (Fig. 2). The color of these fluids was found to depend on the severity of the congestion, and was mostly red in color due to the presence of Erythrocytes (RBCs). The examination of histopathological sections of lungs of exposed animals revealed severe congestion of the arteriolar and venous alveolar membrane walls and the presence of RBCs in inside the lumen. In addition, remarkable congestion was also observed in in the bronchus arteries. Some blood vessels were bleeding and edematous. A widening in the interstitial pulmonary spaces was formed due to the presence of edema. Emphysema of the alveoli and blood vessels congestion are shown in (Fig. 3). Sever congestion of alveoli blood vessels and accumulation of proteinaceous fluid inside the alveoli, while, other main lesions were full of pulmonary vacuoles containing the acidophilus proteinacious fluids (Fig. 4). Similar findings were reported in mice (Frosolono and Pawlowski, 1977, Duiho et al., 2002). Stained – sections of pulmonary alveoli showed regions of different thickness which means the presence of thrombosis inside the alveolar blood vessels surround by edema can be seen in (Fig.5). Based on the

results of this study, it may be concluded that the main cause mortality of phosgene exposure is the induced of the non – cardiogenic lung edema which may be related to the direct contact of phosgene with pulmonary alveoli which acts in turn upon the blood – air barrier increasing the degree of permeability of this barrier. This has been confirmed in this study by finding diffusion of some large molecules such as albumen through blood air barrier which is normally semi – permeable. This was further confirming when inside the alveoli reacted with hematotoxylin - eosin stain.as indicated by light pink color. Such alteration in the nature of this barrier causes.

Table 1: The percentage ratio of the dry weight to wet weight of lungs of exposed and group of rats.

Type of treatment	Mean ± standard error (SE)*
Control group	77.57 ± 0.61
Exposed group	87.05 ± 0.57

*The results are average lungs from 12 animals. The differences seem to be significant on level < 0.05.

