LUNG INDIA (1996), XIV, NO.1 (P24-29)

METHYL ISOCYANATE TOXICITY: A REVIEW OF ANIMAL EXPERIMENTAL STUDIES.

1. SHORT-TERM EFFECTS.

VIJAYAN, VK.
Cardio-Pulmonary Medicine Unit, Tuberculosis Research Centre
Indian Council of Medical Research, Madras 600 031.

(Received Original August 1995; revised February 1996)

ABSTRACT

Methyl isocyanate (MIC) caused the worst Industrial Chemical Disaster in history. Many experimental studies were carried out in rats and guinea pigs to assess the toxicity. The experiments are reviewed in the light of reported acute (short term) effects.

Introduction

Forty two metric tons of methyl isocyanate (MIC) stored in tank No.610 of the Union Carbide Factory at Bhopal leaked on 2/3rd of December 1984, leading to the world's worst chemical industrial disaster (1-3). Bhopal, the capital city of Madhya Pradesh, is centrally located in India and had a population of about 800,000 people in 1984. The leakage of MIC resulted in the immediate death of approximately 2500 people and in the exposure of an estimated population of 2,00,000(4,5). The Union Carbide Corporation established the factory at Bhopal for the manufacture of a carbamate pesticide, Sevin (carbaryl) in 1969. An estimated population of 100, 000 lived within one kilometer radius of the plant at the time of tragedy (4). Methyl isocyanate is the primary raw material for the manufacture of the pesticide, Sevin. The manufacturing process for Sevin involves the reaction of a slight excess of alpha-naphthol with MIC in the presence of a catalyst in carbon tetrachloride solvent. Methyl isocyanate ($CH_3N=C=O$) is produced by reaction of phosgene ($COCl_2$) with monomethyl amine (MMA, CH_3NH_2) to produce methyl- carbamoyl chloride (MCC, $CH_3NHCOCl$) and hydrogen chloride (HCl). MCC is then pyrolized to yield methyl isocyanate and HCl. The chemical reactions involved in the production of MIC via phosgene are as follows:

1. $2C + O_2$ ------> $2CO$
2. $CO + Cl_2$ ------> $COCl_2$
3. $COCl_2 + CH_3NH_2$ ------> $CH_3NHCOCl + HCl$
4. $CH_3NHCOCl$ ------> $CH_3NCO + HCl$

Methyl isocyanate

Methyl isocyanate is a clear, colourless liquid with a pungent odour. It has a molecular weight of 57.05, boiling point at atmospheric pressure of 760 mm Hg, of 39.1°C (102.4°F), specific gravity at 20°C of 0.9599, freezing point of -80°C, and vapour pressure at 20°C of 348 mm Hg (6,7,8). Contact with water causes an exothermic reaction resulting in the formation of carbon dioxide, methylamine gases and N, N'-dimethyl urea. The reaction is enhanced by acids, alkalis and amines. When MIC is pyrolyzed at temperatures of 427-548°C, decomposition products such as hydrogen cyanide (HCN), oxides of nitrogen and carbon monoxide are formed (6,7). MIC is highly irritant to the skin, eyes and mucus membranes of the respiratory tract. The irritant property is based on its reactivity with water which enables it to penetrate tissues and interact with protein. A safe level of exposure for a period of eight hours was estimated to be about 0.02 ppm for humans(7). No odour is detected at 2 ppm, but subjects experience eye, nose and throat irritation and lacrimation. At 4 ppm, symptoms of irritation are more marked. Exposure is unbearable at 21 ppm. Because the threshold limit value (TLV) suggested by the American Congress of Governmental Industrial Hygienists (ACGIH) is 0.02 ppm and is less than the mucus membrane irritation threshold (>0.4 ppm) and the odour threshold (> 2 ppm), MIC is considered to have poor warning properties (9, 10).

Purified MIC will react with itself under the influence of a catalyst to form a cyclic trimer or a high molecular weight polymer. Strong bases such as sodium hydroxide, sodium methoxide and sodium acetate, certain metal chlorides such as ferric chloride and stannic chloride catalyze trimerisation. Since the reaction is quite exothermic, contamination of MIC with traces of the catalysts can cause violent reactions. Highly purified MIC will polymerize spontaneously to a linear polymer/trimer. Water reacts exothermically to produce heat and carbon dioxide. As a result, the tank pressure will rise rapidly if MIC is contaminated with water. The reaction may begin

slowly, especially if there is no agitation, but it will become violent. Aqueous sodium hydroxide solution will react with MIC quite rapidly.