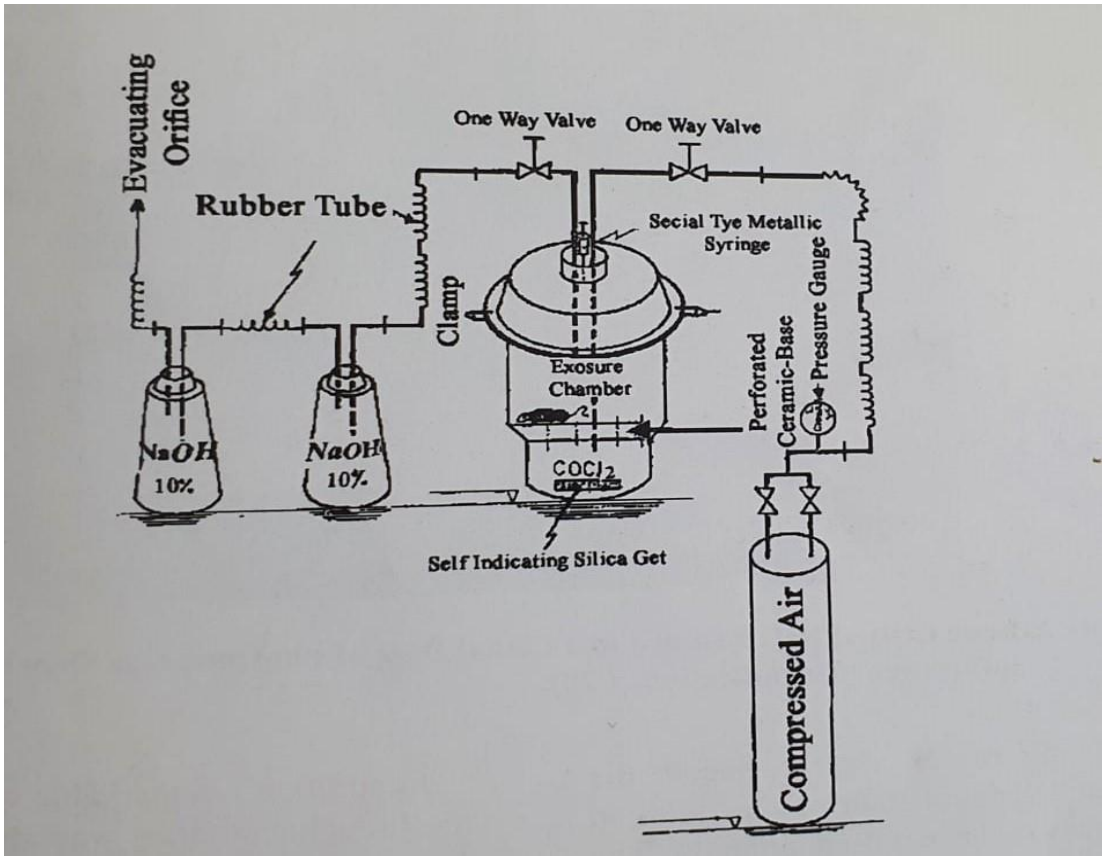

Figure 1: Schematic Diagram o Static Exposure System.

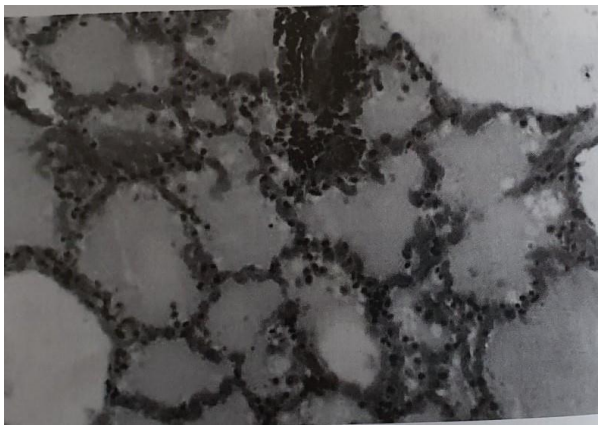

Figure 2: Cellular infiltration of Lung cells of rats exposed to acute high concentration of phosgene gas.

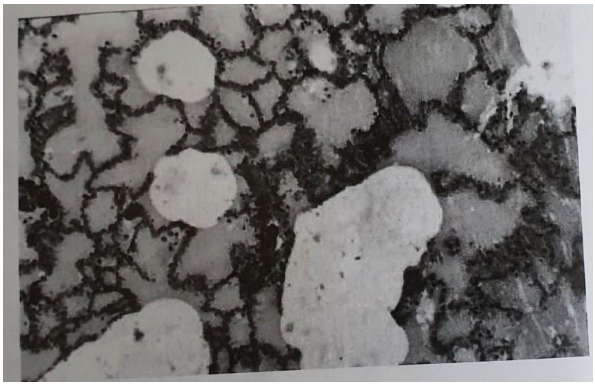

Figure 3: Emphysema of the alveoli and congestion of blood vessels of lungs of rats exposed to acute lethal concentration of phosgene gas.

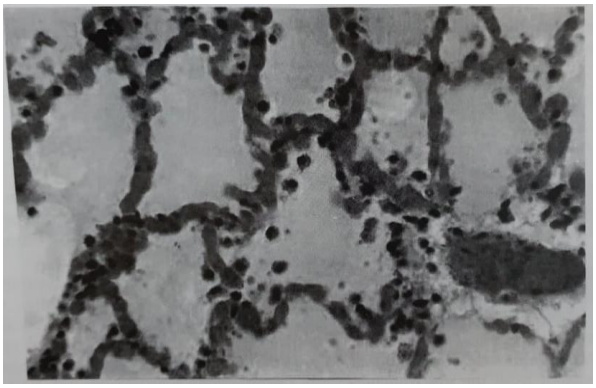

Figure 4: Severe congestion of alveolar blood vessels and accumulation of the proteinaceous fluid inside the alveoli of lung cells of rats expose to a lethal concentration of phosgene gas within 2 minutes.