The following compounds were identified in the residue samples taken from various locations in tank number 610 (3).

1. Methyl isocyanate trimer (MICT)
2. Dimethyl isocyanurate (DMI)
3. Dimethyl urea (DMU)
4. Trimethyl urea (TMU)
5. Dione
6. Trimethyl biuret (TMB)
7. Tetramethyl biuret (TRMB)
8. Monomethyl amine (MMA)
9. Dimethyl amine (DMA)
10. Trimethyl amine (TMA)
11. Chloride
12. Metallic ions (Fe, Cr, Ni, Mo, Na, Ca, Mg).

Short-term effects.

Even though detailed toxicological evaluations are available for isocyanates such as toluene diisocyanate (TDI) (11) used extensively in the plastic industry, methyl isocyanate (MIC) does not have large toxicology data base. At the time of the Bhopal gas disaster, there was only one published paper on the toxicology of MIC, concerning short-term effects following acute exposure to animals and humans (10). Many people died at Bhopal within minutes to a few hours from exposure to MIC. To examine the probable causes of rapid mortalities, the following pathophysiologic mechanisms have been suggested and investigated in experimental animals (12, 13):

1. an inhibition of cholinesterase activity,
2. an alteration of haemoglobin function,
3. development of disseminated intravascular coagulation,
4. activation of the complement systems resulting in adult respiratory distress syndrome,
5. gas exchange impairment in the lung,
6. morphologic alteration of the lung compromising gas exchange and,
7. Cyanide poisoning

All MIC inhalation exposures in experimental animals were acute, of short duration (mainly 15 minutes) and high in concentration (ranging 25-3506 ppm). Early mortality was defined as death occurring within four hours following a single MIC exposure of 15 minutes duration (12). There was no early mortality in rats exposed upto 3506 ppm, but 6% of guinea pigs exposed to 225 ppm had early mortality. Following exposure to 1000 ppm for rats and 225 ppm for guinea pigs, 69% of rats and 100% of guinea pigs died between four and sixteen hours. Thus guinea pigs were more susceptible than rats to MIC exposure (12). LC50 (95% confidence limit) values were 171 (range 114-256) ppm for rats and 112 (range 61-204) ppm for guinea pigs. For both species, the most important clinical signs during exposure were lacrimation, blepharospasm and mouth breathing (12).

1. Inhibitation of Cholinesterase activity in MIC exposed animals.

Previous *in vitro* studies with toluene diisocyanate and hexamethylene diisocyanate had demonstrated an inhibition of plasma cholinesterase (ChE) activity (14). Thus, the first hypothesis to explain the early mortality was that exposure to MIC caused a massive inhibition of ChE activity. MIC vapor exposure in *vitro* (100, 500, 1000 or 2000 ppm) to human, rat and guinea pig packed erythrocytes had shown that erythrocyte ChE activities were inhibited by MIC in a concentration-related manner. However, a similar inhibition of ChE was not observed in either animals receiving an intravenous dose of liquid MIC or those exposed by inhalation (15). Thus, the hypothesis, inhibition of ChE activity, could not be considered as a major contributing factor in the cause of mortality in MIC exposed animals.

2. Alteration of haemoglobin function.

The second hypothesis was that death occurred due to carbamylation of haemoglobin (Hb). It had been noticed previously that the carbamylation of the beta-chains of sickled haemoglobin (HbS) with cyanates or isocyanates inhibits erythrocyte sickling (16). Cyanates and isocyanates are also known to increase the oxygen affinity of haemoglobin of persons with sickle cell anaemia, returning their haemoglobin oxygen affinity to the range of normal haemoglobin (16-19). It was postulated that carbamylation of normal haemoglobin could increase oxygen affinity to a point where oxygen could not be released in peripheral circulation and result in hypoxia at the tissue level. Concentration-related qualitative changes in Hb molecules were demonstrated in erythrocytes exposed to MIC *in vitro* (15). Alteration of the electrophoretic mobility of Hb noticed in this study may reflect qualitative