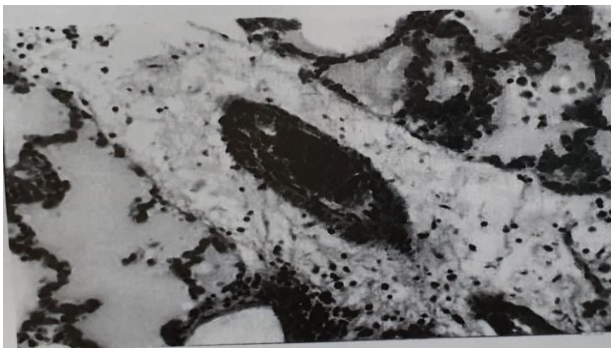

Figure 5: Thrombosis inside the alveolar blood vessels surrounded by non – cardiogenic edema of rats exposed to a lethal concentration of phosgene gas delivered within 2 minutes.

CONCLUSION

In this study we focused of the pulmonary injury associated with the acute inhalation exposure to phosgene gas. The results suggest that the lungs are the main target of phosgene gas. An acute inhalation exposure to a lethal concentration of phosgene at a dose level of 868 ppm for 2 minutes can induce 100 % lethality, mainly due to alteration in the semi - permeability nature of blood – air barrier of alveoli leading to non– cardiogenic edema as the main cause of mortality.

REFERENCES

1. ATSDR- Agency for Toxic Substances and Disease Registry, page last reviewed October, 2014.
2. Baker FJ and Silverton RE. Introduction to medical laboratory technology, 5th edn., chapter 13, Butterworth London, UK, 1976.
3. Castleman BI and Ziem GE. American conference of governmental industrial hygienists: Low threshold of credibility .Am. J. Indust. Med, 1994; 26: 133 – 143.
4. Chasis H. Phosgene: Review of literature on the effect of exposure in man and experimental animals. Contract W – 49- 036 – CWS, 1944; 1.
5. CHSR (center for hazardous substance – research), Kansas State University USA. 2006; 2.
6. Clay JR; Rossing RG. Histopathology of exposure to phosgene: an attempt to produce pulmonary emphysema experimentally. Arch. Pathol, 1964; 78:

544–551.
7. Coman DR, Bruner HD, Horn RC, et al. Studies on experimental phosgene poisoning: The pathogenic anatomy of phosgene poisoning with special reference to the early and late phases. Am. J. Pathol, 1947; 23: 1037 - 1074.
8. Currie DW Hatch EG, Frosolono FM. Pulmonary alteration in rats due to phosgene inhalation. Fundamental and Applied Toxicology, 1987; 8: 107–114.
9. Diller WF and Zante, R. Dosis-wirkungs-beziehungen bei phosgeneinwirkung auf mensch und tier. Zbl. Arbeitsmed, 1982; 32: 360–368. [PubMed]
10. Diller WF, Bruch J, Dehnen W. Pulmonary changes in rat following exposure following low phosgene exposure. Arch Toxicol, 1985; 5: 184–190.
11. Duncan DB. Multiple range and multiple F tests. Biometrics, 1955; 1: 11–42.
12. Duniho SM, Martin J, Jeffry S, et al. Changes in lung histopathology and bronchoalveolar lavage parameters in mice exposed to chocking agent gas phosgene. Toxicol. Pathol., 2002; 30(3): 339–349.
13. Franch S and Hatch GE. Pulmonary biomedical effects of inhaled phosgene in rats. J. Toxicol. Environ Health, 1986; 19: 413 – 423 [PubMed]
14. Fronsolono MF and Pawlowski R. Effect of phosgene on rat lungs after single high – level exposure. Arch Environ. Health, 197; 271 - 277.
15. Ghio AG and Hatch GE. Tolerance to phosgene is associated a neutrophilic influx into the rat lung. Am. J. Respir. Crit. Care Med, 1996; 153(3): 1064 – 1071.
16. Gross P, Rinehart WE, and Hatch T. Chronic pneumonia caused by phosgene. Arch. Environ. Health, 1965; 10: 768-775[PubMed]
17. Gutch M Jain N, Agrawal A, et al. Acute accidental phosgene poisoning . BMJ Case Report, 2012; 2012: 1120115233.
18. Hardison LS, jr Wright E, and Pizon AF.Phosgene exposure : a case of accidental industrial exposure. J Med Toxicol, 2014; (1): 51-56.
19. Hobson ST, Casillas RP, Richieri RA. Development of an acute short – term exposure – model for phosgene Toxicol Mech Methods, 2019; (8): 604 – 615.
20. IIPCS(Int. Prog. on Chem. Safety) :Poisons information:monograph419:phosgene,july,16,2007 :http://www.inchem.org/document/pims/chemical/pim419.htm.
21. Jacobs BM. The analytical toxicology of industrial inorganic poisons.- Interscience publisher- 1967, London, UK.
22. Li W, Pauluhn J. Phosgene – induced acute lung injury(ALI) differences from chlorine - induced injury and attempts to translate toxicology to clinical medicine Clin Transl Med, 2017; 6 : 19.
23. Li W Pauluhn J. Phosgene – induced lung edema: comparison of clinical criteria for ncreased extravascular lung water content and lavage – protein in rats and dogs.Toxicol Lett., 2019; 305: 32 – 39.
24. Lim SE, Yang, Jang AS, et al. Acute lung injury after phosgene inhalation. Korean J Intern Med, 1996; 11(1): 87 – 92.
25. Lipsett MJ, Shusterman DJ, and Beard RR. Phosgene In: Industrial hygiene and toxicology, 4th edn.Vol. II, Part F, Clayton GD and Clayton FE, (Editors) , J Willy & Sons Inc, pages: 4557- 4563.
26. Mehlman MA. 1987.Health effects and toxicity of phosgene: Scientific review. Def. Sci J., 1987; 37(2): 269 – 279.
27. NCR-US.(National Research Counsel - US) Phosgene : physical and chemical properties: National, Academic Press (US), 1984.27.
28. OSHA – 1986 Methods No. 61. Phosgene. Salt Lake City, Utah, USA.
29. Pauluhn J. Acute – nose only exposure of rats to phosgene – Part – II. Concentrations X time dependence of changes in bronchoalveolar lavage during follow – up period of 3 months. Inhal Toxicol, 2006; (9): 595 – 607.
30. Pauluhn J. Carson A, Costa DL, et al. Workshop summary: Phosgene – induced pulmonary toxicity revisited: appraisal of early and late markers of pulmonary injury from animal models with emphasis on human significance. Inhalation Toxicology, 2007; 19(10): 789 – 810.
31. Pawlowski R and Frosolono M. Effect of phosgene on rat lungs after single high – level exposure II. Ultra – structural. Arch Environ Health, 1977; 32(6): 278 – 83. alterations.
32. Polednak AP. Mortality among men occupationally exposed to phosgene in 1943 – 1945. Environ Res., 1980; 22: 357 – 367.
33. Santos C Olveira RC, Serra P, et al. Pathophysiology of acute fibrinous and organizing pneumonia – Clinical and morphological spectra .Pathophysiology, 2019; 26(3): 213 – 217.
34. Sittig M. Handbook of toxic and hazardous chemicals and carcinogenesis,2nd edn., Noyes Publications , Park Ridge, NJ,USA., 1985.
35. Tobias JM, Postel S, Patt HM,et al. Localization of the site of action of a pulmonary irritant(Diphosgene). Amer. J. Physiol., 1948; 158: 173.
36. Vaish AK, Consul S, Agrawal A et al. Accidental phosgene gas exposure: A review of 10 cases. J Eerg Trauma Shock, 2013; 6(4): 271 – 275.
37. Wyatt JP and Allister CA. Occupational phosgene poisoning: a case report and review .Emergency Medicine Journal, 1995; 12: 212 – 213.
38. Zwart A, Arts JHE, Houweling- Klockman JM., et al. Determination of concentration of concentration-time - mortality relationships to replace LC50 values. Inhalation Toxicol, 1990; 2: 105 – 117.