changes in the Hb but need not necessarily be the result of carbamylation of Hb. The electrophoretic mobility of HbS is known to be altered following treatment with organic isocyanates such as MIC(20). MIC-treated HbS specimens show an increase in cathodic migration, with a mobility similar to that of HbA or HbF. This alteration is thought to be the result of an increase in negative charge, consistent with a reaction of the organic isocyanate with the beta-chain groups. However, no change in Hb electrophoretic mobility could be demonstrated *in vivo* in either MIC-injected or inhalation exposed animals (15). Authors, therefore, concluded that the hypothesis, a chemical alteration in the Hb molecule as an important factor causing death of animals exposed to MIC, was not proved (15).

However, using radiolabelled MIC (Label on isocyanate fraction, CH_3-N=^{14}C=O) and dosing female Wistar rats by inhalation and intraperitoneal routes, Bhattacharya et al (21) noticed that MIC carbamylates globin, blood and liver proteins. The radiolabel was also distributed in its active form in brain, liver, kidney and lung tissues upto 10 days after intraperitoneal injection. Similar pattern was found for animals dosed via inhalation. Methylamine, a breakdown product of MIC did not carry the label. The authors concluded that MIC is capable of crossing the blood tissue barrier, binds proteins and is distributed in its active from throughout the body (21). Carbamylation of proteins and peptides in vitro was also demonstrated by other workers as well (22, 23). Slatter et al studied biotransformation of MIC in the rat and demonstrated that glutathione conjugation was a major metabolic pathway for isocyanates, suggesting that multi-system toxicity could result from the ability of MIC conjugates to revert spontaneously to free MIC under physiological conditions (24).

3. Disseminated intravascular coagulation.

The third hypothesis regarding the cause of death, was intravascular coagulation. Experiments performed by administering liquid MIC intravenously had suggested that intravascular coagulation could occur and that there was an increase in creatine phosphokinase (CPK) (15). Thus, the finding of intravascular coagulation coupled with the observation of increased blood creatine phosphokinase levels in animals suggested that MIC caused a condition of localised intravascular coagulation resulting in myocardial ischaemia and mortality (15). The demonstration of intravascular coagulation in the injected animals also suggested that the formation of microthrombi might be responsible for the gross lung lesion. The microthrombi might have also caused damage to the myocardium. In order to substantiate these findings as evidence of the cause of death from intravascular coagulation there should be histologic evidence of fibrin deposition, an increase in the myocardial isoenzyme creatine phosphokinase (CPK-MB) and a decline in platelet numbers. However, the increase in CPK in animals was not due to an increase in the CPK - MB fraction. Thus, the cause of a significant increase in CPK appears to be due to either generalized muscular injury associated with hypoxia or pulmonary changes associated with oedema (15). These findings coupled with normal platelet numbers and the absence of fibrin deposition in the vessels of the lung do not support the hypothesis that intravascular coagulation is the cause of immediate death (15).

4. Complement systems.

It had been postulated that MIC exposure would cause complement activation leading to adult respiratory distress syndrome and would result in immediate mortality. Activation of complement system is an important patho-physiologic mechanism in adult respiratory distress syndrome (25,26). Since the release of C5a anaphylatoxin can be lethal in certain conditions (27), complement activation by MIC was investigated to determine the role of complement activation in MIC toxicity (28). The *in vitro* exposure of human or guinea pig serum and matched EDTA plasma samples or *in vivo* exposure of guinea pigs to MIC vapour induced profound alterations in the complement system. The complement alterations resulted in reduction of key complement component functional activities (28). The human serum samples exposed to MIC showed significant reductions in Factor B, C2, C4, C3, C5 and total haemolytic complement CH50 activity levels. C6 functional activity was unaffected. However *In vivo* studies in guinea pigs have actually shown that the extent of complement activation was greater in the guinea pigs that did not die compared to that in guinea pigs which died (28). Authors, therefore, suggest that intravascular complement activation, though an important consequence of MIC exposure, may actually help delay the onset of death in these animals (28). There is, thus, no conclusive proof that complement activation is the immediate cause of death.

Increases in haemoglobin and haematocrit as well as reticulocytes observed in animals exposed to MIC vapour may be due to the result of respiratory distress. Neutrophilia observed in both rats and

guinea pigs exposed to high concentrations of MIC vapour may be due to the stress of MIC exposure or the activation of the complement cascade (15, 28, 29).

5. Gas exchange in the lungs.

Guinea pigs exposed to MIC at concentration of 240 to 628 ppm had a marked reduction in PaO_2 and pH and an elevated tracheal pressure during artificial ventilation (30). The low PaO_2 was only slightly elevated when animals were ventilated with 100% O_2, suggesting that some areas of the lung appeared to receive no ventilation but still had abundant perfusion. Thus, MIC inhalation caused severe pulmonary blood shunting and ventilation / perfusion imbalance. This, in turn, led to hypoxaemia, metabolic acidosis and tissue hypoxia which could produce death. The observed functional disturbances in gas exchange are consistent with bronchial or bronchiolar obstruction resulting from sloughed epithelial cells, secretions and other debris, possibly derived from the airways proximal to the obstruction (30). None of the artificially ventilated animals died during the study suggesting that prolonged survival may be possible after MIC exposure if assisted ventilation, oxygen therapy and treatment of the metabolic acidosis are applied at an early enough time (30).

6. Morphologic alteration of the lung.

Rats (at concentrations of 100,600 and 1000 ppm) and guinea pigs (at concentrations of 25,125,225 and 675 ppm) exposed to MIC vapour for short intervals (averaging 15 minutes) had shown changes in the conducting airways of the lung as well as the parenchyma (the alveolar ducts and alveoli). The changes found in the bronchioles consisted of lifting the epithelium in sheets from the basement membrane and necrosis of the remaining epithelial cells. This resulted in an influx of plasma proteins and fibrin deposition leading to plugging of many of the major airways. Deep in the lung, fibrin was found in many of the alveoli, indicating damage to the alveoli (31). The airway obstruction and subsequent atelectasis resulted in changes in the blood gases and pH (30). These changes cause a shift in the oxygen dissociation curve of haemoglobin (32) that very likely caused further tissue hypoxia, leading to the death of the animal. Nimery et al (33) had also reported destruction of epithelia in the upper airways when rats were exposed to varying concentrations of MIC vapour in a static exposure chamber for periods upto one hour.

7. Cyanide poisoning.

Hydrogen cyanide (HCN) has been implicated in the acute injury caused by the gas leak (13). A causative role for HCN in MIC toxicity was put forth because of the observation of the cherry red colour of the blood and viscera of the victims, increased urinary thiocyanate levels in some survivors and reported symptomatic relief by administration of sodium thiosulfate (4,13,34). Blake and Ijadi-Maghsoodi (35) and Battacharya et al (36) had shown that pyrolysis of MIC at high temperatures (350°C to 540°C) resulted in the formation of HCN and other degradation products. However, in *in Vitro*, MIC itself had been shown to produce "cherry-red blood" due to formation of methylamine (37, 38). Using an assay capable of detecting sublethal concentration of cyanide (CN), Bucher et al could not detect any cyanide in the blood of rats exposed to lethal concentration of MIC either immediately, or two days after exposure (39). Survival of rats given single or repeated doses of cyanide antidote (sodium nitrite, sodium thiosulfate) did not differ significantly from controls. Authors concluded that cyanide did not appear to be involved in the acute toxicity of methyl isocyanate (39). Nimery et al (40) and Alarie et al (41) were also not able to substantiate the claim of cyanide poisoning in experiments with laboratory animals.

The above mentioned animal studies suggest that the major cause of immediate death was profound hypoxia resulting from blockage of airways by necrotic epithelial cells, mucus, fluid and fibrin. Even though pyrolysis of MIC at high temperatures produces HCN (35, 36) and other degradation products(3), there are no conclusive proof from experimental studies that HCN is the immediate cause of death. However, experimental studies have demonstrated that MIC crosses the blood-tissue barrier and binds to blood and tissue proteins. Thus MIC inhalation can cause multi-system toxicity.

REFERENCES

1. Dhara R. Health effects of the Bhopal gas leak: a review. Epidemiologia e prevenzione n 1992; *52*: 22-31.

2. Indian Council of Medical Research. Health effects of the Bhopal Gas Tragedy. April, 1986; pp 1-16.(Official document)

3. Council of Scientific and Industrial Research. Report on Scientific studies on the factors related to Bhopal toxic gas leakage. December, 1985; pp 41-90.(Official document)

4. Mehta PS,Mehta AS, Mehta SJ and Makhijani AB. Bhopal Tragedy's Health effects: a review of methyl isocyanate toxicity. J Am Med Ass. 1990; *264*: 2781-7.

5. Narayan T. Health impact of Bhopal Disaster. An epidemiological perspective. Economic and Political weekly 1990; *25*: 1835-46.

6. Smith AB and Zenz C. Methyl Isocyanate. In: Zenz C ed. Occupational Medicine. Principles and Practical applications. Chicago. Year Book Medical Publishers Inc; 1988: 745-6.

7. Occupational Health Guidelines for Methyl isocyanate. Washington,DC: US Dept. of Health and Human Services; September 1979: 1-4. NIOSH Publication 81-123.

8. Union Carbide Corporation. Methyl isocyanate. Technical Report F-41443 A. Union Carbide Corporation, New York, 1976.

9. American Conference of Governmental Industrial Hygienists. Threshold limit values for chemical substances in the work environment adopted by ACGIH with intended changes from 1985-86, ACGIH, Cincinnati, OH, 1985.

10. Kimmerle G and Eben A. Toxicity of methyl isocyanate and how to determine its quantity in air. Arch Toxikol 1964; *20*: 235-41.

11. Karol MH. Respiratory effects of inhaled isocyanates. Crit Rev Toxicol 1986; *16*: 349-79.

12. Dodd DE, Frank FR, Fowler EH, Troup CM and Milton RM. Biological effects of short-term, high-concentration exposure to methyl isocyanate. 1. Study objectives and inhalation exposure design. Environ Health Perspect 1987; *72*: 13-9.

13. Round the world-From our Correspondents, India: Long-term effects of MIC. Lancet 1989; *1*: 952.

14. Brown WE, Green AH, Karol MH and Alarie YC. Inhibition of cholinesterase activity by isocyanates. Toxicol Appl Pharamacol 1982; *63*: 45- 52

15. Troup CM, Dodd DE, Fowler EH and Frank FR. Biological effects of short-term, high-concentration exposure to methyl isocyanate. 2. Blood chemistry and hematologic evaluations. Environ Health Perspect 1987; *72*: 21-8.

16. Lee CK. Methyl isocyanate as an anti-sickling agent and its reaction with haemoglobin. J Biol Chem 1976; 251: 6226-31.

17. Cerami A and Manning JM. Potassium cyanate as an inhibitor of the sickling of erythrocytes in vitro. Proc Nat Acad Sci (USA) 1971; *68*: 1180-3.

18. Cerami A, Manning JM, Gillette PN, De Furia F, Miller D, Graziano JH and Peterson CM. Effect of cyanate on red blood cell sickling. *Fed Proc* 1972; *32*: 1668-72.

19. Gillette P, Peterson CM, Lu YS and Cerami A. Sodium cyanate as a potential treatment for sickle cell disease. N Engl J Med 1974; *290*: 654 - 60.

20. Wilson E and Winter WP. Use of Citrate agar electrophoresis in evaluation of antisickling agents. J Nat Med Assoc 1981; *73*: 111-6.

21. Battacharya BK, Sharma SK and Jaiswal DK. In vivo binding of (^{14}C) methyl isocyanate to various tissue proteins. Biochem Pharmacol 1988; *37*: 2489-93.

22. Pearson PG, Slatter JG, Rashed MS, Han D-H and Baillie TA. Carbamylation of peptides and proteins in vitro by S-(N-methyl-carbamoyl) glutathione and S-(N-methyl carbamoyl) cysteine, two electrophilic S-Linked conjugates of methyl isocyanate. Chem Res Toxicol 1991 ; *4*: 436-44.

23. Ramachandran PK, Gandhe BR, Venkateswaran KS, Kaushik MP, Vijayaraghavan R, Agarwal GS, Gopalan N, Suryanarayanan MVS, Shinde SK and Sriramachari S. Gas Chromatographic studies of the carbamylation of haemoglobin by methyl isocyanate in rats and rabbits. J. Chromatogr 1988; *426*: 239-47.

24. Slatter JG, Rashed MS, Pearson PG, Han D-G and Baillie TA. Biotransformation of methyl isocyanate in the rat. Evidence of glutathione conjugation as a major pathway of metabolism and implications for isocyanate-mediated toxicities. Chem Res Toxicol 1991; *4*:157-61.

25. Andreadis N and Petty TL. Adult respiratory distress syndrome: Problems and progress. Am Rev Respir Dis 1985; *132*: 1344-6.

26. Till GO and Ward PA. Systemic complement activation and acute lung injury. Fed Proc 1986; *45*: 13-8.

27. Hugli TE and Muller-Eberhard H.J. Anaphylatoxins: C3a and C5a. Adv Immunol 1978; *26*: 1-53.

28. Kolb WP, Savary JR, Troup CM, Dodd DE and Tarmerius JD. Biological efects of short-term, high-concentration exposure to methyl isocyanate. VI. In vitro and in vivo complement activation studies. Environ Health Perspect 1987; *72*; 189-95.

29. Hammerschmedt ED, Weaver LJ, Hudson ID, Craddock PR and Jacob HS. Association of complement activation and elevated plasma C5a with adult respiratory distress syndrome. Lancet 1980: *1*: 947-9.

30. Fedde MR, Dodd DE, Troup CM and Fowler EH. Biological effects of short-team, high concentration of exposure to methyl isocyanate. III. Influence on gas exchange in the guinea pig lung. Environ Health Perspect 1987; *72*:29-33.

31. Fowler EH, Dodd DE and Troup CM. Biological effects of short-term, high-concentration exposure to methyl isocyanate. V. Morphologic evaluation of rat and Guinea pig lung. Environ Health Perspect 1987; *72*: 39-44.

32. Maginniss LA, Szewczak JM and Troup CM Biological effects of short-term, high concentration exposure to methyl isocyanate IV. Influence on the oxygen-binding properties of guinea pig blood. Environ Health Perspect 1987; *72*: 35-8.

33. Nimery B. Dinsdale D, Sparrow S and Ray D. Effects of methly isocyanate on the respiratory tract of rats. Br J Indust Med 1985; *42;* 799-805.

34. Acharya VN, Naik SR and Potins AV. Sequential study of thiocyanate levels in Bhopal water following methyl isocyanate gas leakage. J. Postgrad Med 1986; *32*: 192-4.

35. Blake PG and Ijadi-Maghsoodi S. Kinetics and mechanism of thermal decomposition of MIC. Int J Chem Kinet 1982; *14*: 945-52.

36. Bhattacharya BK. Malhotra RC and Chattopadhyay DP.

Inhibition of rat brain cytochrome oxidase activity by pyrolised products of methly isocyanate. Toxicol Lett 1987 *37*; 131-4.

37. Salmon HG. Bright red blood of Bhopal victims: Cyanide or MIC? Br. J Indust Med 1986; *43*: 502-4.

38. Anderson N. Long-term effects of methyl isocyanate. Lancet 1989; *1*: 1259.

39. Bucher JR. Gupta BN, Adkins Jr B, Thompson M, Jameson CW, Thigpen JE and Schwetz BA. Toxicity of inhaled methyl isocyanate in F 344/N Rats and $B6C_3F1$ Mice. 1. Acute exposure and recovery studies Environ Health Perspect 1987; *72*: 53-61.

40. Nemery B, Sparrow S and Dinsdale D. Methyl isocyanate: thiocyanate does not protect: Lancet 1985; *2* 1245-6.

41. Alarie Y. Ferguson JS, Stock MF, Weyel DA and Schaper M. Sensory and pulmonary irritation of methyl isocyanate in mice and pulmonary irritation and possible cyanidellike effects of methyl isocyanate in guinea pigs. Environ Health Perspect 1987; *72*: 159-67.

Correspondece / request for reprints :

Dr. V.K. Vijayan, Deputy Director and Head, Cardio-Pulmonary Medicine Unit, Tuberculosis Research Centre, ICMR, Madras-600 031.